ROLE OF NIGELLA SATIVA IN OPIOID DEPENDENCE

BY

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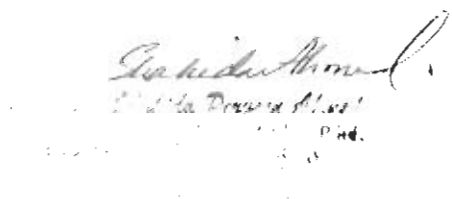

UNIVERSITY OF KARACHI

2004

Dedicated

to

MY TEACHERS

Prof. Dr. Shahida P. Ahmed,

Dr. Kausar Aamir

AND MY CHILDREN

Bilal Badshah, Hadiya Shahzadi, and

Huda Rani

ACKNOWLEDGMENT

All praises be to Almighty Allah the Omnipotent, the Omnipresent whose kindness and help made me able to complete this work and made hardships coming in my way, easy to be ignored. Words are helpless to express thanks and gratitude to Allah

I feel great privilege and pleasure in acknowledging my deepest sense of gratitude to the most inspiring supervisor and a great scholar of Pharmacology Prof. Dr. Shahida P. Ahmed, Professor and Chairperson, Department of Pharmacology, for her encouragement, generous supervision, and valuable suggestions during the course of present study.

I am also thankful to Dr. Kausar Aamir, Chairperson, Department of Pharmacology and Therapeutics, BMSI, JPMC, Karachi, for her kind cooperation in all odds and evens. Without her guidance it would have been difficult for me to start and complete this task.

I also wish to acknowledge for the mutual cooperation of other faculty staff of the Pharmacology department Prof. Dr. Asif Bin Rehman, Dr. Rafeeq Alam Khan, and Raheela Najam to pursue this work on an excellent way.

I feel pleasure to thank my friends and family members for their moral help and encouragement especially Dr. Shah Murad Mastoi and Dr. Poonam Chand, Incharge Rehabilitation Centre, Malir, Muhammad Arshad, ASI Police, and Mr. M. Haroon of the Pharmacology department, University of Karachi, for their valuable support.

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خلاصہ

آپیوئڈ دست نگری (Opioid Dependence) ایک پرانا مرض ہے جو مریض کی ذہنی روش میں ایسی تبدیلیاں پیدا کر دیتا ہے کہ مریض کے دوا کا استعمال ترک کرنے کے بعد بھی طویل عرصہ برقرار رہتی ہیں- یہ التوائی ذہنی تبدیلیاں اس دست نگر شخص کو بیماری کے واپس لوٹ آنے کے خطرے سے دوچار رکھتی ہیں- دوا کے سمی اثرات کی صفائی اور علامات کے خاتمے کا عمل کامیاب ثابت ہوسکتا ہے لیکن درون خرابی کا علاج نہیں ہوتا گویا یہ درست طریقہ علاج نہیں ہے- میتھاڈون (Methadone) اور نالٹریگزان (Naltrexone) سے دیکھ بھال آپیوئڈ دست نگری کے طویل المدتی علاج کی ایک عمومی پریکٹس ہے لیکن ان دواؤں کے اپنے منفی اثرات ہیں کیونکہ کوئی واحد دوا کسی ایک فرد کے آپیوئڈ دست نگری کے علاج میں موزوں نہیں- اس لئے اہم یہ ہے کہ معالجین دستیاب معالجاتی کارندوں کی متفرق اقسام استعمال میں لائیں-

کیلشیم کی ترسیل روکنے والے (Calcium Channel Blockers) مثلاً Nemodipine, Nifedipine, Diltiazam, Verapamil اور Felodipine قلبی شریانی بیماریوں مثلاً بلند فشار خون، دوران خون کی بیماریوں Ischaemic Heart Desease دل کی دھڑکن کے عارضوں Arrhythmias میں مفید ہیں- کیلشیم چینل بلاکرز پر تحقیق سے ثابت ہوا ہے کہ یہ دوائیں متنوع بیماریوں کے علاج کی اہلیت رکھتی ہیں جن میں دمہ، دستوں کی بیماری، قبل از وقت زچگی اور مرکزی اعصابی نظام کی بیماریوں مثلاً مرگی اور آپیوئڈ دست نگری شامل ہیں- جدید دوائیں نہ صرف مہنگی ہیں بلکہ دنیا کی آبادی کی اکثریت کی پہنچ سے باہر ہیں اور ان کے متعدد مابعد اثرات بھی ہیں- اس لئے ایسی دواؤں کی تلاش ضروری ہے جو مقامی ذرائع سے حاصل ہوسکیں اور جن کے مطلوبہ معالجاتی اثرات فطری طور پر قابل مشاہدہ ہوں-

Nigella Sativa (کلونجی) قدیم زمانے سے بہت سی بیماریوں کے علاج میں زیر استعمال ہے- کیلشیم کی ترسیل کو روکنے میں اس کے موثر ہونے پر کافی تحقیقات ہوچکی ہیں- کیلشیم کی ترسیل آپیوئڈ دست نگری کے علاج میں مستعمل ہے اس لئے Nigella Sativa (کلونجی) کو اس مطالعہ میں استعمال کیا گیا ہے- یہ مطالعہ 50 مریضوں پر کیا گیا اور انہیں دو گروہوں میں تقسیم کر دیا گیا- مریضوں کو پہلے 12 دن کے لئے داخل کیا گیا اور اس کے بعد 12 ہفتوں تک فی ہفتہ زیر علاج رکھا گیا-

ہر مریض کو داخلے کے پہلے اور دوسرے دن Placebo منہ زبانی دی گئی اس کے بعد Nigella Sativa (کلونجی) کا استعمال تیسرے دن سے آٹھ ہفتوں کے لئے شروع ہوا- پھر ہر دوا کی خوراک نویں اور دسویں ہفتوں میں گھٹائی گئی اور آخری دو ہفتوں میں کوئی علاج نہیں کیا گیا-

یہ مشاہدہ میں آیا کہ Nigella Sativa (کلونجی) نے قدیم آپیوئیڈ عدم استعمال کی علامت کو تیزی سے درست کرنے میں غیر معمولی تاثیر دکھائی- یہ بھی مشاہدہ میں آیا کہ کلونجی نے مرض کے لوٹ آنے اور دوا کی اشتہاء میں کمی کو نمایاں طور پر روکا- اس سے یہ نتائج اخذ کئے جاتے ہیں کہ کلونجی آپیوئیڈ دست نگری کے طویل مدتی علاج میں موثر ہے اور سفارش کی جاتی ہے کہ اب زیادہ تعداد میں مریضوں پر مزید طویل مدت کے مابعد مطالعے کئے جائیں-

SUMMARY

Opioid dependence is a chronic disorder that produces changes in brain pathways that remain long after the patient stops taking the drug. These protracted brain changes put the dependent person at greater risk of relapse. Detoxification can be successful in cleansing the person of drugs and withdrawal symptoms; it does not address the underlying disorder, and thus is not the adequate treatment. Maintenance with methadone or naltrexone is the usual practice in the long-term management of opioid dependence but both drugs have their own disadvantages because no single medication is appropriate for every individual for treating their opioid dependence, it is important that clinicians have a variety of the therapeutic agents available to them.

Calcium channel blockers, such as verapamil, diltiazem, nifedipine, nimodipine, and felodipine are useful drugs being used in cardiovascular disorders, such as hyper-tension, arrhythmias, and ischaemic heart disease. Research on calcium channel blockers has proved their therapeutic potential in a variety of disorders such as asthma, diarrhoea, premature labour, and diseases of central nervous system such as epilepsy, and opioid dependence. Modern drugs are not only expensive and beyond the reach of majority of the population of world but also have multiple side effects. Hence there is a need to explore such drugs from

indigenous sources and to observe if combination of desired therapeutic efficacy exists in nature.

Nigella Sativa is in use for the treatment of variety of ailments since ancient times. Research has based its many effects on their efficacy of blocking calcium channels. As calcium channels have been tried for the treatment of opioid dependence, so Nigella Sativa was used in this study. This study was carried out on 50 patients who were divided into two groups. Patients were admitted for 12 days and then weekly followed up for 12 weeks.

Each patient received placebo orally during day-1 and day-2 of admission. Thereafter Nigella Sativa was given to the patients from day-3 of admission to eighth week. Then the dose of each drug was tapered off during 9^{th} and 10^{th} weeks and then no treatment was given during last two weeks.

It was observed that Nigella Sativa showed a rapid improvement in signs and symptoms of acute opioid abstinence. It was also observed that Nigella Sativa prevented the development of significant craving and relapse. It is concluded that Nigella Sativa is effective in long term management of opioid dependence and it is suggested that further long term follow up studies may be designed with greater number of patients.

INTRODUCTION

For centuries, people have resorted to narcotics, stimulants, and intoxicants to relieve pain, to minimize anxiety and sorrow, and for sensuous enjoyment. In some cultures the psychic effects of the simple preparations of plants and herbs were attributed to magic or supernatural powers and this belief still prevails in some remote uncivilized areas of the world. As far back as 5,000 BC, the people of the lower Mesopotamia cultivated the poppy plant in order to extract a juice they called "gil" which means "joy" or "rejoicing". Its medicinal properties became known to both Persia and Egypt about 1550 BC and spread throughout the Roman world and later eastern world. It was the Greeks who gave to this wonder-product the name "opion", a diminutive of "opos" which meant "vegetable juice" (Brown, 1961).

Indeed opioids are the oldest psychoactive substance known to humanity. Apparently opium juice was used as a general sedative or sleep was later introduced into China by merchants from the Middle East. It became popular in Europe during the renaissance (Smith et al., 1996).

Opium is obtained from the unripe seed, capsules of "poppy" plant "Papaver Somniferum". The milky juice is dried and powdered to make opium which contains a number of alkaloids. Morphine, the major opioid drug present in opium, was isolated in the beginning of the 19th century.

Throughout this period, opium was viewed as a highly beneficial medicine. One of the earliest warnings against its addictive liabilities was raised by the Chinese emperor Tao Kuang, who banned opium smoking. An attempt to forbid the import of opium into China by the authorities, led to the so-called "opium war" between England and China, with the result that opium trade was permitted. In America, thousands of soldiers developed serious opioid habits during the civil war, when the drug, readily administered through the newly invented hypodermic syringe, was commonly used during treatment of battle injuries (Macht, 1915; Smith et al., 1996).

OPIOIDS

It is the term applying to all agonists and antagonists with morphine-like activity as well as to naturally occurring and synthetic opioid peptides. Opiates are drugs derived from opium and include morphine, codeine and wide variety of semi-synthetic cogeners derived from them and from thebaine, another component of opium. Endorphin is a generic term referring to the three families of endogenous opioid peptides enkephalins, dynorphins, and β-endorphins.

Opioid Receptors

Direct evidence that opioids are recognized by specific receptors came from binding studies by Synder and his colleagues in 1973, though the existence of specific antagonists had earlier suggested that such receptors must exist. Various pharmacological observations implied that

more than one type of receptor was involved, the original suggestions of multiple receptor types arising from in vivo studies of the spectrum of actions (analgesia, sedation, pupillary constriction, bradycardia etc) produced by different drugs. It was also found that some opioids, but not all, were able to relieve withdrawal symptoms in morphine-dependent animals, and this was interpreted in terms of distinct receptor subtypes. The conclusion (Dhawan et al., 1996) from these and many subsequent pharmacological studies, now confirmed by receptor cloning, is that three types of opioid receptor, termed μ, δ, and κ mediate the main pharmacological effects of opiates (Smith et al., 1996).

The first definitive evidence for multiple opioid receptors was obtained by Martin and coworkers who studied the pharmacology of morphine and cogeners in chronic spinal dogs their findings appeared to be consistent with the existence of at least three types of opioid receptors, which they named mu (μ) (for morphine), kappa (κ) (for ketocyclazocine), and sigma (σ) (for SKF-10047, N-allyl-normetazocine). These drugs exhibited quite different pharmacological profiles and were unable to replace each other in the suppression of withdrawal symptoms in dogs treated chronically with one of them, separate receptors appeared to be the simplest explanation (Gilbert and Martin, 1976; Martin et al., 1976).

The discovery of enkephalins by Hugnes and Kosterlitz led to postulate of another receptor type with preference for these opioid

pentapeptide. The first evidence for this comes from work in Kosterlitz's laboratory with isolated organ systems. Enkephalins were much less effective than morphine in inhibiting electrically evoked contractions of the isolated guinea pig ileum, whereas the reverse was true in the isolated mouse vas deferens. The enkephalin preferring receptor that seemed to predominate in the latter tissue was named delta (δ) (for deferens). Further support for this hypothesis came from competition binding studies and from the finding that the receptors in the mouse vas deferens were significantly more resistant to naloxone than those in the guinea pig ileum, i.e. 10 times as much naloxone was required for the same degree of reversal of opioid inhibition of contraction. The receptors that predominate in the guinea pig ileum were similar to Martin's μ receptor and were therefore assumed to be the same receptor (Lord et al., 1977).

The σ receptor is very interesting but is perhaps not truly an opioid receptor, since actions mediated via this receptor are not reversed by the opiate antagonist naloxone an operational definition of opioid receptors that is widely accepted. There is considerable evidence suggesting that the σ receptors are binding sites for another abused drug, phencyclidine, also known as "angel dust" or PCP (Zukin and Zukin, 1979). Several additional types of receptors have been postulated, most notably a specific receptor for β endorphin called ε (Epsilon).

Another receptor for enkephalins, different from δ receptors, called ι (iota), because it seemed to predominate in intestines. Subtypes of opioid receptors, such as μ_1 and μ_2; δ_1 and δ_2; and κ_1 and κ_2 have also been proposed (Schulz et al., 1979; Oka et al., 1980; Dhawan et al., 1996; Narita and Tseng, 1998).

Apart from occurring as separate molecules, brain μ and δ opioid receptors have also been suggested to function as a μ-δ receptors complex. In slices of rat neostriatum, activation of this complex, which displays an affinity profile for opioid ligands different from non-associated μ and δ opioid receptors, has been shown to inhibit dopamine (DA) D1-receptor stimulated adenylate cyclase activity (Schoffelmeer et al., 1992; Rothman et al., 1993; Schoffelmeer et al., 1993).

Some compounds bind to opioid receptors but do not induce analgesia or other effects of opioids. In fact, when given with morphine or other active opioids, they inhibit analgesia and other opioid effects. These substances known as opioid antagonists, include synthetic alkaloids such as naloxone and naltrexone. The structure of these drugs differ from that of morphine and other agonists primarily in that they possess relatively bulky substituents on the pyridine nitrogen. It is believed that these bulky N-substituents prevent normal interaction of compound with an appropriate site on the receptor responsible for the effects of opioids. Therefore, while the antagonist can bind to the receptor, it cannot trigger

the subsequent biochemical processes necessary to mediate the effects of opioids; it can however, prevent the action of endogenously released or exogenously administered opioids. Some opioids have both agonist and antagonistic effects. Several different opioid receptor types are present in brain and are thought to mediate somewhat different pharmacological effects. Mixed agonist, antagonist, such as pentazocine and nalorphine, act at more than one of these receptors.

Finally, several other opioids, known as partial agonist can have both agonist and antagonist effects for a different reason though they induce analgesia, they do not achieve the full analgesic effect seen with morphine and related opioid agonists. Thus when given in conjunction with the latter, they compete with morphine, reducing its effects to some degree. Buprenorphine is one such partial agonist (Smith et al., 1996).

ENDOGENOUS OPIOID PEPTIDES

Like the opioid alkaloids, the endogenous opioid peptides are extremely diverse, and include both natural and synthetic substances. They can be grouped into three major classes: β-endorphin (from endogenous morphine) and related peptides, enkephalins (from the Greek "in the head"), and dynorphin (from the Greek "dynamis" means "power") and related peptides. Members of each class derived from a genetically distinct, larger precursor molecule, that is β-endorphin from propiomelano-cortin (POMC), enkephalins from pro-enkephalin A, and dynorphin from pro-enkephalins B (pro-dynorphin) (Mansour et al., 1988).

The discovery of POMC was of considerable importance. It was the first protein precursor found to give rise to several different and seemingly unrelated biologically active peptides. In addition to being the precursor of the endorphins, this protein gives rise to adrenocorticotrophin (ACTH) and a family of melanocyte stimulating hormones (α, β, and γ-MSH), as indicated by its name coined by Sidney Udenfriend. The intermediate lobe of pituitary is the major source of POMC, and β-endorphin is the major opioid peptide derived from this precursor. It exists mainly in the pituitary gland and the CNS (Robert and Herbert, 1977).

Pro-enkephalin contains one copy of leu-enkephalin, four copies of met-enkephalin, and two copies of c-terminal extended met-enkephalin peptides, a heptapeptide and an octapeptide. Proenkephalin was first discovered in bovine adrenal cortex, where enkephalin bio-synthesis was elucidated by Udenfriend and collaborators. It has been cloned and sequenced from bovine and human tissue prodynorphin, the last of the opioid peptide precursors to be characterized has been isolated from various mammalian tissues, including brain and spinal cord, pituitary, adrenal and reproductive organs. All of the opioid peptides derived from this protein, dynorphin A and B and α neo-endorphins and β neo-endorphin, are C terminal extensions of leu-enkephalin (Comb et al., 1982; Kakidani et al., 1982; Udenfriend and Kilpatrick, 1984).

The anterior and neuro-intermediate lobes of the pituitary gland are major sites of POMC biosynthesis. In brain, there are two distinct nuclei that contain POMC neurons, the arcuate nucleus of the hypothalamus and the nucleus tractus solitarius. Widespread projections from these neurons are present throughout the brain. Pro-enkephalin containing neurons are widely distributed throughout the brain and consists of both local circuits and long projection neuron. Prodynorphin containing cell bodies have a characteristic widespread distribution throughout the CNS. Prodynorphin containing neurons have both short and long projection pathways (van Ree et al., 1999).

ENDOGENOUS OPIOID ALKALOIDS

Although most endogenous opioids are peptides, several laboratories have reported the detection of opioid alkaloids in some mammalian systems. Morphine and codeine have been identified in rat brain and in bovine hypothalamus and adrenal; morphine has also been detected in toad skin. In addition, an enzyme that catalyzes a critical step in the synthesis of morphine in the opium poppy has been detected in rat liver. Since no endogenous opioid peptide with high selectivity for mu-opioid receptors has been reported, it has been proposed that morphine is the endogenous ligand for this type of receptor. However, the physiologic significance of endogenous morphine has not yet been established (Spector et al., 1985; Donnerer et al., 1987; Weitz et al., 1987).

MECHANISM OF ACTION OF OPIOIDS

The opioid peptides are thought to exert their action at neural synapses as either neurotransmitters or neuromodulators. It is likely that the peptides act as neurotransmitters, i.e. by altering (generally decreasing) the trans-synaptic potential, when their receptors are localized pre-synaptically. On the other hand, evidence suggests that many opioid receptors are localized post-synaptically. In this case, the peptides modulate the release of a neurotransmitter, which can be one of the classical amines, such as acetylcholine, norepinephrine, dopamine, or serotonin, or another peptide, such as substance P or neurotensin. All three major types of opioid receptor are coupled to G proteins. G proteins, in turn, can couple the receptors either to second messenger systems or directly to ion channels. It is thought that slower effects of opioids may be exerted via an inhibition of the enzyme adenylate cyclase, which synthesizes the second messenger cAMP (cyclic adenosine 3', 5', monophosphate). The level of cAMP affects the activity of an enzyme that is able to phosphorylate proteins (cAMP activated protein kinase A). The phosphorylation of synaptic proteins would have relatively immediate effects. It is also possible that other proteins that act on gene expression can be phosphorylated, resulting in a down-regulation or up-regulation of gene transcription. This could be responsible for some of the very long lasting changes produced by opiates (Childers, 1991; Simon, 1997).

The rapid effects are most likely due to direct effect on ion channels. μ and δ opioid receptors open potassium channels, which results in reduction of calcium conductance. The activation of κ-receptors found to reduce calcium conductance by closing calcium channels. It has recently been found that all three types of opioid receptors can act by both mechanisms, i.e. they can open potassium channels or close calcium channels (Gross and Macdonald, 1987; North et al., 1987).

DRUG ABUSE/ADDICTION

Tolerance and physical dependence do not imply abuse or addiction. Abuse and addiction are behavioural syndromes that exist along a continuum from minimal use to abuse to addictive use, while tolerance and physical dependence are biological phenomena that can be defined precisely in the laboratory and diagnosed accurately in the clinic (O'Brien, 1996).

Drug abuse may refer to "the use, usually by self-administration of any drug in a manner that deviates from the approved medical or social patterns within a given culture". Drug dependence may be a syndrome manifested by a behavioural pattern in which the use of a given psychoactive drug or class of drugs is given much higher priority than other behaviours that once had higher value. In its extreme form drug dependence is associated with the need for continued drug exposure (compulsive drug use), and it exhibits the characteristic of a chronic

relapsing disorder. Addiction can be regarded as a severe degree of drug dependence that is an extreme on the continuum of involvement with drug use. The system of diagnosis for mental disorders published in DSM-IV (diagnostic and statistical manual-IV) by the American Psychiatric Association (1994) uses the term substance dependence instead of addiction for the overall behavioural syndrome. Substance dependence is defined as "a cluster of symptoms indicating that the individual continuing use of the substance despite significant substance related problems". Withdrawal symptoms and tolerance can be present but are not a condition sine qua non for the diagnosis of substance dependence. Substance abuse, a less severe diagnosis, involves a pattern of adverse consequences from repeated use that does not meet criteria for substance dependence (Edwards et al., 1981; Jaffe, 1990; O'Brien, 1996).

For more than a century, three major hypotheses have been put forth account for continued opioids use. This first is that after a period of opioids use for whatever reason, people became physically dependent and then continue use to avoid the distress of withdrawal. A second hypothesis is that people continue to use opioids because they like the effects (e.g. euphoria) produced. "Positive reinforcing effects" is the preferred term to explain repeated drug taking in absence of physical dependence. The third major hypothesis is that, for some people even on initial are, opioids alleviate some pre-existing dysphoric or painful affective

state. Hence for these individuals opioids use is repeated not because of desire for euphoria but to relieve some psychological distress that is not being caused by withdrawal (i.e. it is used for self-medication) (Jaffe and Jafe, 1989).

The transition from the controlled use to dependence may be referred to as initiation of drug dependence. It has been suggested that initially the use of a particular drug is related to its ability to produce effects of well being and euphoria. Environmental variables and/or individual characteristics contribute to whether or not an individual becomes dependent on the drug. At this point a basic emotional feature may have been altered by repeated drug use, which in turn is responsible for the need to experience the effect of the drug again and again.

This need is basically of a psychic nature. Psychic dependence has been defined as "a condition in which a drug produces a feeling of satisfaction and a psychic drive that requires periodic or continuous administration of the drug to produce pleasure or to avoid discomforts". Besides development of psychic dependence, physical dependence can contribute to compulsive drug use but it is not necessary for continued use. Although the nature of psychic and physical dependence is different, both are considered a prior to result from adaptive changes of neural systems in the brain in response to repeated drug use and/or exposure (Eddy et al., 1965).

TOLERANCE

Tolerance can be defined as the reduction in the response to the drug after repeated administrations, thus a higher dose is required to produce the same effect that was once obtained at a lower dose (O'Brien, 1996).

PHYSICAL DEPENDENCE

Physical dependence is a state that develops as a result of the adaptation (tolerance) produced by a resetting of homeostatic mechanisms in response to repeated drug use. It manifests itself by intense physical disturbances when the administration of drug is suspended (Eddy et al., 1965).

WITHDRAWAL (ABSTINENCE) SYNDROME

The appearance of a withdrawal syndrome, when administration of the drug is terminated, is the only actual evidence of physical dependence. Withdrawal signs and symptoms occur when drug administration in a physically dependent person is abruptly terminated. Withdrawal symptoms have at least two signs: first, the removal of the drug of dependence and second, the central nervous system hyper arousal due to re-adaptation to the absence of the drug of dependence. Pharmacokinetic variables are of considerable importance in the amplitude and duration of the withdrawal syndrome. Withdrawal symptoms are characteristic for a given category of drugs, and they tend to be opposite to the original effects produced by the drug before tolerance developed (O'Brien, 1996).

PROTRACTED WITHDRAWAL

Withdrawal symptoms, in general, are most intense during the first week of cessation of opiate use, yet both subjective and physiologic disturbances can continue for weeks to months after initiation of abstinence "protracted withdrawal", as this phenomenon has been called, is almost always associated with craving for the linquished substances and has important clinical relevance because it may contribute to relapse (Himmelsbach, 1942).

The term "protracted withdrawal" has been used to refer to: (i) a general set of symptoms that are still present after acute withdrawal is completed, (ii) a milder and longer-lasting version of acute withdrawal, and (iii) cellular processes initiated in response to substance use in order to maintain homeostasis in the central nervous system. Symptoms that persist after completion of acute withdrawal are either opposite in direction to the intoxication syndrome or unrelated in direction to the syndrome. Opposite symptoms are milder and longer-lasting versions of acute withdrawal and are time-limited. These opposite symptoms of withdrawal can be reversed by administration of the original substance (Schuckit, 1989).

Unrelated symptoms are associated with chronic exposure to substances in various ways. For example, symptoms of memory impairment on organic mood disorder may reflect lingering toxic effects;

these are not reversible but, rather, are worsened by re-administration of the substance.

Depression or anxiety disorders constitute another form of unrelated persistent post use syndrome. Also, apathy or dysphoria may persist as psychological reactions (i.e. adjustment disorders) that emerge in the context of newly achieved abstinence. These various subjective symptoms may be immediately reversible with the re-introduction of the drug (as in "self-medication") but are not, themselves, related to the pharmacologic action of the drug (Schuckit, 1992).

Evidence for Protracted Mood and Behavioural Symptoms

An important issue in relapse is craving. Craving is defined as a "compelling use" that intrudes upon the drug user's thoughts, affects the user's mood, and compels alteration in his or her behaviour. This compelling use has also been described as an "urgent and overpowering desire" or an "irresistible impulse" to use the substance. Classically, desire for a drug, or craving, has been used as an indicator of withdrawal. Determinants of craving may, at times, be independent of physiologic opiate withdrawal and may arise in response to conditioned cues (Brozarth and Wise, 1984; Wise, 1988).

Evidence for Protracted Physiologic Symptoms

Kolb was the first to report that some narcotic addicts continued to complain of fatigue, palpitation, and restlessness for six to nine months

after acute withdrawal. Himmelsbach (1942) found that three to nine months of total abstinence were necessary for complete recovery of "normal" functions, including weight control, sleep, basal metabolic rate, temperature, respiration, blood pressure, and haematologic variables (sedimentation rate, haematocrit). The abstinence syndrome that emerged following withdrawal after chronic administration of morphine has two phases. The early or primary phase is characterized by an increase in blood pressure, pulse rate, body temperature, respiratory rate, and pupil diameter, and these signs last for four to ten weeks. The protracted or secondary phases first begin to emerge between sixth and ninth weeks after complete withdrawal and persisted through the twenty-sixth to thirtieth week. It is characterized by decreased blood pressure, pulse rate, body temperature, and pupillary diameter (Martin and Jasinski, 1969).

Morphine withdrawal displays aggressive behaviour that can persist for several weeks. This aggressive behaviour is attributed to increase dopaminergic supersensitivity, which is continued in attenuated form for upto four weeks. There is hypersensitivity to dopaminergic and cholinergic agonists and decreased responsiveness to antagonists of the same neurotransmitter systems three to four months after morphine detoxification.

The physiological mechanism implicated in the development of opiate dependence and the expression of withdrawal syndrome consists

of adaptive changes that include processes of homologous regulation, affecting the endogenous opioid system, and heterologous regulation that affect other neurotransmitter systems.

Numerous non-opioid neurotransmitters have been proposed to participate in this heterologous regulation. Thus, changes in catecholaminergic, serotonergic, cholinergic, GABAergic or peptidergic transmission have been reported during chronic opiate administration, and at the moment of spontaneous or naloxone-precipitated morphine abstinence.

Many of the pharmacological and biochemical studies have been focused on one of these neurotransmitters, the noradrenergic system, which seems to have an important role in the expression of somatic signs of opioid withdrawal. This involvement is supported by several lines of evidence based on biochemical changes reported in noradrenergic transmission during opiate dependence and withdrawal and on the pharmacological responses induced on opiate withdrawal by the administration of adrenergic compounds. Much of the evidence of the noradrenergic involvement in opioid dependence has been derived from studies of the locus caeruleus. The locus caeruleus is the largest cluster of noradrenergic innervation of the limbic system, cerebral and cerebellar cortices, and quantitatively smaller source of innervation of hypothalamic, spinal cord and other brain stem nuclei. Multiple receptors, including

opioid and noradrenaline receptors seem to converge onto single effectors and to exert a synergic action in this brain structure thus both mu opioid and α_2 adrenoceptor agonists have been demonstrated to hyperpolarize locus caeruleus neurons by opening membrane potassium channels (Maldonado, 1997).

Acute morphine administration is associated with a decrease of noradrenaline in the brain and adrenaline in the adrenal glands, together with an increased urinary output of catecholamines, suggesting a releasing effect of morphine under acute conditions. During chronic morphine treatment, catecholamines in brain and adrenals tend to return to control levels and the urinary excretion stabilizes at a slightly elevated rate, which is maintained as long as morphine is given. It is suggested that adaptation to long-term chronic exposure of morphine is associated with a reduced liberation of catecholamines, compared to acute conditions, and a stimulated synthesis.

Spontaneous and precipitated morphine withdrawal is associated with a reduction of noradrenaline in the brain, adrenaline in the adrenal glands, and an increased urinary output of catecholamines. During morphine abstinence brain content of 3-methoxy-4-hydroxy-phenerthyleneglycol (MHPG), the principal metabolite of brain noradrenaline is increased in several brain regions innervated by the locus caeruleus and is correlated with the severity of withdrawal behaviour (Crawley et al., 1979; Swann et al., 1983; Charney et al., 1984).

ROLE OF CALCIUM IN OPIOID TOLERANCE AND OPIOID DEPENDENCE

The informational role of Ca^{++} in biologic systems is well established. It is accepted that elevation of the free intracellular calcium levels and binding of calcium to calcium modulated proteins serves to link many membrane-initiated events with cellular responses, considerable interest, therefore, attaches to defining the sources of mobilized calcium and their links to membrane receptors.

Several sources of calcium appear to be used in excitation-contraction coupling including extracellular, membrane-bound and bound or stored intracellular calcium. However, the use of a single source or single translocation route is probably exceptional and within a given tissue, different stimulants may employ different calcium sources. Furthermore, the source of calcium appears often to differ between the individual components (fast and slow) of the response to a single stimulant (Bolton, 1979).

Macromolecular proteins that transverse the lipid bilayer and selectively permit ions to move from one side of this barrier to the other are referred to as channels. It is probable that at least two distinct categories of trans-membrane calcium channels exist. One, the potential-dependent channel (PDC), is activated with decreasing membrane potential and is responsible for action potential generation of other

channel; the receptor operated channel (ROC), may serve to admit calcium with either moderate or no membrane depolarization or with stimulant action in depolarized preparations. The aqueous pore within each channel is provided with a selectivity filter that defines the type of ion that can pass through that type of channel. Since ion channels can be opened or shut, the channel also has voltage sensors, i.e. charged regions of the channel proteins that determine whether the channel "gates" are opened or shut.

When a propagated wave of depolarization approaches the membrane region containing the calcium channel, reduction of membrane potential (a decrease in the electronegativity in the cell interior) causes the activation gate to open, permitting calcium to cross the membrane and pass into the cells. The gate closes when the interior of the cell has again become electronegative, i.e. when the resting level of transmembrane potential has been restored. Since the movement of calcium through these channels is controlled by electrical potentials, they have been termed "voltage dependent channels" (Braunwald, 1982).

Activation of adrenergic receptors does not appear to increase calcium influx by increasing the size of calcium channels on the rates at which their gates open or close, but rather appears to recruit an additional number of active channels. It has been proposed that when endogenous adrenergic nervous activity is low or blocked by an adrenergic receptor

blocker, a certain proportion of the calcium channels are unable to open in response to depolarizing stimulus. According to this theory, physiologic stimuli or drugs that activate adrenergic receptors elevate cyclic AMP levels which facilitate the transfer of a phosphate in ATP to form a phosphoester bond with one of the proteins in an inactive calcium channel, permitting the channel to participate in calcium entry into the cell. As a consequence, adrenergic influences increase calcium influx across the sarcolemma; the channels acted upon by receptor-mediated events are termed "receptor-operated" channels (Braunwald, 1982).

The extracellular concentration of calcium is high. The intracellular concentration of free calcium is low due to membrane pumps and intracellular storage sites. The cytosolic calcium concentration is increased by various contractile stimuli. Thus many hormones and neuro-hormone increase calcium influx through so called "receptor operated" channels, while high concentration of potassium and depolarizing electrical stimuli increase calcium influx through voltage sensitive or potential operated channels. In neurons, some endocrine cells, smooth and cardiac muscle, calcium entry can contribute significantly to the cell action potential. Calcium entry in such cells is an essential link in the processes of stimulus/secretion and stimulus/contraction coupling (Miller and Freedman, 1984).

The evidence suggesting an important role for calcium in the actions of narcotic analgesics has accumulated (Yamamoto et al., 1978).

Morphine has been shown to reduce calcium levels in discrete brain regions of rat (Cardenas and Ross, 1965 cited by Opmeer et al., 1980) and to inhibit synaptosomal calcium uptake in mouse and rat brain (Baeyens et al., 1987).

The antinociceptive response of morphine was antagonized in mice by intracerebroventricular injection of calcium, although this response was potentiated by the calcium chelator EGTA [Ethylenebis (oxyethylenenitrilo) tetraacetate] (Harris et al., 1975).

Since synaptic vesicles are known to contain neurotransmitters and as increased intrasynaptosomal calcium levels are required for neurotransmitter release (Rubin, 1974 cited by Harris et al., 1977), these selective effects on synaptic vesicles suggest that an acute action of morphine might be to interfere with neurotransmitter release (Harris et al., 1977). Indeed, narcotic agonists have been found to inhibit the release of a variety of neurotransmitters, including acetylcholine in guinea pig ileum (Dingledine and Golstein, 1976) and nor-epinephrine in cat nictating membrane (Henderson et al., 1975). As calcium reduces analgesic actions of morphine (Harris et al., 1975), the accumulation of vesicular calcium during chronic morphine treatment could conceivably reduce the inhibitory effects of narcotics on neurotransmitters release, resulting in tolerance (Harris et al., 1977). The opiate withdrawal syndrome may also be related to the elevation of vesicular calcium during tolerance and

dependence development as sudden removal of morphine could lead to increased neurotransmitter release resulting in autonomic hyperactivity, which characterizes opiate withdrawal (Jaffe, 1975). Also in guinea-pig ileum, chronic exposure to opioids may involve the activation of L-type calcium channel, which would indicate that intracellular calcium may be one of the final pathways through which myenteric neurons adapt to the chronic opioid exposure (Garaulet et al., 1996).

Calcium Antagonists Receptors

Receptors binding studies have classified the way in which calcium channel blockers act. Upto now it has been possible to label binding sites most reliably with dihydropyridines such as [H^3] nitrendipine (Murphy et al., 1982; Bolger et al., 1982). Direct comparison of the potencies of numerous dihydropyridines in blocking calcium-induced contractions of smooth muscle and in binding to the same muscle tissue has established that the labeled sites are pharmacologically relevant (Bolger et al., 1982). The relation of the receptor sites to calcium channels was clarified by the observation that [H^3] nitrendipine binding is abolished in the absence of calcium but can be restored by physiological concentrations of calcium or other divalent cations that mimic the calcium actions (Gould et al., 1982).

Pharmacologic studies indicate that the drugs of the verapamil and diltiazem class act at sites different from those of the dihydropyridines. This is consistent with the failure of these drugs to compete directly for [H^3] nitrendipine binding.

CALCIUM CHANNEL BLOCKING AGENTS

The drugs that interfere with the entry of calcium into the cell are variously termed as calcium antagonists; calcium channel blockers, calcium entry blockers and slow channel blockers. They exert their action by blocking calcium movements at the level of the plasma membrane. They have provided the basic scientists with powerful new tools for the study of role of this ion in normal as well as pathologic states and have provided the clinicians with several important new therapeutic agents for use in a variety of diseases (Fleckenstein, 1977; Triggle and Swamy, 1983).

History of Calcium Antagonists

The discovery of calcium antagonists as a new principle of coronary drugs reaches back to 1964, when it was reported that two new compounds, later given the generic names verapamil (Iproveratril) and prenylamine, mimicked the cardiac effects of simple calcium withdrawal. They diminished calcium dependent high energy phosphate utilization, contractile force, and oxygen requirement of the beating heart without impairing the sodium dependent action potential parameters.

In an extensive search for other calcium antagonists, a considerable number of substances that also met these criteria were identified, i.e. D650 (gallopamil), nifedipine, niludipine, nimodipine, perhexiline, fendiline, terodiline.

There has been some terminological confusion in recent years regarding these drugs, besides the original designation "calcium antagonist" introduced in 1969. Certain synonyms were proposed such as "slow-channel blocker", "calcium-channel-blocker", "calcium-entry-blocker", "calcium blocker" etc. All these alternative terms refer to the fact that the fundamental action of these drugs consists of a dose related selective inhibition of the transsarcolemmal inward calcium current through the so-called "slow channels" (Fleckenstein, 1983).

PLANT REMEDIES IN INDIGENOUS MEDICAL SYSTEM

During the last few decades, there has been a resurgence of interest in plants as sources of medicines and of novel molecules for use in the elucidation of physiological/biochemical phenomena. There are a number of reasons for this.

First, there is a genuine expectation in developing countries that their drug problems can be alleviated through a sensible scientific exploitation of medicinal plants, some of which have been used for generations by indigenous populations. Then there is a worldwide green revolution, which is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs. Furthermore, underlying this upsurge of interest in plants, is the fact that many important drugs in use today were derived from plants or from starting molecules of plant origin: digoxin/digitoxin, the vinca alkaloids, reserpine and tubocurarine are some important examples. Plants have also yielded molecules, which are extremely valuable tools in the characterization of enzymes and the classification of receptor systems: physostigmine, morphine, muscarine, atropine, nicotine, and tubocurarine are important examples. Some scientists thus expect that the plant kingdom holds the key to the understanding of complex human biochemistry/pathology and the cure of man's perplexing diseases. The initial optimism, engendered

by the idea that a sophisticated understanding of receptor systems and of the biochemistry of disease would pave the way to predictable drug development, has not been realized. Therefore, laboratories around the world are engaged in the screening of plants for biological activity with therapeutic potential. One major criterion for the selection of a plant for such study is traditional healers' claims for its therapeutic usefulness. It is thus worth reflecting on the cultural environment in which traditional healers use plant remedies, as well as the methods of plant use, in order to strengthen the research design.

CAUSES OF ILLNESS AND THE USE OF PLANT REMEDIES IN INDIGENOUS SYSTEMS OF MEDICINE

Virtually every human society evolved an indigenous health care system to cope with illness. In western technologically advanced societies, traditional pre-science notions of the causes of disease and how to manage it have given way to modern ideas based on scientific biomedical theories. In the less technologically developed societies, traditional modes of thought still dominate the forms of medical practice seen in those societies. It is imperative that we do not ignore the thought processes behind these systems for two reasons. First, it is the continued use of plants for the treatment of disease in these systems that have invigorated our interest in phytotherapy; second, the experience crystallized in cultural

practices of medicine can often be of value in the biomedical scientist's search for understanding of complex aspects of healing.

There is a great variation between traditional societies in their perception of the causes of serious illness, and the extent to which these beliefs are articulated. For example, there are ancient records on traditional Chinese medicine, the Indian ayurvedic and Pakistani unani systems. The theories of these Asian systems have been refined and elaborated over several millenniums. The fundamental concepts of Chinese medicine are embedded in Confucianism and Taoism which by 600 BC stood as two fully evolved philosophies (Chow, 1984). These bodies of knowledge have been systematized so that, for example, Colleges of Ayurvedic Medicine now exist in parts of the world, and acupuncture, practiced in traditional Chinese medicine, is now an accepted method in biomedicine. On the other hand, the African therapeutic systems, though just as ancient, have remained more informal, less organized, based as they are on oral traditions (Okpako, 1991).

In spite of the different levels of articulation, indigenous traditional systems share certain common attributes. The most important of these is the tendency to see man as an integral part of nature and to regard a harmonious relationship with the rest of nature as being essential for good health. Most traditional medical systems therefore emphasize the holistic

nature of their approach to the management of illness. They tend to be concerned, not with specific diseases but with the state of illness, which is believed to be brought about by an imbalance, a disharmony in the elements that govern the integrity of the individual in his/her particular cultural environment. In the Chinese system, this idea is highly developed in the doctrine that illness is caused by an imbalance in the elaborate opposites, yin and yang. Treatment is aimed at restoring the body to a state of harmony. In the African perception, the spiritual component of the human existence is greatly emphasized. Illness is believed to be due to disharmony between the sufferer, his spiritual (ancestors and gods) and social worlds. To re-establish harmony by means of suitable sacrifices and ritual exposure of hidden guilt is therefore a major objective in the treatment of serious illness. The second attribute shared by traditional systems of medicine is the use of plant remedies (herbology). Again the Asian pharmacopoeias of plant remedies are well worked and developed over a period of several millenniums. In these systems, the use of plants in the treatment of illness is in the context of beliefs as to the cause of disease. Therefore, plants are selected not so much on the basis of their chemical constituents, but on the basis of their perceived ability to restore harmony, and often informally, according to the doctrine of signatures. Respectively, little is known of the thought processes underlying the use of plants in traditional African medicine. All one can say with some certainty

is that the use of plants in this system is also within the context of the cultural beliefs as to what causes illness. Therefore, if one is to undertake a phytotherapeutic analysis of the plants used in traditional medicine, one must work with a consciousness of the culture in which the remedies are used.

Selecting Plants for Investigation

The number of species of higher plants on this planet is estimated to be between 370,000 and 500,000. All higher plants elaborate chemical secondary metabolites that are of potential medicinal interest. Therefore, the determination of the criteria for selecting plants for phyto-therapeutic investigation is perhaps as important an exercise as the investigation itself. The following selection criteria is being followed worldwide:

1. Selection Based on Traditional Usage

   This is a popular basis for selecting plants for investigation, especially in societies where traditional medicine of some sort is a major form of health care. The reasons are obvious. The investigation is easily justified, a clear objective is pursued through identification of plant material and there is a suitable biological model.

   If success is claimed traditionally, in the treatment of a particular disease, it is expected to find a chemical constituent in the plant extract with an appropriate pharmacological activity.

This expectation is based on the principle of selective toxicity, which is applicable in modern medicine but not in traditional systems. The extract of the plant is screened not just for biological activity, but also on as wide range of other models as possible. The Madagascar rose periwinkle, which was eventually found to contain the powerful anti-cancer agents, the vinca alkaloids, was reputed to be a cure for diabetes mellitus in traditional medicine. Also the extract of the roots of Fagara xanthoxyloides (originate in Yoruba) was originally investigated for anti-microbial activity, consistent with its use as chewing stick (chewed to a brush and used to clean the teeth), and the observation that its users tended to be free of dental caries; but in laboratory tests the extract showed anti-sickling activity. This then sparked off much research into Fagara for anti-sickling potential, for which the plant is famous today.

2. Poisonous Plants

Poisonous plants are known to indigenous populations, but because these are not usually used as medicines traditionally, searching for drugs in plants does not often include them as a group for phyto-chemical investigation. A search for highly specific and potent compounds that can be used as drugs in modern medicine or as probes for the elucidation of biological phenomena is likely to be more productive among poisonous plants than in plants used regularly traditionally.

Many of the most important drugs of plant origin used in medicine today come from poisonous plants, e.g. tubocurarine (arrow poison); atropine (poison); picrotoxin (fish poison); muscarine (poisonous mushrooms); dicoumarol (poisonous clover); physostigmine (ordeal poison). Other compounds of current medicinal interest obtained from plants are a series of tumour-promoting and pro-inflammatory phorbol esters (from poisonous members of the order Euphorbiales).

The approach in screening extracts of poisonous plants would be to test in a random battery of biological models in search of an interesting activity. The text can be more focused if the nature of the poisoning caused by the plant is known, e.g. muscle paralysis.

Although it is not possible to foresee the therapeutic benefit of this kind of investigation, it is justifiable on scientific grounds or even as explanation for the mechanism of the poisoning and a possible way to counteract it.

Selection Based on Chemical Composition

It may be decided in the laboratory, for example, because of facilities available to it, to extract a certain class of compounds, such as alkaloids, for investigation. Then different species of plants which are known to contain alkaloids, whether or not these have been used

traditionally, are extracted and screened on as wide a range of models as possible. This approach is greatly helped by chemotaxonomic information relating different classes of compounds to different plant species.

Screening for a Specific Biological Activity

Another way to proceed is to decide on a set of pharmacological laboratory models for a disease and test extracts of plants selected according to any criteria on the models for possible therapeutic usefulness in the treatment of the disease. The models may be designed to search for anti-cancer, anti-hypertensive, anti-inflammatory or cholesterol-lowering activity, etc. On the basis of computerized data-banks, it is possible to say that one species of plant is more likely to yield the activity of interest than another. In the absence of a data-bank, plants can be screened at random. Results from such procedures suggest that for anti-cancer activity random selection is no less productive than selection based on traditional claims.

Combination of Criteria

Plants used in traditional medicine and which are also known to contain particular types of compounds, e.g. alkaloids or glycosides, may be investigated. This approach depends on the available chemical expertise and facilities (Williamson et al., 1998).

NIGELLA SATIVA

Nigella Sativa Linn belongs to family Ranulaceae. The herb is widely known in different parts of the world and its seeds are used as condiment. In subcontinent it is known as "kalonji" and its Arabic name is "Habatul Sauda" (Nandakarni, 1976). In the west it is known as "Black Cumin" (Duke, 1992). There is a HADITH of HAZRAT MUHAMMAD (PEACE BE UPON HIM) that, ***"black seed is treatment of every disease but death".***

Based on this Hadith the black seeds are widely used for different ailments. In Arab folk and in South Asia it is used for asthma, chronic headache, migraine, chest congestion, dysmenorrhoea, infection (both fungal and bacterial), obesity, paralysis, hemiplegia, back pain, rheumatism, hypertension, anti-abortion, and gastrointestinal problems, like, dyspepsia, flatulence, diarrhoea.

It has been proved scientifically in in-vitro and in in-vivo studies that volatile oil of Nigella Sativa seeds inhibited the spontaneous movements of rat and guinea pig uterine smooth muscle and also the contractions induced by oxytocin stimulation (Aqel and Shaheen, 1996).

Hexane extract of the seeds of Nigella Sativa Linn prevented pregnancy in Sprague-Dawley rats treated orally at 2 gm/kg daily dose on day-1 to day-10 post coitum (Keshri et al., 1995).

Samples of the expressed fixed oil from different sources of Nigella Sativa seeds were examined by thin-layer and gas chromatography for content of fixed oils and thymoquinone and these substances were tested as possible inhibitors of eicosanoid generation and membrane lipid peroxidation. The crude fixed oil and pure thymoquinone both inhibited the cyclooxygenase and 5-lipoxygenase pathways of arachidonate metabolism in rat peritoneal leukocytes. These pharmacological properties of the oil support the traditional use of Nigella Sativa and its derived products as a treatment for rheumatism and related inflammatory diseases (Houghton et al., 1995).

The effects of the volatile oil of the black seed (Nigella Sativa) on the arterial blood pressure and heart of methane-anaesthetized rats were investigated and the effects were compared with those of its constituent thymoquinone. Administration of volatile oil decreased the arterial blood pressure and the heart rate. The results suggested that volatile oil-induced cardiovascular depressant effects were mediated mainly centrally via indirect and direct mechanisms that involved both 5-hydroxy tryptaminergic and muscarinic mechanisms. The direct mechanisms may be due to the presence of thymoquinone in the volatile oil. The volatile oil seemed to possess the potential of being a potent centrally acting anti-hypertensive agent (EL-Tahir et al., 1993).

The effect of the volatile oil of the black seed Nigella Sativa on the respiratory system of the methane-anaesthetized guinea pig was investigated and compared with those of its constituent thymoquinone. Intravenous administration of volatile oil in the dose range induced dose-dependent increase in the respiratory rate and intra-tracheal pressure.

Removal of thymoquinone from volatile oil may provide a potential centrally acting respiratory stimulant (EL-Tahir et al., 1993).

Nigellone is the carboxyl polymer of thymoquinone, isolated from Nigella Sativa Linn seeds. The polymer is far less toxic but retains much of the pharmacologic properties of thymoquinone, which is the active principle. Investigations, carried out on rat peritoneal mast cells in vitro, show that nigellone in relatively low concentrations is very effective in inhibiting histamine release induced by the secretagogues: antigen in sensitized cells. The mechanism of action seems to be through decreasing intracellular calcium by inhibiting its uptake and stimulating the efflux, and by an inhibition on protein kinase C. There is also indication for a mild inhibition of oxidative energy metabolism contributing to some inhibition of the release (Chakaravarty, 1993).

The active principle of Nigella Sativa seeds containing certain fatty acids was studied for anti-tumour activities against Ehrlich ascites carcinoma (EAC), Daltons lymphoma ascites (DLA), and sarcoma-180 (S-180) cells. In vitro cytotoxic studies showed 50% cytotoxicity to Ehrlich

ascites carcinoma, Daltons lymphoma ascites, and sarcoma-180 cells. Tritiated thymidine incorporation studies indicated the possible action of an active principle at DNA level. In vivo EAC tumour development was completely inhibited by the active principle (Salomi et al., 1992; Medenica et al., 1997).

Filter paper discs impregnated with the diethyl ether extract of Nigella Sativa seeds caused concentration dependent inhibition of gram positive bacteria represented by staphylococcus aureus. Gram negative bacteria represented by pseudomonas aeruginosa and Escherichia coli and a pathogenic yeast candida albicans. The extract showed anti-bacterial synergism with streptomycin and gentamicin and showed additive anti-bacterial action with spectinomycin, erythromycin, tobramycin, doxycycline, chloramphenicol, nalidixic acid, ampicillin, lincomycin, and sulphamethoxyzole trimethoprim combination (Hanafy and Hatem, 1991; Agarwal et al., 1979; El-Fatatry, 1975).

Anticestodal effect of Nigella Sativa seeds was studied in children infected naturally with the respective worms. The activity was judged on the basis of percentage reduction in the faecal eggs per gram (EPG) counts. Single oral administration of 40 mg/kg of Nigella Sativa, equivalent amount of its ethanolic extract and 50 mg/kg of niclosamide reduced the percentage of EPG counts not significantly different from each other. Therefore, it is conceivable that the plant contains active principle effective

against nematodes and cestodes. The crude drug did not produce any adverse side effects in the doses tested (Akhtar and Riffat, 1991).

It is reported that plant mixture extract comprising of Nigella Sativa, Mynh, Gum olibanum, Gum asafoetida, and aloe to have a blood glucose lowering effect. In study with streptozotocin diabetic rats, focused on the mechanism of action, specifically on (a) hepatin gluconeogenesis, (b) activity of ketogluconeogenic enzymes, pyruvate carboxylase, and phosphoenol pyruvate carboxykinase. Similar studies using a biguanide, phenformins, have been conducted to compare the mode of action of these two components.

The rate of gluconeogenesis in isolated hepatocytes as well as activity of PC and PEPCK in liver homogenates is significantly lowered following treatment with the plants extract. Anti-diabetic action of the plant extract may at least partly be mediated through decreased hepatic gluconeogenesis. The extract may prove to be a useful therapeutic agent in the treatment of non-insulin dependent diabetes mellitus (Al-Awadi, 1991).

Nigella Sativa is used in Arab folk medicine as a diuretic and hypotensive plant. An oral dose of Nigella Sativa extract and furosemide increased significantly, the diuresis by 16 and 30 percent respectively after 15 days of treatment; urinary excretion of chlorine, sodium, and potassium and urea is also increased. Simultaneously, the mean arterial pressure

decreased respectively by 22 and 18% in the Nigella Sativa treated rat and nifedipine treated rat. The diuretic activities observed in the spontaneously hypertensive rat treated with Nigella Sativa seeds may be partially responsible for its diuretic action; it seems that other pathways may also be involved in their cardiovascular effects (Zaui et al., 2000).

Thymoquine, the active constituent of Nigella Sativa, was tested in isolated rat hepato-protective agent against tert-butyl hydro-peroxide toxicity. TBHP was used to produce oxidative injury in isolated rat hepatocytes and caused progressive depletion of intracellular glutathione, loss of cell viability as evidenced by trypan blue uptake and leakage of cytosolic enzymes, alanine transaminase and aspartic transaminase. Pre-incubation of hepatocytes with ImM of either thymoquinone or silybin, which is a known hepato-protective agent, resulted in the protection of isolated hepatocytes against TBHP induced toxicity evidenced by decreased leakage of ALT and AST. Both thymoquinone and silybin prevented TBHP induced depletion of glutathione to the same extent (Daba and Rehman, 1998).

The antinociceptive effects of Nigella Sativa oil and its major component, thymoquinone, were examined in mice the P.O. administration of Nigella Sativa oil dose-dependently suppressed the nociceptive response. The results suggest that Nigella Sativa oil and thymoquinone produce antinociceptive effects through indirect activation

of the supraspinal mu (1) and kappa-opioid receptor subtypes (Fatah et al., 2000).

Phytochemical studies on seeds revealed the presence of volatile oil (1.5%), fixed oil (37.5%), nigellin, melanthin and thymoquinone. The volatile oil consists mainly of carvone (45-60%), carvene, cymene and thymoquinone (Salama, 1973). Nigellidine, nigellimine, and nigellicine are the alkaloids isolated from the black seeds, these are devoid of pharmacological effects (Rehman et al., 1985, 1992, 1995).

The crude extract of Nigella Sativa seeds was studied in vitro for its possible spasmolytic and bronchodilator activities to rationalize these medicinal uses. In isolated rabbit jejunum preparations, Ns.Cr caused a dose-dependent relaxation of spontaneous contraction. Ns.Cr also inhibited potassium-induced contractions in a similar dose range, suggestive of calcium channel blockade. The data indicate that the crude extract of Nigella seeds exhibits spasmolytic and bronchodilator activities mediated possibly through calcium channel blockade and this activity is concentrated in the organic fraction (Gilani et al., 2001).

The seeds of Nigella Sativa contain a yellowish white volatile oil (0.5-1.6%) fixed oil (35.6-41.6%), proteins, aminoacids e.g. albumin, globulin and valine reducing sugars, mucilage, alkaloids, organic acids, tannins, resins, toxic glucoside, melarbin, bitter principles, glycosidal saponins, melanthin resembling helleborin, melanthigenin (1%), ash, moisture, and Arabic acid.

The seeds have also been found to contain fats, crude fibre, minerals e.g. Fe, Cu, Zn, P, Ca and vitamins like thiamine, niacin, pyridoxine and folic acid, they also possess nutritional value (Takruri and Dameh, 1998).

Nigella Sativa seeds yield esters of fatty acids, e.g. oleic acid, linoliec acid, and dehydrostearric acid, higher terpenoids, aliphatic alcohols, and α-β unsaturated hydroxyl ketones (Kapoor, 1990).

Free sterols, steryl esters, steryl glucosides and acylated steryl glucosides were isolated from the seed oil (Menounos et al., 1986).

A novel alkaloid, nigellicine, an isoquinoline alkaloid, nigellimine, and an indazole alkaloid, nigellidine, were also isolated from the seeds of Nigella Sativa (Rehman et al., 1985, 1992, 1995).

The active constituents of the seeds include the volatile oil consisting of carone, and unsaturated ketone, terpene or d-limonene also called carnone and cymene (Kapoor, 1990). The crystalline active principle, nigellone, is the only constituent of the carboxyl fraction of the oil. Pharmacologically active constituents of the volatile oil are thymoquinone, dithymoquinone, thymohydroquinone, and thymol (Ghosheh et al., 1999).

Keeping its rich composition and traditional uses in view, it has been extensively researched scientifically and many of its traditional uses have been verified, and its mode of action in connection of several biological abnormalities has been found.

Since the ancient times, the plant is being used for several ailments, as in infectious diseases or metabolic disorders. It has also been used traditionally as spice, carminative, condiment, aromatic, stimulant, diuretic, stomachic, liver tonic and digestive. It has also been found useful in loss of appetite, vomiting, and puerperal diseases. It is also used commercially as emmenagogue and galactagogue and stimulant of uterine contractions.

It is also used as natural remedy for amenorrhoea and dysmenorrhoea. It has remained in use for hepatic and digestive disorders as well as in chronic headache and migraine. Its traditional uses also include obesity, dyspnoea, eczema, pityriasis, mercury poisoning, sores and leprosy (Evans, 1996). It is also given in leucoderma, alopecia, eczema, freckles, and pimples (Usman et al., 1997).

PURPOSE OF STUDY

Drug addiction (drug dependence) is a serious health problem, in addition to the huge direct health cost (psychiatric and physical), there are massive costs in terms of crime, loss of earnings and productivity, and social damages. Reducing the extent of drug dependence is one of the major goals of medicine (Nutt, 1996).

Physical dependence and tolerance are hallmarks of opioid type of drugs. It is characterized by abstinence syndrome when opioid intake is abruptly terminated or when opioid antagonist is being used. Nowadays anti-psychotics (chlorpromazine and thioridazine) and anti-depressants (amitriptylline) are commonly used for controlling opioid abstinence syndrome in different hospitals at Karachi but their efficacy is scant. There is as yet no truly safe and effective treatment for the opioid dependent patients to manage withdrawal and craving, and certainly none that has universal applicability (Shulman et al., 1998).

Because no single medication is appropriate for every individual for treating their opioid addiction, it is important that clinicians have a variety of the therapeutic agents available to them. Rational medication therapy begins with an understanding of not only the disease state generally, but also the specific dynamics of addiction process that affect the overall success of treatment (Greenstein et al., 1997).

Opioid detoxification/maintenance therapy remains the primary pharmacological approach for the treatment of opioid dependence. At

present only two medications, methadone and naltrexone are currently approved for the treatment of opioid addiction. However, detoxification from methadone maintenance is slow and frequently accompanied by minor abstinence syndrome. Also opiate agonists have their own potential for abuse and withdrawal symptoms. Naltrexone is a competitive antagonist at the mu-opioid receptors and is used to maintain abstinence in detoxified opioid addicts. It has found limited acceptance by population targeted for treatment, and its successful utilization generally requires highly motivated patients. Hence it necessitates use of a non-opiate treatment for opioid detoxification and dependence.

As Nigella Sativa has been found to have calcium channel blocking ability and calcium channel blockers have been proved to be effective in opioid withdrawal syndrome so purpose of present study was to evaluate the effects of Nigella Sativa in opioid dependence.

MATERIALS AND METHODS

MATERIALS AND METHODS

This study was carried out at the Drug Rehabilitation Centre, RHC Murad Memon Goth, Malir, Karachi and in the Department of Pharmacology, University of Karachi, under the supervision of Prof. Shahida P. Ahmed, Head of the Department of Pharmacology, University of Karachi.

A total of 50 male opiate addicts who were seeking treatment for opioid dependence were consecutively admitted between September 2001 to September 2003. All were admitted for 12 days to treat acute opiate withdrawal syndrome and then treated for opioid dependence as outpatients for 12 weeks.

SELECTION OF PATIENTS

All patients in the study were selected according to the following criteria:

Inclusion Criteria

1. Males between 21 and 45 years of age seeking treatment for opioid dependence for a duration of three months.
2. Following routine clinical criteria indicating opioid addiction were observed:
    a. Self reported duration of opioid dependence of at least four months.
    b. An average of two or more episodes of opioid use per day.

c. Physical evidence of recent intravenous drug use (tracks).

d. Urine toxicology positive for opiates at entry to the study.

e. A rating of two or greater on a self reported level of withdrawal scale 12 hours after the last opioid use.

3. Ability and willingness to give informed consent.

Exclusion Criteria

1. Psychiatric illness e.g. anxiety, neurosis, phobia, obsessive compulsive neurosis, and hysteria.
2. Self reported current dependence on alcohol or other major drug of abuse like sedatives, hypnotics (including benzodiazepines), cocaine, or amphetamines.
3. Acute liver or cardiovascular diseases.
4. Current enrolment in an opiate treatment programme.
5. Any debilitating disease (Mendelson et al., 1996).

MATERIALS

1. Dried black seeds of Nigella Sativa (Kalonji) were purchased from Majeed Brothers, Lajpat Road, Hyderabad, and were cleaned off from adulterant materials and were ground with an electric grinder into coarse powder.
2. Empty capsules manufactured locally were purchased from open market.

3. The frontline opiates test strips obtained from Boehringer Mannheim Pakistan (Pvt) Ltd., Ch-B/Lot No. 28739531 and expiry in February 2004.

Treatment Schedule

The selected patients were divided into two groups:

Group-I

Twenty five patients with opioid dependence were kept on 500 mg Nigella Sativa orally TID.

Group-II

Twenty five patients with opioid dependence were kept on one gram Nigella Sativa TID.

The patients received single blind placebo capsule (orally) containing ferrous sulphate powder of same colour, size and shape for the drug, during day-1 and day-2 of admission. They were observed and rated for the presence and absence of opioid withdrawal signs and symptoms experienced during the previous 24 hours by an observer.

Thereafter each treatment, group received single blind capsule, containing either 500 mg or one gram Nigella Sativa on day-3 of admission (treatment day-1). After the initial administration, patients with a positive response to the drug in terms of opiate withdrawal signs and symptoms and without producing side effects were given the drug upto day-12 of admission (treatment day-10). Diazepam 5 mg was prescribed

for some patients having body aches. The ratings covered the withdrawal signs and symptoms during the previous 24 hours. All the patients received their respective treatment upto day-12 of admission during their stay in hospital. After that patients were discharged on the same treatment and advised to attend OPD weekly upto twelve weeks.

GENERAL PLAN OF THE STUDY (PROTOCOL)

The study was carried out according to the following protocol:

1. Permission was obtained from the Incharge Medical Officer of the Rehabilitation Centre, R.H.C., Memon Goth, Karachi.
2. All the patients met inclusion and exclusion criteria for admission to the study.
3. Consents were obtained from all patients before they were enrolled in the study.
4. On entry in the study, the patients received complete physical examinations including electrocardiograms and laboratory screening tests (complete blood cell count, serum chemistry for hepatic functions and urine analysis) to exclude any pathology.
5. All the patients were admitted to the hospital for 10 days for the treatment of acute opiate withdrawal syndrome.
6. The inpatient records of each group were recorded on a proforma (Appendix-I).

7. The severity of opioid abstinence syndrome of each patient during admission and during follow up was recorded on a proforma especially designed for this study (Appendix-II).

8. All the patients were physically dependent on heroin, with a model dose range "between ¼ and ½ grams" (street doses) per day.

9. To ensure that the patients during the protocol did not covertly ingest other drugs. They were confined to a locked inpatient unit, and visitors were restricted. However, patients could discontinue their participation in the protocol and leave the unit at any time on request without prejudice to their future treatment.

10. Patients were divided into two groups. One group comprising 25 opiate addicts was treated with 500 mg Nigella Sativa and the other group comprising of 25 opioid addicts was treated with one gram Nigella Sativa.

11. The patients received single blind placebo capsule for each drug during day-1 and 2 of admission and they were observed and rated for the presence or absence of opioid withdrawal signs and symptoms experienced during the previous 24 hours.

12. On day-3 of admission single blind treatment with Nigella Sativa was assigned in a random manner.

13. After the initial Nigella Sativa administration, patients with a positive response to drug in terms of opiate withdrawal signs and symptoms and without producing side effects were given drug upto day-12 of admission.
14. Urine samples were collected, on day-1 and 12 of admission and tested immediately for opioids by test strips.
15. Patients were discharged after 15 days of admission and then assessed weekly upto twelve weeks.
16. Each patient received the treatment for eight weeks and then the doses of drug were gradually tapered off during next two weeks that is weeks-9 and 10.
17. During last two weeks, that is, weeks-11 and 12, patients were assessed without given any drug.
18. Urine samples were tested for opioids on weeks-4, 8 and 12 during the follow up period.
19. This study was carried out on 50 patients. Total period of study was two years.

Data was statistically evaluated.

METHOD

Measures

The selected patients were enrolled, data and progress of the patients were recorded as per Appendix-II, which includes the parameters for abstinence as well as protracted withdrawal for opiate dependence.

Subject-Reported Measures

It was in the form of modified subjective opiate withdrawal scale (MSOWS), which contained 38 opiate withdrawal symptoms (Appendix-II). Subjects indicated the degree to which they had experienced each symptom during the past 24 hours on a five point scale in which 0=not at all, 1=a little, 2=moderately, 3=quite a bit, and 4=extremely (maximum possible total score was 152). The ratings for individual item were summed for a total score each scale (Hiltunen et al., 1995).

Observer Rated Measures

It was in the form of objective opiate withdrawal scale (OOWS, Appendix-II) containing 18 observable physical signs. An independent observer observed and rated the presence and intensity of signs on a five point grade scale in which 0=not at all, 1=a little, 2=moderately, 3=quite a bit, and 4=extremely (Hiltunen et al., 1995).

PHYSIOLOGICAL PARAMETERS

It includes the pulse rate, systolic blood pressure, diastolic blood pressure, temperature, respiratory rate, body weight, and caloric intake (Appendix-II) (Martin and Jasinski, 1969).

URINE ANALYSIS MEASURES

Urine samples were collected on days-1 and 12 of admission and then on weeks-4, 8 and 12. All samples were collected under staff observation to deter bogus urine samples and tested immediately for opioids by using one-step dip and read chromatographic test strips (Frontline opiates test strips).

Principle

The test is used for the immunological semi-quantitative detection of opiates in urine. The test principle is based on the GLORIA (Gold labeled Optical-read Rapid Immunoassay) technology. During immersion the strip absorbs the urine volume necessary for chromatography. The urine passes through a zone containing soluble antibody – gold conjugate that binds specifically to opiates. Excess conjugate is retained by an intercepting zone of immobilized morphine so that only the gold conjugate loaded with opiate metabolites reaches the detection zone, which develops a colour between cream.

Ingredients

One test contains:

i. 3.5 μgm monoclonal antibodies to opiates labeled with colloidal gold.

ii. 8.2 μgm morphine polyhapten

TEST PROCEDURE

Test strip is dipped into the urine sample such that the fluid level is between the two blue marks. After 3 to 5 seconds test strip is withdrawn and placed horizontally for chromatography. The result can be read after two minutes. The resulting colour is estimated by using a comparison scale (downward reading). The test strip offers three ranges for an estimation of the concentration on present in the sample:

- < 200 ng/ml negative
- > 200 ng/ml cut off, positive
- >1000 ng/ml highly positive

Stability

Frontline opiates are stable upto the expiry date as specified when stored at +15°C to +25°C.

Interference

Urine samples with acidic values (pH <3) may lead to false negative reading. Urine samples with alkaline values (pH >10) may lead to false positive readings.

STATISTICAL ANALYSIS

The mean was calculated by adding up the observed values and dividing by the total number of observations. This is expressed by the following symbol:

$$\bar{x} = (\sum x) / n$$

Where

$\bar{x}$ signifies the mean, **x** is each of observations, n is the number of observations, $\sum$ the Greek capital sigma denotes "sum of".

Standard deviation (SD) can be calculated by formula:

$$SD = \sqrt{\frac{\sum x^2 - (\sum x)^2 / n}{n-1}}$$

Where

x = Individual observation
n = Number of individual values in series
$\sum$ = Sum of

Standard error of mean (SE) or (SEM) was calculated by the formula:

$$SE = SD / \sqrt{n}$$

Where

SD = Standard deviation
n = Number of observations

Student's 't' test

The statistical significance of difference between the mean values of the two groups was evaluated by student's 't' test.

The value of 't' was calculated by the formula:

$$t = x_1 - x_2 / \sqrt{(SE_1)^2 + (SE_2)^2}$$

Where

$x_1 > x_2$

x_1 = Mean of one group of observations
x_2 = Mean of second group of observations
SE_1 = Standard error of x_1
SE_2 = Standard error of x_2

Degree of freedom (d.f) was calculated as:

$$d.f. = n_1 + n_2 - 2$$

Where

n_1 = Number of observation in one group
n_2 = Number of observation in second group

P Value

The degree of probability was computed by comparing the calculated value of 't' with tabulated values in the table of 't' distribution against the degree of freedom (d.f). The difference in the mean values of the two groups was regarded statistically significant if the P value was equal to or less than 0.05 and non-significant (NS) if the P value was greater than 0.05. It was highly significant if the P value was less than 0.001.

OBSERVATIONS AND RESULTS

OBSERVATIONS AND RESULTS

Total 75 patients were enrolled in the study; 40 patients were in group-I and 35 patients in group-II. Out of these 15 (37.5%) patients were dropped out from group-I during third and fourth weeks of treatment. In group-II, 10 (28.7%) patients were dropped during first and second weeks of treatment.

All patients were men ranging in age from 21-44 years ($x = 33.152 \pm 0.421$). They all expressed interest in discontinuing the use of opioid and gave written consents to join the study that required an abrupt withdrawal from opioids after admission to hospital. They had a mean of 4.957 ± 0.553 years history of opioid consumption (range 1-15 years). All had subjective symptoms and objective signs of opiate withdrawal and urine specimens showing positive results when tested with frontline opiates dipstick-subjects of Nigella Sativa 500 mg and 250 mg treatment showed minor adverse effects.

Our final analysis applies to 50 patients who completed the protocol and were divided at random into two groups. Each group had 25 patients.

- Group-I :: Nigella Sativa 250 mg orally T.I.D.
- Group-II :: Nigella Sativa 500 mg orally T.I.D.

Some patients in both the groups received Diazepam 5 mg for night time sedation.

The results in tables and in appendices show a cumulative score of opiate withdrawal signs and symptoms on day-1 to day-12 of admission and then on week-1 to week-12 as outpatient. The degree of withdrawal signs and symptoms were assessed according to the scoring system described in methodology.

GROUP-I

Twenty-five patients were given a placebo treatment on day-1 and day-2 of admission. Thereafter from day-3 to day-12 of admission the patients received 25 mg Nigella Sativa orally, three times daily.

Placebo had no significant effect on cumulative scores of symptoms of acute withdrawal from opioids. A mean score of 39.4±7.48 was obtained on day-1, which had reached to a peak of 55.08±12.65 on day-3 of admission. However admission of Nigella Sativa 250 mg produced a decrease in opiate withdrawal symptoms from 55.08±12.65 on day-3 to 29.4±5.02 on day-12 of admission (Table-5, Figure-1, Appendix-VII).

TABLE - 5

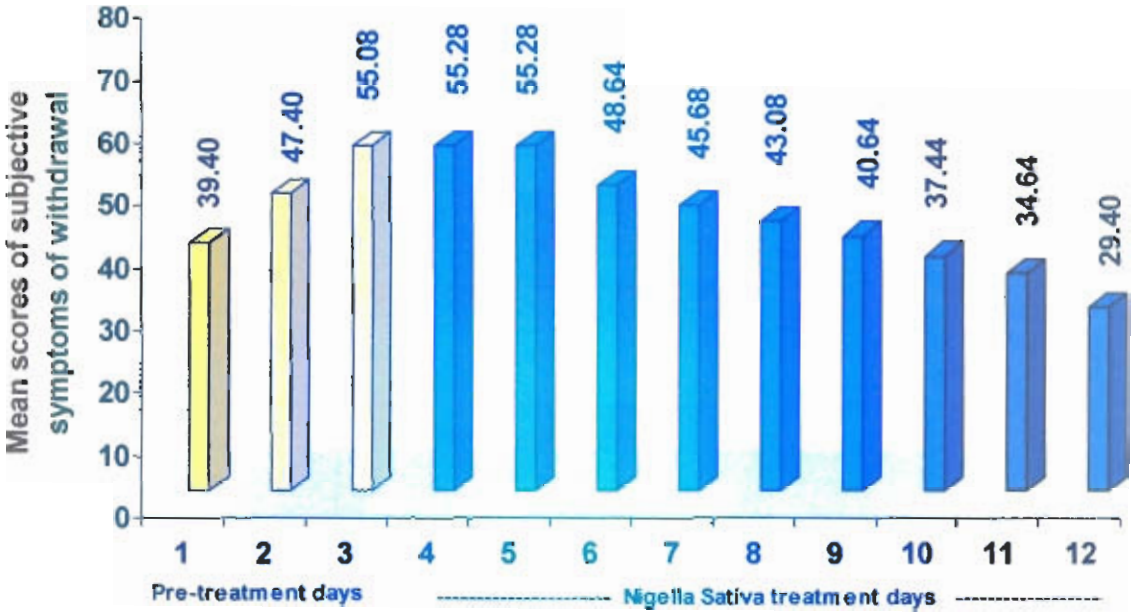
Effects of Nigella Sativa Treatment on Subjective Symptoms of Withdrawal from Opioids in Group-I Patients during 12 days stay in Hospital

	Pre-treatment Day			Nigella Sativa Day								
	1	2	3	4	5	6	7	8	9	10	11	12
Mean ± SEM Scores of Subjective Symptoms of Withdrawal	39.40 ±7.98	47.40 ±7.50	55.08 ±12.65	55.28 ±6.58	55.28 ±6.58	48.64 ±6.54	45.68 ±6.77	43.08 ±6.52	40.64 ±5.91	37.44 ±6.04	34.64 ±5.37	29.40 ±5.02

- Numbers indicate the mean ± SEM scores of 38 symptoms reports of all ratings in a total of 25 patients on each admission day vid e Appendix-VII.

FIGURE - 1

Effects of Nigella Sativa 250 mg Treatment on Subjective Symptoms of Withdrawal from Opioids in Group-I patients during 12 days Stay in Hospital



Effects of Nigella Sativa treatment on the mean±SEM scores of 38 symptoms of withdrawal of the 12 days sample in 25 opioid addicts at days after the third in patient day of withdrawal. Students' t-test comparing change in symptoms from pre-treatment inpatient day-3 to treatment day-12 indicated trend decrease in symptoms to be reported on day-12 of admission.

Similarly placebo had no significant effects on the cumulative score of signs of acute withdrawal from opioids. A mean score of 14.92±3.35 was obtained on day-1 which reached a peak of 19.76±4.58 on day-3 of admission. However, admission of Nigella Sativa 250 mg produced a rapid decrease in opiate withdrawal signs from 19.76±4.58 on day-3 12.12±2.60 on day-12 of admission (Table-6, Figure-2, Appendix-VIII).

TABLE – 6

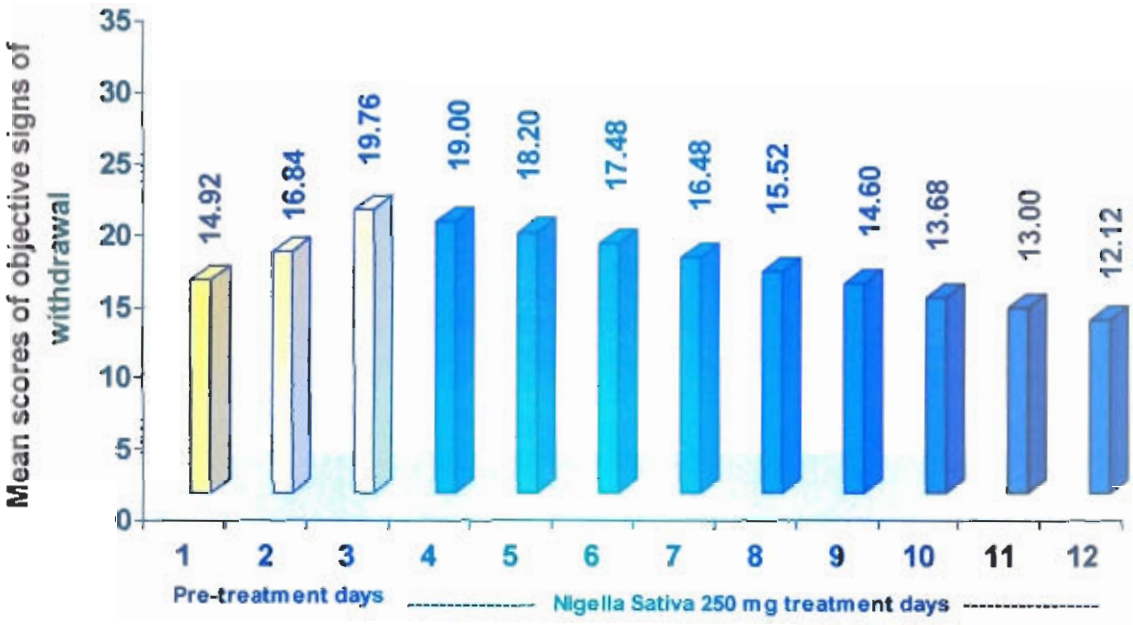
Effects of Nigella Sativa 250 mg Treatment on Objective Signs of Withdrawal from Opioids in Group-I Patients during 12 days stay in Hospital

	Pre-treatment Day			Nigella Sativa Day								
	1	2	3	4	5	6	7	8	9	10	11	12
Mean ± SEM Scores of Objective Signs of Withdrawal	14.92 ±3.35	16.84 ±3.35	19.76 ±4.58	19.00 ±4.22	18.20 ±4.00	17.48 ±3.85	16.48 ±3.54	15.52 ±3.46	14.60 ±3.26	13.68 ±2.76	13.00 ±2.79	12.12 ±2.60

- Numbers indicate the mean ± SEM scores of 18 signs reports of all ratings in a total of 25 patients on each admission day vide Appendix-VIII.

FIGURE – 2

Effects of Nigella Sativa 250 mg Treatment on Objective Signs of Withdrawal from Opioids in Group-I patients during 12 days Stay in Hospital



Effects of Nigella Sativa treatment on the mean±SEM scores of 18 signs of withdrawal of the 12 days sample in 25 opioid addicts at days after the third inpatient day of withdrawal. Students' t-test comparing change in signs from pre-treatment inpatient day-3 to treatment day-12 indicated trend decrease in symptoms to be reported on day-12 of admission.

The physiological parameters including pulse rate, systolic blood pressure, diastolic blood pressure, temperature and respiratory rate were decreased significantly from day-3 to day-12 of admission. There were also increase in body weight and caloric intake from day-3 to day-12 of admission, while the pupillary diameter was increased from 3.28±0.35 on day-3 to 2.70±0.32 on day-12 of admission (Table-6A).

Urine toxicology was also decreased significantly from day-3 to day-12 of admission.

TABLE - 6A

Comparison of Abstinence of Heroin in Group-I patients treated with Nigella Sativa 250 mg Day-3 versus Day-12 Scores of Symptoms and Signs and Physiological parameters

	Day - 3	Day - 12	P value	Percentage
Mean ± SEM scores of subjective symptoms of abstinence	55.08 ± 12.65	29.40 ± 5.02	0.179	53.37
Mean ± SEM scores of objective signs of abstinence	19.76 ± 4.58	12.12 ± 2.60	0.236	61.33
Physiological parameters:				
1. Pulse rate (per minute)	89.52 ± 12.27	82.28 ± 11.90	0.674	91.91
2. Systolic blood pressure (mmHg)	113.68 ± 14.79	103.44 ± 12.02	0.593	99.99
3. Diastolic blood pressure (mmHg)	76.40 ± 8.10	69.20 ± 8.97	0.554	90.57
4. Temperature (°F)	99.13 ± 1.03	98.22 ± 0.73	0.475	99.08
5. Respiratory rate (per minute)	23.36 ± 1.70	21.76 ± 2.08	0.554	93.15
6. Body weight (kilogram)	53.72 ± 7.69	53.32 ± 8.29	0.972	99.25
7. Pupil diameter (mm)	3.28 ± 0.35	2.70 ± 0.32	0.242	82.31
8. Caloric intake (kilo calories)	1.91 ± 0.41	1.98 ± 0.39	0.902	103.66

Nigella Sativa 250 mg treatment regimen used in this study shows a significant effect on withdrawal from opioids as substantiated with clinical condition, physical appearance, and the cumulative scores of withdrawal signs and symptoms daily (Appendices-VII and VIII).

The patient was discharged on the same treatment after 12 days of admission and advised to attend OPD weekly for the follow up.

The cumulative scores of symptoms of protracted abstinence was decreased from 55.08±12.65 on day-3 to 1.16±1.84 after 12 weeks of follow up (Table-7, Figure-3). Similarly, the cumulative score of signs of protracted abstinence was decreased from 19.76±4.58 to 0.20±0.64 after 12 weeks of follow up (Table-8, Figure-4).

TABLE – 7

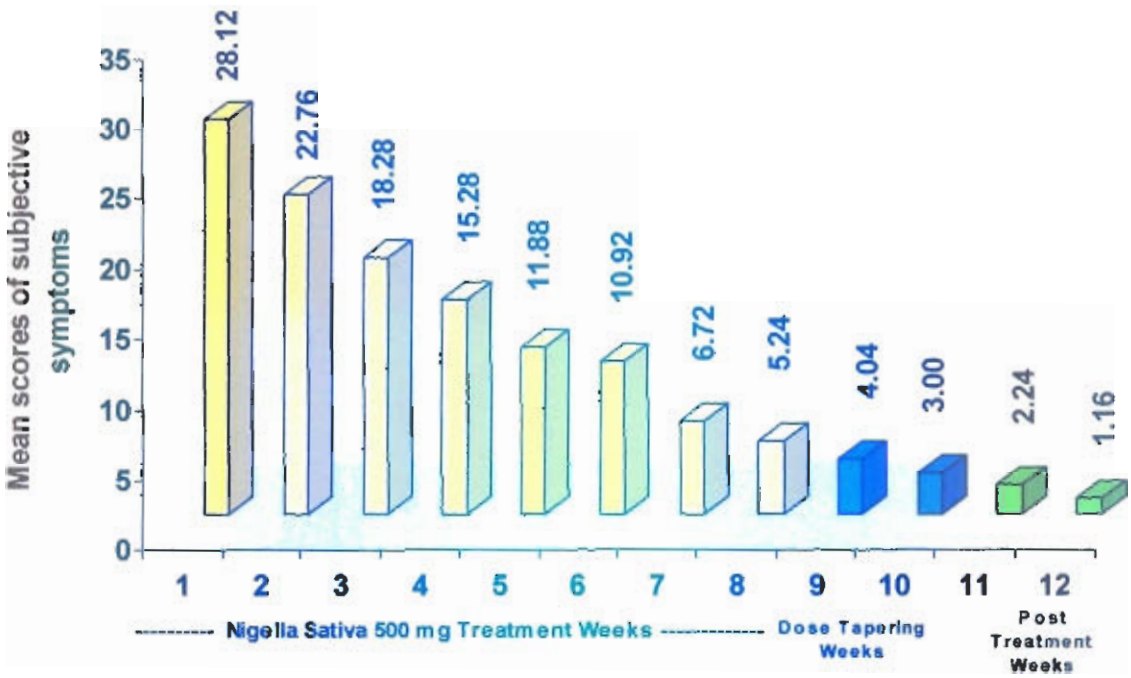
Effects of Nigella Sativa 250 mg Treatment on Subjective Symptoms of Protracted Abstinence in Group-I patients with Opioid dependence during 12 weeks of follow up

	Nigella Sativa 500 mg Treatment Weeks								DTW		PTW	
	1	2	3	4	5	6	7	8	9	10	11	12
Mean ± SEM Scores of Subjective Symptoms of Withdrawal	28.12 ±10.61	22.76 ±6.38	18.28 ±6.27	15.28 ±5.67	11.88 ±5.92	10.92 ±6.81	6.72 ±5.82	5.24 ±5.65	4.04 ±4.97	3.00 ±3.92	2.24 ±2.97	1.16 ±1.84

- Numbers indicate the mean ± SEM scores of symptoms reports of all ratings in a total of 25 patients on each follow up week vide Appendix-VI.

FIGURE – 3

Effects of Nigella Sativa 250 mg Treatment on Subjective Symptoms of Protracted Abstinence in Group-I patients with Opioid Dependence during 12 weeks of Follow up



Effects of Nigella Sativa 250 mg treatment on the mean±SEM scores of subjective symptoms of protracted abstinence in 25 patients after every week during 12 weeks of follow up. Students' t-test comparing change in symptoms from pre-treatment inpatient day-3 to week-12 indicated trend decrease in symptoms to be reported on week-12 of follow up.

TABLE - 8

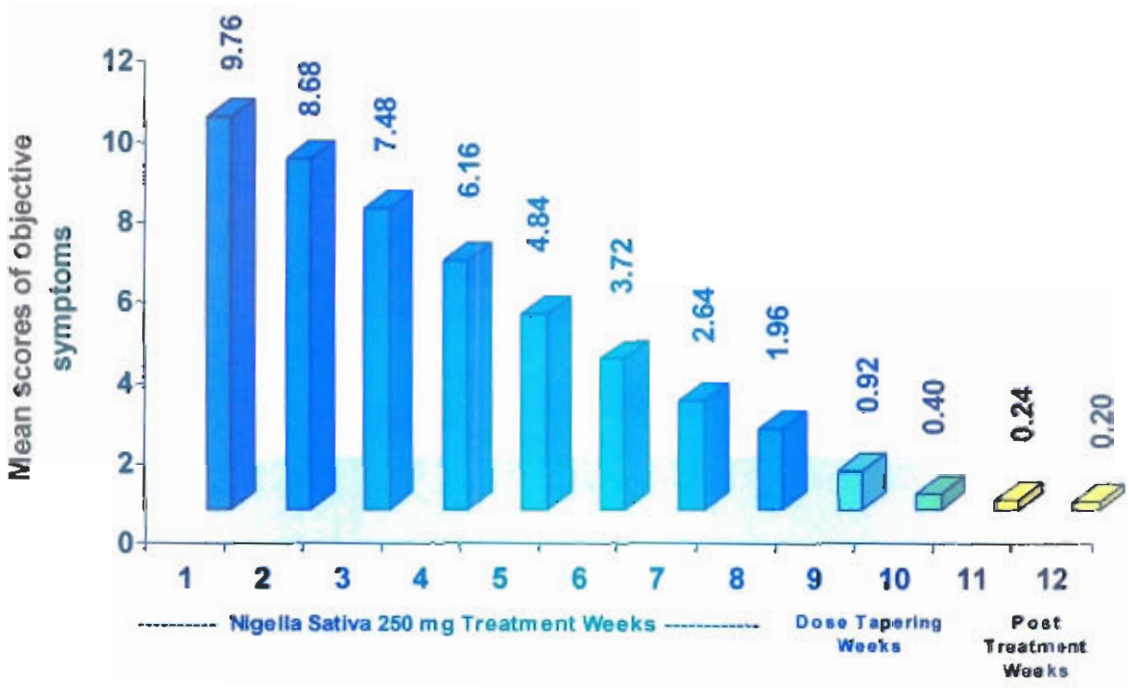
Effects of Nigella Sativa 250 mg Treatment on Objective Signs of Protracted Abstinence in Group-I patients with Opioid dependence during 12 weeks of follow up

	Nigella Sativa 500 mg Treatment Weeks								DTW		PTW	
	1	2	3	4	5	6	7	8	9	10	11	12
Mean ± SEM Scores of Subjective Symptoms of Withdrawal	9.76 ±2.87	8.68 ±2.76	7.48 ±2.97	6.16 ±2.91	4.84 ±2.57	3.72 ±2.22	2.64 ±2.01	1.96 ±1.85	0.92 ±1.32	0.40 ±1.00	0.24 ±0.72	0.20 ±0.64

- Numbers indicate the mean ± SEM scores of signs of reports of all ratings in a total of 25 patients on each follow up week vide Appendix-X.

FIGURE – 4

Effects of Nigella Sativa 250 mg Treatment on Objective Signs of Protracted Abstinence in Group-I patients with Opioid Dependence during 12 weeks of Follow up



Effects of Nigella Sativa 250 mg treatment on the mean±SEM scores of objective signs of protracted abstinence in 25 opioid addicts after every week of 12 weeks of follow up. Students' t-test comparing change in signs from pre-treatment inpatient day-3 to week-12 indicated trend decrease in signs to be reported on week-12 of follow up.

The physiological parameters including pulse rate, temperature, respiratory rate decreased and papillary diameter were increased from day-3 to week-12 of follow up. There was a decrease in systolic and diastolic blood pressure from day-3 to week-12 of follow up. The caloric intake was increased from 1.41±0.41 to 2.70±0.13 after 12 weeks of follow up, while there was not much in body weight during that period (Table-8A).

TABLE – 8A

Comparison of Protracted Abstinence of Heroin dependence in Group-I patients treated with Nigella Sativa 250 mg Day-3 versus Week-12 Scores of Symptoms and Signs and Physiological parameters

	Day – 3	Week – 12	P value	Percentage
Mean ± SEM scores of subjective symptoms of abstinence	55.08 ± 12.65	1.16 ± 1.84	0.050	2.10 ↓
Mean ± SEM scores of objective signs of abstinence	19.76 ± 4.58	0.20 ± 0.64	0.050	1.01 ↓
Physiological parameters:				
1. Pulse rate (per minute)	89.52 ± 12.27	77.28 ± 5.16	0.345	86.32 ↓
2. Systolic blood pressure (mmHg)	113.68 ± 14.79	107.00 ± 10.10	0.711	94.12 ↓
3. Diastolic blood pressure (mmHg)	76.40 ± 8.10	71.04 ± 6.65	0.611	92.98 ↓
4. Temperature (°F)	99.13 ± 1.03	98.13 ± 0.44	0.354	98.99 ↓
5. Respiratory rate (per minute)	23.36 ± 1.70	18.24 ± 1.04	0.117	18.40 ↓
6. Body weight (kilogram)	53.72 ± 7.69	52.82 ± 6.18	0.928	98.32 ↓
7. Pupil diameter (mm)	3.28 ± 0.35	2.03 ± 0.16	0.085	60.97 ↓
8. Caloric intake (kilo calories)	1.41 ± 0.41	2.70 ± 0.13	0.090	191.48 ↑

GROUP-II

Twenty-five patients were given a placebo treatment on day-1 and day-2 of admission. Thereafter from day-3 to day-12 of admission the patients received 500 mg of Nigella Sativa orally three times daily.

Placebo had no significant effect on cumulative scores of symptoms of acute withdrawal from opioids. A mean score of 42.64±12.28 was obtained on day-1 which reaches a peak of 63.2±13.57 on day-3 of admission. However administration of Nigella Sativa 500 mg produced a decrease in opiate withdrawal symptoms from 63.2±13.57 on day-3 to 14.56±8.13 on day-12 of admission (Table-1, Figure-5, Appendix-III).

TABLE - 1

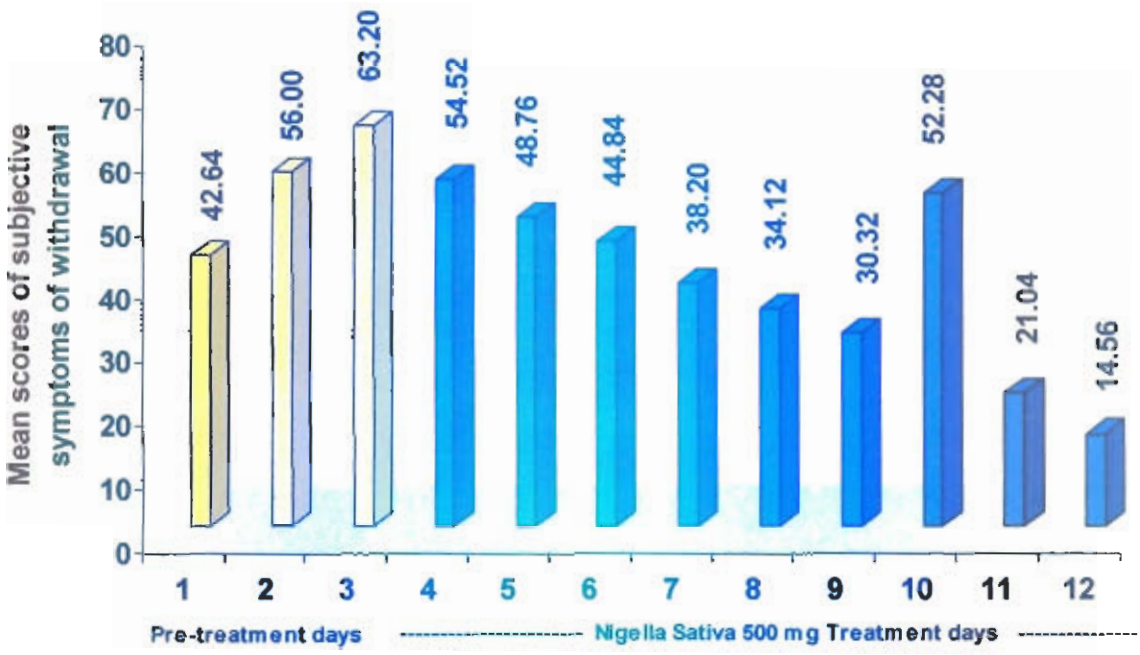
Effects of Nigella Sativa 500 mg Treatment on Subjective Symptoms of Withdrawal from Opioids in Group-II Patients during 12 days stay in Hospital

	Pre-treatment Day			Treatment Day								
	1	2	3	4	5	6	7	8	9	10	11	12
Mean±SEM Scores of Subjective Symptoms of Withdrawal	42.64 ±12.28	56.00 ±12.50	63.20 ±13.57	54.52 ±17.72	48.76 ±17.49	44.84 ±13.61	38.20 ±12.32	34.12 ±11.11	30.32 ±10.04	52.28 ±10.01	21.04 ±9.12	14.56 ±8.13
P value	0.087											
Percentage	34.14%											

- Numbers indicate the mean ± SEM scores of 38 symptoms of all ratings in a total of 25 patients on each admission day vide Appendix-III.

FIGURE – 5

Effects of Nigella Sativa 500 mg Treatment on Subjective Symptoms of Withdrawal from Opioids in Group-II during 12 days stay in Hospital

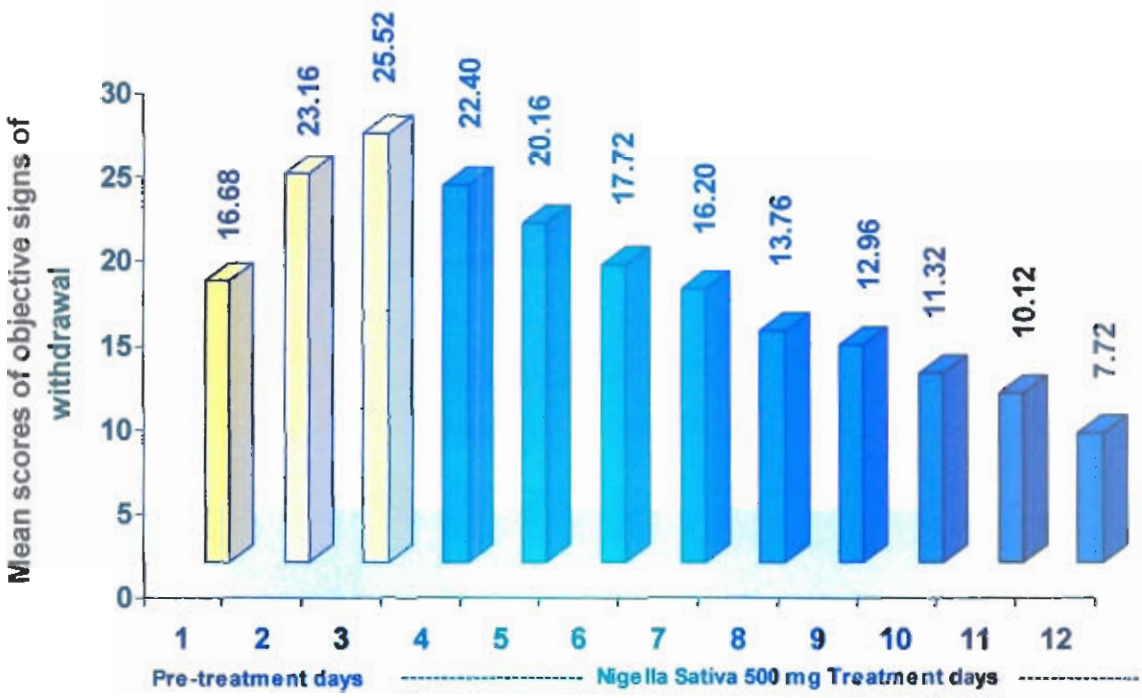


Effects of Nigella Sativa 500 mg treatment on the mean±SEM scores of 38 symptoms of withdrawal of the 12 days sample in 25 opioid addicts at days after the 3rd inpatient day of withdrawal. Students' t-test comparing change in symptoms day-12 indicated trend decrease in symptoms to be reported on day-12 of admission.

Similarly, placebo had no significant effect on the cumulative score of signs of acute withdrawal from opioids. A mean score of 16.68±3.22 was obtained on day-1, which had reached a peak of 25.52±3.08 on day-3 f admission. However administration of Nigella Sativa 500 mg produced a rapid decrease in opiate withdrawal signs from 25.52±3.08 on day-3 to 7.72±2.35 on day-12 of admission (Table-2, Figure-6, Appendix-IV).

FIGURE - 6

Effects of Nigella Sativa 500 mg Treatment on Objective Signs of Withdrawal from Opioids in Group-II patients during 12 days stay in Hospital



Effects of Nigella Sativa 500 mg treatment on the mean±SEM scores of 18 signs of withdrawal of the 12 days sample in 25 opioid addicts at days after the 3rd inpatient day of withdrawal. Students' t-test comparing change in signs from pre-treatment inpatient day-3 to treatment day-10 indicated trend decrease in signs to be reported on day-12 of admission.

A decrease was also observed on physiological parameters including pulse rate, systolic blood pressure, diastolic blood pressure, and respiratory rate from day-3 to 12 ot admission. The temperature was decreased from 98.83±1.06 on day-3 to 98.20±0.49 on day-12 of admission. Pupillary diameter was decreased from 3.34±0.37 on day-3 to 2.12±0.75 on day-12 of admission. There was not much increase in body weight and caloric intake from day-3 to day-12 of admission (Table-2A).

Urine toxicology was significantly decreased from day-3 to day-12 of admission.

TABLE – 2A

Comparison of Abstinence of Heroin in Group-II patients treated with Nigella Sativa 500 mg Day-3 versus Day-12 Scores of Symptoms and Signs and Physiological parameters

	Day – 3	Day – 12	P value	Percentage
Mean ± SEM scores of subjective symptoms of abstinence	42.64 ± 12.28	14.56 ± 8.13	0.175	34.14 ↓
Mean ± SEM scores of objective signs of abstinence	25.52 ± 3.08	7.72 ± 2.35	0.001	*30.25 ↓
Physiological parameters:				
1. Pulse rate (per minute)	90.36 ± 15.66	79.24 ± 9.70	0.442	87.69 ↓
2. Systolic blood pressure (mmHg)	114.40 ± 13.79	106.88 ± 11.74	0.679	93.42 ↓
3. Diastolic blood pressure (mmHg)	79.12 ± 10.24	73.80 ± 7.21	0.673	93.27 ↓
4. Temperature (°F)	98.83 ± 1.06	98.20 ± 0.49	0.478	99.34 ↓
5. Respiratory rate (per minute)	22.80 ± 2.16	21.60 ± 1.52	0.652	94.73 ↓
6. Body weight (kilogram)	54.81 ± 9.94	56.45 ± 9.87	0.907	102.99 ↑
7. Pupil diameter (mm)	3.34 ± 0.37	2.12 ± 0.75	0.232	63.47 ↓
8. Caloric intake (kilo calories)	2.07 ± 0.44	2.23 ± 0.35	0.778	107.72 ↑

*P<0.05

Nigella Sativa 500 mg regimen used in this study shows a significant effect on withdrawal from opioids, as clinical condition and physical appearance of patients showed and the cumulative scores of withdrawal signs and symptoms daily (Appendices-III and IV).

The patient was discharged on same treatment after 12 days of admission and advised to attend OPD weekly for the follow up.

The cumulative scores of symptoms of protracted abstinence was decreased from 63.2±13.57 on day-3 to 0.32±0.90 after 12 weeks of follow up (Table-3, Figure-7).

TABLE – 3

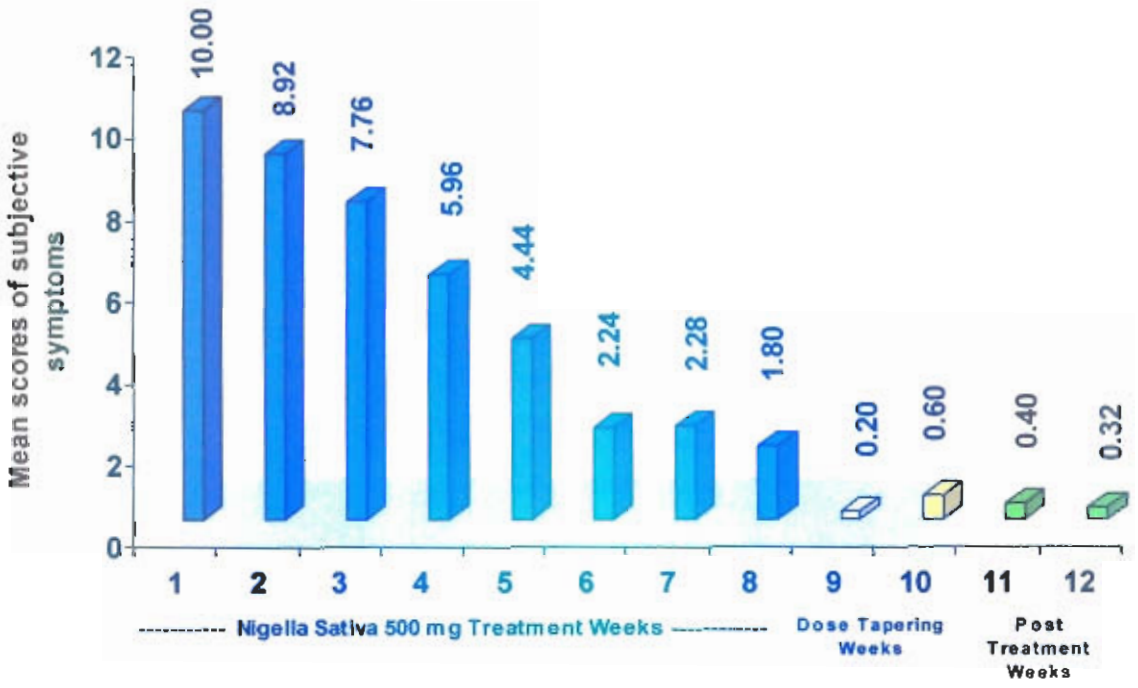
Effects of Nigella Sativa Treatment on Subjective Symptoms of Protracted Abstinence in Group-II patients with Opioid dependence during 12 weeks of follow up

	Nigella Sativa Treatment Weeks								DTW		PTW	
	1	2	3	4	5	6	7	8	9	10	11	12
Mean ± SEM Scores of Subjective Symptoms of Withdrawal	10.00 ±4.54	8.92 ±5.12	7.76 ±4.55	5.96 ±4.15	4.44 ±3.83	2.24 ±3.34	2.28 ±3.64	1.80 ±3.00	0.20 ±1.75	0.60 ±1.35	0.40 ±1.11	0.32 ±0.90

- Numbers indicate the mean ± SEM scores of symptoms of all reports of all ratings in a total of 25 patients on each follow up week vide Appendix-V.

FIGURE – 7

Effects of Nigella Sativa 500 mg Treatment on Subjective Symptoms of Protracted Abstinence in Group-II patients with Opioid Dependence during 12 weeks of Follow up



Effects of Nigella Sativa 500 mg treatment on the mean±SEM scores of subjective symptoms of protracted abstinence in 25 opioid addicts after every week during 12 weeks of follow up.

Similarly, the cumulative score of signs of protracted abstinence was decreased from 25.52±3.08 on day-3 to 0.36±0.63 after 12 weeks of follow up (Table-4, Figure-8).

TABLE – 4

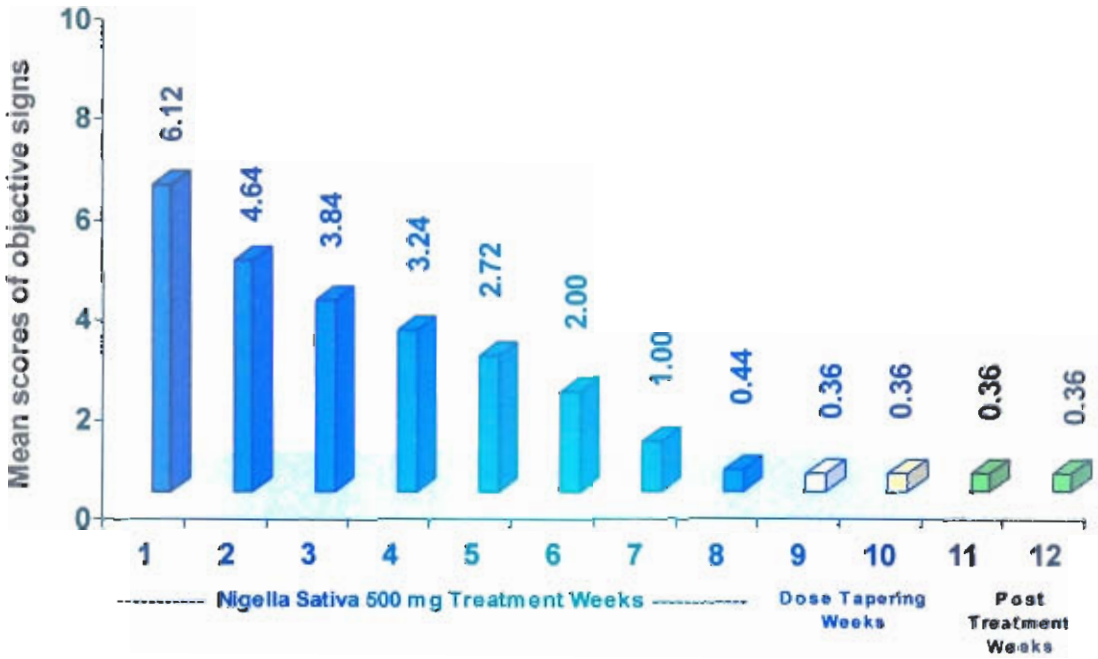
Effects of Nigella Sativa Treatment on Objective Signs of Protracted Abstinence in Group-II patients with Opioid dependence during 12 weeks of follow up

	Nigella Sativa Treatment Weeks									DTW		PTW	
	0	1	2	3	4	5	6	7	8	9	10	11	12
Mean ± SEM Scores of Subjective Symptoms of Withdrawal	7.72 ±2.35	6.12 ±2.75	4.64 ±2.58	3.84 ±2.37	3.24 ±2.31	2.72 ±1.70	2.00 ±2.28	1.00 ±1.08	0.44 ±0.76	0.36 ±0.63	0.36 ±0.63	0.36 ±0.63	0.36 ±0.63

- Numbers indicate the mean ± SEM scores of signs reports of all ratings in a total of 25 patients on each follow up week vide Appendix-VI.

FIGURE – 8

Effects of Nigella Sativa 500 mg Treatment on Objective Signs of Protracted Abstinence in Group-II patients with Opioid Dependence during 12 weeks of Follow up



Effects of Nigella Sativa 500 mg treatment on the mean±SEM scores of objective signs of protracted abstinence in 25 opioid addicts after every week during 12 weeks of follow up. Statistics comparing change in signs from pre-treatment in patient day-3 to week-12 indicated trend of decrease in signs to be reported on week-12 of follow up.

The physiological parameters, including pulse rate, respiratory rate, and pupillary diameter were decreased from day-3 to highly significant levels after 12 weeks of follow up. There were no significant decrease in systolic blood pressure, diastolic blood pressure, and temperature. Similarly, there was an increase in caloric intake but no significant increase in body weight from day-3 to week-12 of follow up (Table-4a).

The urine toxicology remained negative throughout the follow up period as tested on weeks-4, 8 and 12.

TABLE – 4A

Comparison of Protracted Abstinence of Heroin dependence in Group-II patients treated with Nigella Sativa 500 mg Day-3 versus Week-12 Scores of Symptoms and Signs and Physiological parameters

	Day – 3	Week – 12	P value	Percentage
Mean ± SEM scores of subjective symptoms of abstinence	42.64 ± 12.28	0.32 ± 0.90	0.175	0.75 ↓
Mean ± SEM scores of objective signs of abstinence	25.52 ± 3.08	0.36 ± 0.63	0.001	1.41 ↓
Physiological parameters:				
1. Pulse rate (per minute)	90.36 ± 15.66	74.56 ± 5.43	0.337	82.51 ↓
2. Systolic blood pressure (mmHg)	114.4 ± 13.79	109.6 ± 13.06	0.801	95.80 ↓
3. Diastolic blood pressure (mmHg)	79.12 ± 10.24	73.6 ± 9.41	0.693	93.02 ↓
4. Temperature (°F)	98.83 ± 1.06	97.25 ± 3.62	0.523	98.40 ↓
5. Respiratory rate (per minute)	22.80 ± 2.16	21.60 ± 15.93	0.787	94.73 ↓
6. Body weight (kilogram)	54.81 ± 9.94	53.92 ± 8.07	0.945	98.37 ↓
7. Pupil diameter (mm)	3.34 ± 0.37	1.60 ± 0.47	0.005	47.90 ↓
8. Caloric intake (kilo calories)	2.07 ± 0.44	2.69 ± 0.15	0.269	129.95 ↑

H.S. = Highly significant; S = Significant; N.S. = Non-significant

Nigella Sativa 500 mg treatment regimen used in this study showed significant effect on protracted abstinence of opioid as clinical condition of patient and cumulative scores of signs and symptoms of protracted abstinence were observed (Appendices-V and VI).

On comparing the effects of Nigella Sativa 250 mg and 500 mg in controlling the signs and symptoms of acute withdrawal syndrome of opioids, both the drugs decrease the severity of withdrawal in opioid addicts during the period of 10 days stay in hospital.

On day-3 of admission the mean score of opiate withdrawal symptoms in Nigella Sativa 250 mg group was 55.08±12.65, whereas in Nigella Sativa 500 mg group the mean score of opiate withdrawal symptom was 63.2±13.57. So there is no main difference in the score of opiate withdrawal symptoms in two groups on day-3 of admission (Table-9, Figure-9). However on day-12 of admission the mean score of opiate withdrawal symptoms was decreased to 14.56±8.13. Thus the difference in effectiveness of the two drugs in controlling the acute opiate withdrawal symptoms is obvious. Nigella Sativa 500 mg shown to be more effective than Nigella Sativa 250 mg in controlling acute opiate withdrawal symptoms during 12 days of admission in hospital (Table-9, Figure-9).

TABLE – 9

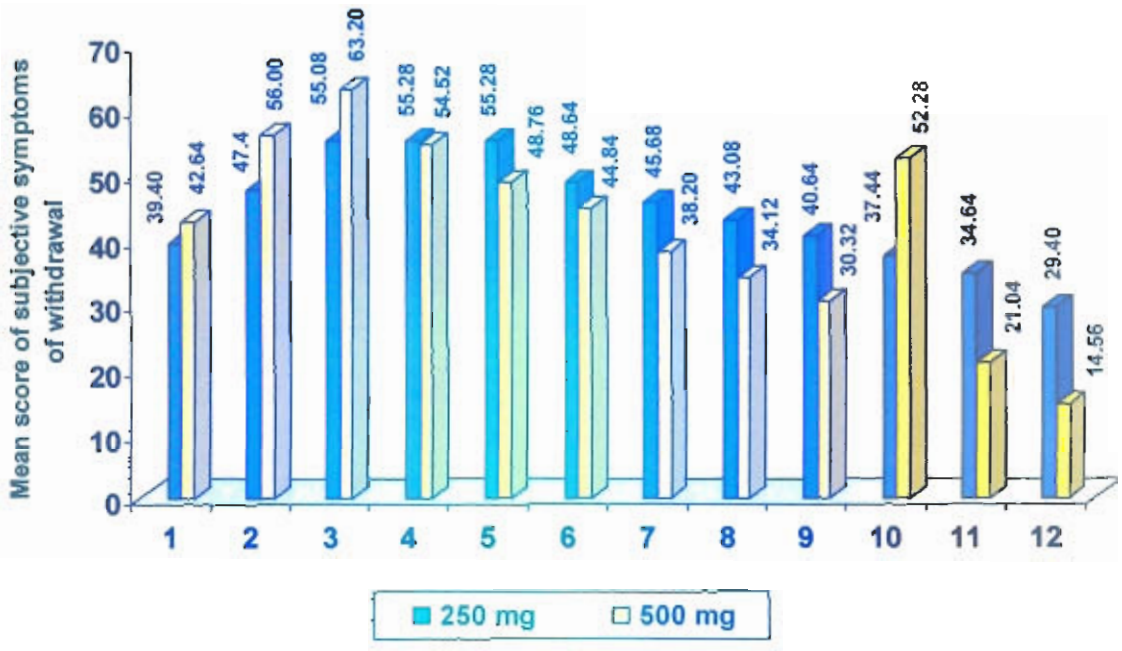
Comparison of Effects of Treatment with Nigella Sativa 250 mg and 500 mg on Subjective Symptoms of Withdrawal from Opioids during 12 days' stay in Hospital

inpatients Day	Mean ± SEM Scores of Subjective Symptoms of Withdrawal			
	Nigella Sativa 250 mg	Nigella Sativa 500 mg	P value	Percentage
1.	39.40 ± 7.98	42.64 ± 12.28	0.641	49.52%
2.	47.4 ± 7.50	56.00 ± 12.50	0.447	
3.	55.08 ± 12.65	63.20 ± 13.57	0.663	
4.	55.28 ± 6.58	54.52 ± 17.72	0.561	
5.	55.28 ± 6.58	48.76 ± 17.49	0.559	
6.	48.64 ± 6.54	44.84 ± 13.61	0.619	
7.	45.68 ± 6.77	38.20 ± 12.32	0.470	
8.	43.08 ± 6.52	34.12 ± 11.11	0.409	
9.	40.64 ± 5.91	30.32 ± 10.24	0.356	
10.	37.44 ± 6.04	52.28 ± 10.01	0.267	
11.	34.64 ± 5.37	21.04 ± 9.12	0.264	
12.	29.40 ± 5.02	14.56 ± 8.13	0.220	
n	25	25		

- Numbers indicate the mean ± SEM scores of 38 symptoms reports of all ratings on each admission day.
- n = No. of Patients

FIGURE – 9

Comparison of Effects of Treatment with Nigella Sativa 250 mg and 500 mg on Subjective Symptoms of Withdrawal from Opioids during 12 days Stay in Hospital



Comparison of effects of treatment with Nigella Sativa 250 mg (n=25) and 500 mg (n=25) on the mean±SEM scores of 38 symptoms of withdrawal of the 12 days sample at days after 3rd in-patient day of withdrawal. Statistical comparison shows obvious difference in two groups.

Similarly, on day-3 of admission the mean score of opiate withdrawal signs in Nigella Sativa 250 mg treatment group was 14.92±3.35, whereas in Nigella Sativa 500 mg group the mean score of opiate withdrawal signs was 16.68±3.22. So there is not much difference in the peak mean scores of opiate withdrawal signs in two groups on day 12 of admission the mean score of opiate withdrawal signs decreased to 7.72±2.35. Thus the difference in effectiveness of the two drugs in controlling the acute opiate withdrawal signs in obvious. Nigella Sativa 500 mg came out to be more effective than 250 mg in controlling acute opiate withdrawal signs during 12 days of admission in hospital (Table-10, Figure-10).

TABLE - 10

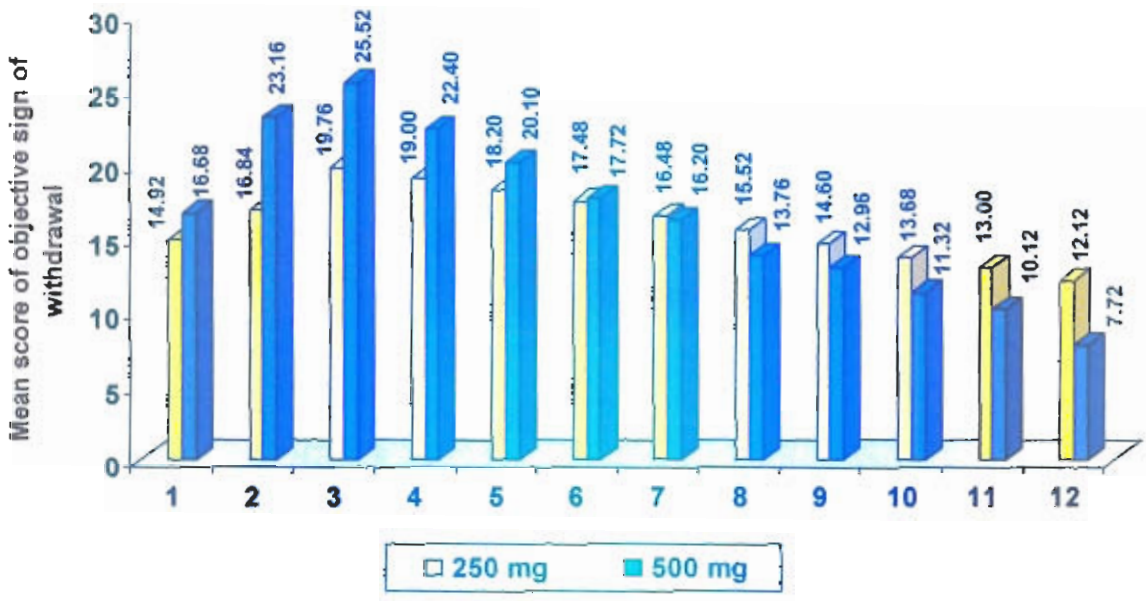
Comparison of Effects of Treatment with Nigella Sativa 250 mg and 500 mg on Objective Signs of Withdrawal from Opioids during 12 days stay in Hospital

Inpatients Day	Mean ± SEM Scores of Objective Signs of Withdrawal		
	Nigella Sativa 250 mg	Nigella Sativa 500 mg	P value
1.	14.92 ± 3.35	16.68 ± 3.22	0.706
2.	16.84 ± 3.35	23.16 ± 3.43	0.193
3.	19.76 ± 4.58	25.52 ± 3.08	0.301
4.	19.00 ± 4.22	22.40 ± 2.88	0.508
5.	18.20 ± 4.00	20.16 ± 2.64	0.526
6.	17.48 ± 3.85	17.72 ± 2.97	0.961
7.	16.48 ± 3.54	16.20 ± 2.43	0.948
8.	15.52 ± 3.46	13.76 ± 3.53	0.723
9.	14.60 ± 3.26	12.96 ± 2.47	0.690
10.	13.68 ± 2.76	11.32 ± 2.54	0.532
11.	13.00 ± 2.79	10.12 ± 2.63	0.456
12.	12.12 ± 2.60	7.72 ± 2.35	0.215
N	25	25	

- Numbers indicate the mean ± SEM scores of 18 signs reports of all ratings on each admission day.
- n = No. of Patients

FIGURE - 10

Comparison of Effects of Treatment with Nigella Sativa 250 mg and 500 mg on Objective Signs of Withdrawal from Opioids during 12 days Stay in Hospital



Comparison of effects of treatment with Nigella Sativa 250 mg and 500 mg on the mean±SEM scores of 18 signs of withdrawal of the 12 days sample at days after 3rd in-patient day of withdrawal. Statistical comparison shows obvious difference in the effectiveness of Nigella Sativa 250 mg and 500 mg.

There were no significant differences in all the physiological parameters of two groups on day-3 of admission. Similarly, there was no significant difference in the physiological parameters including pulse rate, systolic blood pressure, diastolic blood pressure, temperature and body weight of two groups on day-12 of admission (Tables-10a and 10b).

TABLE – 10A

Comparison of Abstinence of Heroin in patients treated with Nigella Sativa 250 mg and 500 mg on Day-3 Scores of Symptoms and Signs and Physiological Parameters

	Nigella Sativa 250 mg	Nigella Sativa 500 mg	P value
Mean ± SEM scores of subjective symptoms of abstinence	55.28 ± 6.58	63.20 ± 13.57	0.474
Mean ± SEM scores of objective signs of abstinence	19.76 ± 4.58	25.52 ± 3.08	0.301
Physiological parameters:			
1. Pulse rate (per minute)	89.52 ± 12.27	90.36 ± 15.66	0.966
2. Systolic blood pressure (mmHg)	113.68 ± 14.79	114.40 ± 13.79	0.972
3. Diastolic blood pressure (mmHg)	76.40 ± 8.10	79.12 ± 10.24	0.836
4. Temperature (°F)	99.13 ± 1.03	98.83 ± 1.06	0.841
5. Respiratory rate (per minute)	23.36 ± 1.70	22.80 ± 2.16	0.839
6. Body weight (kilogram)	53.72 ± 7.69	54.81 ± 9.94	0.931
7. Pupil diameter (mm)	3.28 ± 0.35	3.34 ± 0.37	0.903
8. Caloric intake (kilo calories)	1.91 ± 0.41	2.07 ± 0.44	0.791

TABLE – 10B

Comparison of Abstinence of Heroin in patients treated with Nigella Sativa 250 mg and 500 mg on Day-12 Scores of Symptoms and Signs and Physiological Parameters

	Nigella Sativa 250 mg	Nigella Sativa 500 mg	P value
Mean ± SEM scores of subjective symptoms of abstinence	29.40 ± 5.02	14.56 ± 8.13	0.220
Mean ± SEM scores of objective signs of abstinence	12.12 ± 2.60	7.72 ± 2.35	0.222
Physiological parameters:			
1. Pulse rate (per minute)	82.28 ± 11.90	79.24 ± 9.70	0.844
2. Systolic blood pressure (mmHg)	103.44 ± 12.02	106.88 ± 11.74	0.838
3. Diastolic blood pressure (mmHg)	69.20 ± 8.97	73.80 ± 7.21	0.691
4. Temperature (°F)	98.22 ± 0.73	98.20 ± 0.49	0.982
5. Respiratory rate (per minute)	21.76 ± 2.08	21.60 ± 1.52	0.951
6. Body weight (kilogram)	53.32 ± 8.29	56.45 ± 9.87	0.809
7. Pupil diameter (mm)	2.70 ± 0.32	2.12 ± 0.75	0.407
8. Caloric intake (kilo calories)	1.98 ± 0.39	2.23 ± 0.35	0.638

Even on comparing the results after the completion of study period, that is, after 12 weeks of follow up, there was no significant difference in the mean scores of symptoms of protracted abstinence in two groups (Table-11, Figure-11). Similarly, there was no significant difference in the mean scores of signs of protracted abstinence in two groups (Table-12, Figure-12). Also the difference in physiological parameters of two groups after 12 weeks of follow up was not significant (Table-12a).

TABLE – 11

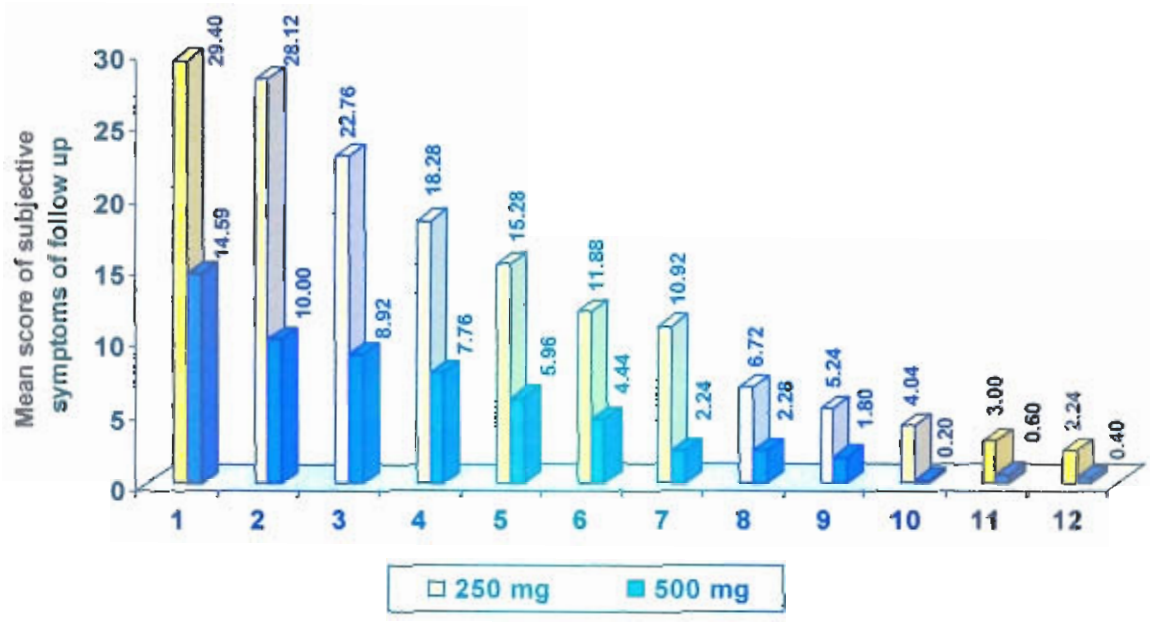
Comparison of Effects of Treatment with Nigella Sativa 250 mg and 500 mg on Subjective Symptoms of Protracted Abstinence in patients with Opioid Dependence during 12 weeks of follow up

Weeks	Mean ± SEM Scores of Subjective Symptoms		
	Nigella Sativa 250 mg	Nigella Sativa 500 mg	P value
0.	29.40 ± 29.40	14.50 ± 8.13	
1.	28.12 ± 10.61	10.00 ± 4.54	0.219
2.	22.76 ± 6.38	8.92 ± 5.12	0.097
3.	18.28 ± 6.27	7.76 ± 4.55	0.180
4.	15.28 ± 5.67	5.96 ± 4.15	0.191
5.	11.88 ± 5.92	4.44 ± 3.83	0.310
6.	10.92 ± 6.81	2.24 ± 3.34	0.292
7.	6.72 ± 5.82	2.28 ± 3.64	0.426
8.	5.24 ± 5.65	1.80 ± 3.00	0.468
9.	4.04 ± 4.97	0.20 ± 1.75	0.400
10.	3.00 ± 3.92	0.60 ± 1.35	0.453
11.	2.24 ± 2.97	0.40 ± 1.11	0.452
12.	1.16 ± 1.84	0.32 ± 0.90	0.526
n	25	25	

- Numbers indicate the mean ± SEM scores of symptoms reports of all ratings on each follow up.
- n = No. of Patients

FIGURE - 11

Comparison of Effects of Treatment with Nigella Sativa 250 mg and 500 mg on Subjective Symptoms of Protracted Abstinence in Patients with Opioid dependence during 12 weeks of Follow up



Comparison of effects of treatment with Nigella Sativa 250 mg and 500 mg on the rnean±SEM scores of subjective symptoms of protracted abstinence after every week during 12 weeks of follow-up. Statistical comparison on day-3 two groups and week-12 in two groups indicate no significant difference.

TABLE – 12

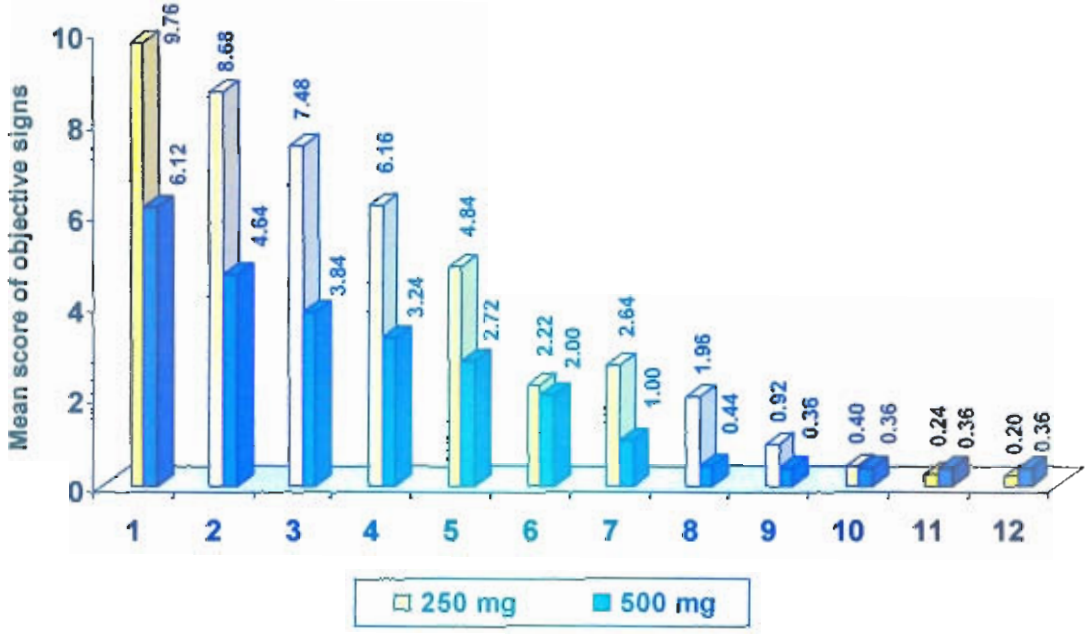
Comparison of Effects of Treatment with Nigella Sativa 250 mg and 500 mg on Objective Signs of Protracted Abstinence with Opioid Dependence during 12 weeks of follow up

Weeks	Mean ± SEM Scores of Objective Signs		
	Nigella Sativa 250 mg	Nigella Sativa 500 mg	P value
1.	9.76 ± 2.87	6.12 ± 2.75	0.364
2.	8.68 ± 2.76	4.64 ± 2.58	0.289
3.	7.48 ± 2.97	3.84 ± 2.37	0.342
4.	6.16 ± 2.91	3.24 ± 2.31	0.435
5.	4.84 ± 2.57	2.72 ± 1.70	0.411
6.	2.22 ± 3.72	2.00 ± 2.28	0.728
7.	2.64 ± 2.01	1.00 ± 1.08	0.402
8.	1.96 ± 1.85	0.44 ± 0.76	0.390
9.	0.92 ± 1.32	0.36 ± 0.63	0.541
10.	0.40 ± 1.00	0.36 ± 0.63	0.855
11.	0.24 ± 0.72	0.36 ± 0.63	0.901
12.	0.20 ± 0.64	0.36 ± 0.63	0.859
N	25	25	

- Numbers indicate the mean ± SEM scores of signs reports of all ratings on each follow up week.
- n = No. of Patients

FIGURE - 12

Comparison of Effects of Treatment with Nigella Sativa 250 mg and 500 mg on Objective Signs of Protracted Abstinence in Patients with Opioid dependence during 12 weeks of Follow up



Comparison of effects of treatment with Nigella Sativa 250 mg and 500 mg on the mean±SEM scores of objective signs of protracted abstinence after every week during 12 weeks of follow-up. Statistical comparison in change in signs from in-patient day-3 for each group indicated a trend of decrease in signs to be reported on week-12 of follow up. Comparison in signs on day-3 in two groups and week-12 in two groups indicate no significant difference.

TABLE – 12A

Comparison of Protracted Abstinence of Heroin Dependence in patients treated with Nigella Sativa 250 mg and 500 mg on Week-12 Scores of Symptoms and Signs and Physiological Parameters

	Nigella Sativa 250 mg	Nigella Sativa 500 mg	P value
Mean ± SEM scores of subjective symptoms of abstinence	1.16 ± 1.84	0.32 ± 0.90	0.534
Mean ± SEM scores of objective signs of abstinence	0.20 ± 0.64	0.36 ± 0.63	0.858
Physiological parameters:			
1. Pulse rate (per minute)	77.28 ± 5.16	74.56 ± 5.43	0.718
2. Systolic blood pressure (mmHg)	107.00 ± 10.10	109.60 ± 13.06	0.875
3. Diastolic blood pressure (mmHg)	71.04 ± 65.65	73.60 ± 9.41	0.846
4. Temperature (°F)	98.13 ± 0.44	97.25 ± 3.62	0.628
5. Respiratory rate (per minute)	18.24 ± 1.09	21.6 ± 15.93	0.651
6. Body weight (kilogram)	52.82 ± 6.18	53.92 ± 8.07	0.914
7. Pupil diameter (mm)	2.03 ± 0.16	1.60 ± 0.47	0.374
8. Caloric intake (kilo calories)	2.70 ± 0.13	2.69 ± 0.15	0.972

H.S. = Highly significant; N.S. = Non-significant

DISCUSSION

DISCUSSION

Heroin dependents are those who continue to use heroin in the face of difficulties they know or believe to be caused by its use such as health, legal and inter-personal difficulties. They typically use heroin daily, develop tolerance to its effects, and experience withdrawal symptoms on abrupt cessation of use. About one quarter of people who have ever used heroin developed dependence (Anthony and Helzer, 1995).

Dependence does not end when the drug is removed from the body (detoxification) or when the acute post-drug taking illness dissipates (withdrawal). Rather, the underlying addictive disorder persists, and this persistence produces a tendency to relapse to active drug taking (O'Brien and McLeVan, 1996).

Methadone maintenance treatment (MMT) is the most extensively researched form of maintenance treatment for opioid dependence. The effectiveness of treatment for opioid dependence would ideally be assessed through randomized controlled trials (RCTs). Only five such trials have ever taken place in the 35 years since MMT was introduced. All five trials involved small number of patients who were rarely followed for longer than one year. The effectiveness of MMT in observational studies of community treatment programmes has not been as impressive as that in the RCTs, indicating that methadone treatment in routine use is not a panacea for opioid dependence. About half of those who enter treatment

left within 12 months, and some of those continued to one heroin and other illicit drugs though much less frequently than before they entered treatment (Hall et al., 1998).

Maintenance with pure opioid antagonists such as naltrexone has been shown to be effective in opioid dependent people for whom failure to comply with treatment has major personal consequences (e.g. opioid dependent health professionals) but pure opioid antagonists have not proven popular with the wider population of opioid dependent people, in whom low rates of compliance have been a major difficulty. Naltrexone has almost no agonist effects and will not satisfy craving or relieve protracted withdrawal symptoms. For these reasons, naltrexone treatment does not appeal to the average heroin addict especially those with less motivation to remain opioid free (O'Brien, 1996).

The study, we proposed, was the first project launched in the Department of Pharmacology and Therapeutics, University of Karachi. The objective for this project was to search non-opiate treatment of opioid dependence for long term management; a treatment which would be more safe, less hazardous and more acceptable to opioid addicts.

Prior to this few studies were conducted at Basic Medical Sciences Institute, J.P.M.C., Karachi, in which role of calcium channel blockers was observed in the treatment of opioid dependence syndrome. These studies were carried out by Baloch (1991), Mahesar (1994), Salat (1998), and

Ansari (1999) in which effect of calcium channel blocker in treatment of opioid dependence was evaluated in animal and patients.

Baloch (1991) and Mahesar (1994) observed the effect of verapamil and felodipine in morphine dependent animals subjected to naloxone in vivo and vitro. They observed that calcium channel blockers were effective in reducing the abstinence in vivo and in vitro effects. These observations led to pilot project on morphine addict patients.

First clinical study was conducted by Salat in 1998, who compared the effects of calcium channel blocker, verapamil with thioridazine and amitriptyline, contemporary treatment prevalent in Karachi in the management of acute opioid abstinence syndrome.

He observed that verapamil showed a highly significant improvement in signs and symptoms of abstinence. The patients, who were admitted during the study with previous history of opioid abstinence without treatment, expressed that they did not experience these withdrawal effects. He concluded that verapamil therapy is safe, effective, and more pronounced in treating the acute opioid withdrawal syndrome than amitryptyline and thioridazine.

Second clinical study was done by Ansari (1999) in which the effects of verapamil and clonidine compared with thioridazine and chlorpromazine in opioid abstinence syndrome were observed. He observed that the effects of verapamil and clonidine to decrease the signs

and symptoms of acute withdrawal from opioids were highly significant when compared with chlorpromazine and thioridazine.

Hence, in the light of previous clinical studies, this single blind study was proposed to observe the effects of Nigella Sativa in long term management of opioid dependence. Each patient received treatment initially for 12 days during his stay in hospital. Then each patient was advised for weekly follow up as out-patient and the same treatment was continued till eight weeks, then the dose of drug was gradually tapered off during next two weeks period after that patient was followed up further for two weeks without giving any drug.

A placebo for the drug was given on day-1 and 2 of admission, only to observe and confirm the opiate withdrawal syndrome on first three days of admission. Nigella Sativa was administered in dose of 250 mg and 500 mg three times daily, in the two groups. Each group comprised of 25 patients. Nigella Sativa produced a decrease in opiate withdrawal signs and symptoms.

Nigella Sativa 250 mg reduced the subjective symptoms from pretreatment day-3 scoring rate of 55.08±12.65 to 29.4±5.02 at day-12. Similarly, objective signs were also reduced from pretreatment day-3 scoring rate of 19.76±4.58 to 12.12±2.60 at day-12.

Regarding the physiological parameters decrease was observed in pulse rate, systolic blood pressure, diastolic blood pressure, and

temperature, but all were within normal physiological ranges. Respiratory rate and pupil diameter also decreased. Both body weight and caloric intake increased.

Similarly, Nigella Sativa 500 mg was given to 25 patients three times daily throughout the treatment period after abrupt discontinuation of opioid administration. It also produced rapid decrease in opiate withdrawal signs and symptoms. The subjective symptoms were reduced from pretreatment day-3 scoring rate of 63.2±13.57 to 14.56±8.13 at day-12. Similarly objective signs were also reduced from pretreatment day-3 scoring rate of 25.52±3.08 to 7.72±2.35 at day-10.

Maintenance with opioid agonists methadone or L-α-methadol (LAAM), antagonist (naltrexone) or partial agonist (bupremorphine) is the usual practice in the long term management of opioid dependence, but all these drugs have their own disadvantages. It has been proved in many invitro studies that calcium channels/blockers, modulate the opioid receptors or release of endogenous opiopeptins in one or other way (Hernandez et al., 1993; Martinez et al., 1993; Smart and Lambert, 1996; Sher et al., 1996; Spampinato et al., 1994; Simmons, 1995; Fields and Sarne, 1997; Schwartz and Katki, 1990; Vonvoigtlander et al., 1987; Bongiani et al., 1986).

We found only one in vivo study (Shulman et al., 1998), in which the calcium channel blockers, verapamil or nifedepine were used in only

three patients for 2-8 weeks after detoxification. It was observed that calcium channel blockers prevent the development of significant craving and prevent the relapse. There was an increased sense of well being, manifested as less anxiety, clear thoughts, more satisfying sleep (often without sedatives), and a greater desire and capacity to participate in social and sporting activities. None of the patients suffered severe calcium channel blockers evoked adverse effects and none required cessation of calcium channel blocking agent.

There were 20 symptoms in the opiate withdrawal questionnaire used in previous studies with minimum score of zero to maximum 80. Grading of intensity of symptoms were from 0-4 with increasing severity. While the state used in our study had 38 subjective symptoms with a maximum score of 152. The additional symptoms were added for the more comprehensive assessment of the state of acute withdrawal as well as for assessing the state of protracted withdrawal after the acute abstinence. The additional symptoms were such as decrease in appetite, alertness, cheerfulness, calmness, patience, relaxation, and clear thinking, disorientation, carefreeness, drug craving, dysphoric mood, feelings of anxiety, increased sensitivity to pain, low psychomotor speed, pounding heart, sadness and shooting up.

Similarly, there were only six signs in opiate withdrawal questionnaire in previous two studies with minimum score of zero to maximum 24.

Grading of intensity of signs were from 0-4 with increasing severity. While the scale used in our study had 18 objective signs with a maximum score of 72. The additional signs were added for the more comprehensive assessment of the state of acute withdrawal as well as for assessing the state of protracted withdrawal after the acute abstinence. The additional signs were such as air, hunger, anorexia, insomnia, mydriasis, and tremor.

As it was proved in an in vitro study that Nigella Sativa is having calcium channel blocking effect (Gilani et al., 2001), along with analgesic, spasmolytic, anti-microbial, and anti-diarrhoeal etc., effects so this drug was used in this study. It is concluded that this drug is effective in long term management of opioid dependence.

It not merely cures the opioid dependence but also cures the infections and weakness from which majority of addicts suffer. It is suggested that further long-term follow up studies are needed to evaluate the benefit of this drug in maintaining the patients opioid free. In addition, various biochemical and physiological evidences are required to further strengthen the effectiveness of this non-opiate drug in long-term management of opioid dependence.

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APPENDICES

APPENDIX – I

OPIATE WITHDRAWAL SCALES – SEVERITY OF OPIOID WITHDRAWAL SIGNS

Name of Hospital:_____ Name:_____
Date of Admission _____ Indoor No._____ Bed # _____
Dated: _____

(B) Objective opiate withdrawal scale (OOWS)

1.	Air hunger	[0]	[1]	[2]	[3]	[4]
2.	Anorexia	[0]	[1]	[2]	[3]	[4]
3.	Cold flashes (shivering observed)	[0]	[1]	[2]	[3]	[4]
4.	Diarrhoea	[0]	[1]	[2]	[3]	[4]
5.	Emesis	[0]	[1]	[2]	[3]	[4]
6.	Hot flashes	[0]	[1]	[2]	[3]	[4]
7.	Insomnia	[0]	[1]	[2]	[3]	[4]
8.	Lacrimation	[0]	[1]	[2]	[3]	[4]
9.	Muscle Twitches	[0]	[1]	[2]	[3]	[4]
10.	Mydriasis	[0]	[1]	[2]	[3]	[4]
11.	Pilo-erection	[0]	[1]	[2]	[3]	[4]
12.	Restlessness (frequent shift of position)	[0]	[1]	[2]	[3]	[4]
13.	Rhinorrhoea	[0]	[1]	[2]	[3]	[4]
14.	Signs of abdominal	[0]	[1]	[2]	[3]	[4]
15.	Anxiety (foot shaking or trembling)	[0]	[1]	[2]	[3]	[4]
16.	Sweating	[0]	[1]	[2]	[3]	[4]
17.	Tremor	[0]	[1]	[2]	[3]	[4]
18.	Yawning	[0]	[1]	[2]	[3]	[4]

Physiologic Parameters

1. Pulse rate per minute:_____
2. Systolic blood pressure:_____
3. Diastolic blood pressure:_____
4. Temperature:_____
5. Respiratory rate:_____
6. Body weight:_____
7. Pupil diameter:_____
8. Caloric intake:_____

APPENDIX – II

OPIATE WITHDRAWAL SCALES – SEVERITY OF OPIOID WITHDRAWAL SYMPTOMS (SUBJECTIVE)

Name of Hospital:_____ Name:_____
Date of Admission:_____ Indoor # _____ Bed # _____
Dated:_____

1.	Abdominal cramps and sick to stomach	[0]	[1]	[2]	[3]	[4]
2.	Aching bones, joints and muscles	[0]	[1]	[2]	[3]	[4]
3.	Alertness, clear thinking, cheerfulness	[0]	[1]	[2]	[3]	[4]
4.	Apathy, dysphoria, disorientation, memory impairment	[0]	[1]	[2]	[3]	[4]
5.	Backache	[0]	[1]	[2]	[3]	[4]
6.	Bothered with noise	[0]	[1]	[2]	[3]	[4]
7.	Calmness, patience, relaxation	[0]	[1]	[2]	[3]	[4]
8.	Carefreeness	[0]	[1]	[2]	[3]	[4]
9.	Cold flashes	[0]	[1]	[2]	[3]	[4]
10.	Constipation	[0]	[1]	[2]	[3]	[4]
11.	Diarrhoea	[0]	[1]	[2]	[3]	[4]
12.	Drug craving	[0]	[1]	[2]	[3]	[4]
13.	Fatigue	[0]	[1]	[2]	[3]	[4]
14.	Feeling of Anxiety	[0]	[1]	[2]	[3]	[4]
15.	Goose flesh	[0]	[1]	[2]	[3]	[4]
16.	Hot flashes	[0]	[1]	[2]	[3]	[4]
17.	Increased sensitivity to pain	[0]	[1]	[2]	[3]	[4]
18.	Insomnia	[0]	[1]	[2]	[3]	[4]
19.	Irritability	[0]	[1]	[2]	[3]	[4]
20.	Loss of appetite	[0]	[1]	[2]	[3]	[4]
21.	Libido	[0]	[1]	[2]	[3]	[4]
22.	Low psychomotor speed	[0]	[1]	[2]	[3]	[4]
23.	Muscle twitching	[0]	[1]	[2]	[3]	[4]
24.	Muscle cramp	[0]	[1]	[2]	[3]	[4]
25.	Nausea and vomiting	[0]	[1]	[2]	[3]	[4]
26.	Palpitation	[0]	[1]	[2]	[3]	[4]
27.	Pounding heart	[0]	[1]	[2]	[3]	[4]
28.	Restlessness	[0]	[1]	[2]	[3]	[4]
29.	Running nose	[0]	[1]	[2]	[3]	[4]
30.	Sadness, depression	[0]	[1]	[2]	[3]	[4]

Continued.....

31.	Salivation	[0]	[1]	[2]	[3]	[4]
32.	Sexual intercourse time	[0]	[1]	[2]	[3]	[4]
33.	Shaking or tremulous	[0]	[1]	[2]	[3]	[4]
34.	Skin clammy and damp	[0]	[1]	[2]	[3]	[4]
35.	Shooting up and aggressive behaviour	[0]	[1]	[2]	[3]	[4]
36.	Sneezing	[0]	[1]	[2]	[3]	[4]
37.	Sweating	[0]	[1]	[2]	[3]	[4]
38.	Tearing eyes	[0]	[1]	[2]	[3]	[4]
39.	Tension	[0]	[1]	[2]	[3]	[4]
40.	Yawning	[0]	[1]	[2]	[3]	[4]

APPENDIX – III

Cumulative scores of Subjective Symptoms of withdrawal from Opioids in Group-II patients treated with Nigella Sativa 500 mg during 12 Days stay in Hospital

NOP	Pre-treatment Day			Treatment Day								
	1	2	3	4	5	6	7	8	9	10	11	12
1.	56	70	72	62	49	45	31	30	30	29	26	22
2.	53	73	74	74	52	46	36	34	32	28	25	18
3.	40	72	80	76	74	70	58	40	38	36	34	34
4.	53	73	77	77	74	72	70	68	60	54	48	40
5.	75	80	91	88	80	66	60	52	43	37	30	22
6.	54	65	79	70	64	54	48	40	36	30	28	12
7.	56	65	80	76	60	58	40	36	32	28	20	10
8.	57	70	78	70	62	60	54	50	48	40	32	16
9.	33	47	52	40	40	32	30	24	22	22	20	14
10.	46	58	67	65	36	30	30	28	26	24	22	12
11.	47	59	64	60	52	50	48	46	38	32	26	12
12.	33	50	55	52	52	46	39	38	27	23	17	13
13.	46	57	59	54	50	48	30	30	30	28	22	20
14.	40	49	49	46	44	42	32	30	26	23	19	15
15.	38	42	48	47	40	39	37	30	26	20	16	08
16.	30	46	55	53	47	40	30	25	25	23	20	12
17.	47	48	55	54	48	40	36	34	30	28	20	14
18.	40	52	57	55	50	48	37	28	27	24	18	12
19.	45	62	65	64	60	48	36	30	29	25	22	13
20.	35	46	65	60	54	50	46	41	37	12	12	08
21.	22	32	38	30	30	28	22	20	20	18	12	08
22.	29	41	45	31	27	22	21	18	16	12	10	06
23.	27	41	43	35	31	25	25	25	14	10	07	06
24.	26	48	65	48	40	30	30	30	22	14	10	09
25.	38	54	67	45	37	32	29	26	24	12	10	08
N	25	25	25	25	25	25	25	25	25	25	25	25
Mean	42.64	56	63.2	54.52	48.76	44.84	38.2	34.12	30.32	52.28	21.04	14.56
SD	12.03	12.24	13.29	17.36	17.13	3.36	12.07	10.89	9.83	9.80	8.93	7.96
SEM	12.28	12.50	13.57	17.72	17.49	13.61	12.32	11.11	10.04	10.01	9.12	8.13

Key: NOP = No. of Patients

APPENDIX – IV

Cumulative scores of Objective Signs of withdrawal from Opioids in Group-II patients treated with Nigella Sativa 500 mg during 12 Days stay in Hospital

NOP	Pre-treatment Day			Treatment Day								
	1	2	3	4	5	6	7	8	9	10	11	12
1.	18	24	27	20	17	14	10	10	08	07	07	07
2.	20	22	30	26	21	18	18	15	13	12	11	10
3.	17	21	26	25	20	12	12	11	11	11	10	09
4.	19	20	21	20	19	18	17	15	14	14	15	15
5.	15	22	24	20	18	17	17	17	14	12	12	10
6.	17	23	24	19	18	17	15	10	07	07	06	06
7.	19	20	22	19	18	16	15	14	11	10	07	06
8.	20	23	25	19	18	17	15	14	12	10	09	08
9.	14	20	22	20	17	13	13	12	11	10	07	06
10.	16	26	30	27	26	25	19	18	16	15	14	12
11.	13	26	28	26	24	15	14	11	10	09	08	07
12.	12	27	29	25	22	20	18	16	15	14	14	05
13.	10	29	29	26	22	18	16	15	14	12	10	09
14.	20	25	28	24	22	20	18	17	16	15	12	09
15.	18	14	20	18	16	15	14	14	12	10	09	04
16.	20	21	22	20	19	17	16	14	12	10	08	06
17.	20	22	26	20	18	16	15	14	12	09	07	06
18.	15	22	23	21	20	17	16	15	14	13	12	07
19.	12	24	26	25	23	22	19	18	17	15	14	06
20.	19	27	28	22	18	18	16	12	12	08	08	07
21.	14	28	28	26	24	22	20	16	15	14	12	08
22.	20	29	30	26	24	22	20	17	16	15	13	09
23.	16	20	22	22	19	17	17	13	13	09	09	06
24.	21	22	24	22	20	18	18	14	14	10	09	07
25.	12	22	24	22	21	19	17	16	15	12	10	08
Mean	16.68	23.16	25.52	22.40	20.16	17.72	16.20	13.76	12.96	11.32	10.12	7.72
SD	3.15	3.36	3.02	2.82	2.58	2.91	2.38	3.46	2.42	2.49	2.58	2.30
SEM	3.22	3.43	3.08	2.88	2.64	2.97	2.43	3.53	2.47	2.54	2.63	2.35

Key: NOP = No. of Patients

APPENDIX – V

Cumulative Scores of Subjective Symptoms of Protracted Abstinence in Group-II patients treated with Nigella Sativa 500 mg for Opioid dependence during 12 weeks of Follow up

NOP	Nigella Sativa Treatment Week								DTW		PTW	
	1	2	3	4	5	6	7	8	9	10	11	12
1.	15	12	14	13	14	13	15	10	0	0	0	0
2.	14	12	10	9	9	6	4	0	0	0	0	0
3.	12	10	9	8	6	4	5	3	3	1	1	1
4.	13	9	8	7	6	5	4	3	3	0	0	0
5.	20	15	13	12	9	8	7	6	2	2	2	1
6.	8	8	4	2	2	1	0	0	0	0	0	0
7.	11	11	11	11	10	9	9	7	4	2	0	0
8.	7	4	2	1	1	1	0	0	0	0	0	0
9.	6	10	10	7	4	4	3	0	0	0	0	0
10.	14	14	14	14	12	11	9		7	6	5	4
11.	10	8	6	5	4	3	2	1	0	0	0	0
12.	9	10	10	7	4	1	0	0	0	0	0	0
13.	17	9	9	2	2	2	0	0	0	0	0	0
14.	14	5	1	0	0	0	0	0	0	0	0	0
15.	15	4	4	4	4	2	0	0	0	0	0	0
16.	16	2	2	2	0	0	0	0	0	0	0	0
17.	3	4	4	4	0	0	0	0	0	0	0	0
18.	7	23	12	10	6	0	0	0	0	0	0	0
19.	9	8	8	2	2	2	0	0	0	0	0	0
20.	5	6	6	6	4	4	4	2	2	2	0	0
21.	11	13	13	8	4	2	2	2	0	0	0	0
22.	8	3	3	1	1	0	0	0	0	0	0	0
23.	9	17	16	9	3	1	0	0	0	0	0	0
24.	5	1	0	0	0	0	0	0	0	0	0	0
25.	7	5	5	5	4	2	2	2	2	2	2	2
Mean	10	8.92	7.76	5.96	4.44	2.24	2.28	1.8	0.2	0.6	0.4	0.32
SD	4.45	5.01	4.46	4.07	3.75	3.27	3.57	2.93	1.71	1.32	9.09	0.88
SEM	4.54	5.12	4.55	4.15	3.83	3.34	3.64	3.00	1.75	1.35	1.11	0.90

Key: NOP = No. of Patients

APPENDIX – VI

Cumulative Scores of Objective Signs of Protracted Abstinence in Group-II patients treated with Nigella Sativa 500 mg in Opioid dependence during 12 weeks of Follow up

NOP	Nigella Sativa Treatment Week								DTW		PTW	
	1	2	3	4	5	6	7	8	9	10	11	12
1.	4	4	3	2	1	1	0	0	0	0	0	0
2.	6	8	5	3	2	1	1	0	0	0	0	0
3.	8	6	4	4	3	2	1	1	1	1	1	1
4.	12	9	7	5	4	2	1	0	0	0	0	0
5.	7	6	6	5	5	5	2	0	0	0	0	0
6.	9	5	4	4	3	3	1	1	1	1	1	1
7.	5	4	4	3	3	1	1	1	1	1	1	1
8.	6	6	4	4	4	4	3	3	2	2	2	2
9.	4	5	5	3	3	1	0	0	0	0	0	0
10.	3	1	1	0	0	0	0	0	0	0	0	0
11.	6	2	2	2	1	1	0	0	0	0	0	0
12.	1	0	0	0	0	0	0	0	0	0	0	0
13.	8	5	3	2	1	1	0	0	0	0	0	0
14.	10	9	9	9	8	4	2	1	1	1	1	1
15.	7	6	6	5	5	5	2	0	0	0	0	0
16.	6	2	2	2	1	1	1	0	0	0	0	0
17.	5	5	3	2	1	1	0	0	0	0	0	0
18.	4	4	4	4	4	4	4	2	2	2	2	2
19.	7	6	6	5	5	5	2	0	0	0	0	0
20.	9	6	3	3	3	1	1	1	1	1	1	1
21.	11	9	9	9	8	4	2	1	0	0	0	0
22.	4	3	2	2	2	2	1	0	0	0	0	0
23.	2	2	1	1	0	0	0	0	0	0	0	0
24.	3	1	1	0	0	0	0	0	0	0	0	0
25.	6	2	2	2	1	1	0	0	0	0	0	0
Mean	6.12	4.64	3.84	3.24	2.72	2	1	0.44	0.36	0.36	0.36	0.36
SD	2.70	2.52	2.32	2.26	1.67	2.23	1.05	0.75	0.62	0.62	0.62	0.62
SEM	2.75	2.58	2.37	2.31	1.70	2.28	1.08	0.76	0.63	0.63	0.63	0.63

Key: NOP = No. of Patients

APPENDIX – VII

Cumulative scores of Subjective Symptoms of withdrawal from Opioids in Group-I patients treated with Nigella Sativa 250 mg in During 12 days stay in Hospital

NOP	Pre-treatment Day			Treatment Day								
	1	2	3	4	5	6	7	8	9	10	11	12
1.	40	44	46	46	40	35	33	30	28	26	24	22
2.	50	54	60	58	58	54	50	48	44	40	38	30
3.	28	30	50	48	46	40	38	36	34	30	28	26
4.	38	40	46	46	40	35	33	30	28	26	24	22
5.	48	48	52	50	48	46	40	38	38	36	32	30
6.	50	54	60	59	58	52	50	48	44	40	38	30
7.	53	57	70	64	60	56	50	46	42	36	30	28
8.	48	56	65	64	60	58	54	50	48	44	40	36
9.	38	50	58	56	50	48	46	42	40	38	36	23
10.	34	50	60	58	52	52	50	48	44	40	38	30
11.	40	52	70	68	62	58	56	54	50	48	34	28
12.	46	60	74	72	68	60	54	50	44	30	30	22
13.	30	40	55	54	50	48	48	44	42	40	39	30
14.	30	40	58	56	40	38	30	30	30	28	26	20
15.	26	34	58	56	54	52	50	48	46	44	40	30
16.	26	36	50	50	50	48	48	42	40	38	37	36
17.	44	51	57	56	50	50	49	47	46	42	40	38
18.	48	53	53	52	48	46	44	40	40	38	37	35
19.	40	47	50	49	48	46	44	43	40	35	34	30
20.	36	48	58	56	50	48	40	38	37	35	34	30
21.	31	43	57	54	54	50	48	47	46	45	40	32
22.	37	50	61	58	56	52	50	48	48	47	45	39
23.	38	54	54	52	50	48	47	44	38	36	32	30
24.	40	46	53	52	50	50	48	46	40	36	34	28
25.	46	48	50	48	46	46	42	40	39	38	36	30
Mean	39.4	47.4	55.08	55.28	55.28	48.64	45.68	43.08	40.64	37.44	34.64	29.4
SD	7.82	7.35	12.39	6.45	6.45	6.41	6.63	6.39	5.79	5.92	5.26	4.92
SEM	7.98	7.50	12.65	6.58	6.58	6.54	6.77	6.52	5.91	6.04	5.37	5.02

Key: NOP = No. of Patients

APPENDIX – VIII

Cumulative scores of Objective signs of withdrawal from Opioids in Group-I patients treated with Nigella Sativa 250 mg during 12 days stay in Hospital

NOP	Pre-treatment Day			Treatment Day								
	1	2	3	4	5	6	7	8	9	10	11	12
1.	20	23	30	27	25	24	23	23	22	20	20	19
2.	18	17	28	27	25	23	23	23	21	20	20	20
3.	15	16	26	25	24	23	19	18	17	16	15	11
4.	19	23	25	24	24	23	22	20	19	18	17	15
5.	18	20	22	22	21	20	19	17	15	14	14	12
6.	15	18	20	21	20	18	17	14	13	13	12	11
7.	18	19	23	22	19	18	15	13	13	12	12	11
8.	14	16	18	17	17	15	15	15	14	14	14	14
9.	15	16	19	18	15	14	13	12	11	11	11	10
10.	10	12	16	16	15	15	14	13	13	12	12	12
11.	18	20	22	21	21	21	19	18	17	15	14	13
12.	17	19	21	20	20	20	18	16	15	14	13	12
13.	20	20	24	23	22	21	19	18	17	15	14	13
14.	12	14	18	17	17	17	18	16	15	14	12	12
15.	16	18	20	19	19	19	19	18	13	12	11	11
16.	15	16	18	18	18	17	15	14	14	13	12	10
17.	10	12	14	14	14							
18.	12	15	17	16	16	15	14	13	12	12	11	11
19.	11	13	14	14	14	14	14	14	12	11	10	10
20.	18	20	22	21	20	20	19	18	17	15	14	12
21.	16	18	18	17	17	17	16	16	18	16	15	13
22.	12	14	15	15	15	15	15	15	15	13	10	10
23.	8	10	12	12	11	11	11	11	11	11	11	11
24.	12	18	18	16	14	12	12	11	10	10	10	10
25.	14	14	14	13	12	11	10	10	10	10	10	10
n	25	25	25	25	25	25	25	25	25	25	25	25
Mean	14.92	16.84	19.76	19	18.2	17.48	16.48	15.52	14.6	13.68	13	12.12
SD	3.28	3.28	4.49	4.13	3.91	3.77	3.47	3.39	3.2	2.70	2.74	2.55
SEM	3.35	3.35	4.58	4.22	4	3.85	3.54	3.46	3.26	2.76	2.79	2.60

Key: NOP = No. of Patients

APPENDIX – IX

Cumulative scores of Subjective symptoms of Pre-treated Abstinence in Group-I patients treated with Nigella Sativa 250 mg for Opioid Dependence during 12 weeks of follow up

NOP	500 mg Nigella Sativa Week								DTW		PTW	
	1	2	3	4	5	6	7	8	9	10	11	12
1.	30	26	24	22	20	16	14	12	10	8	4	2
2.	26	26	20	18	18	17	16	14	14	12	8	4
3.	12	12	10	9	8	6	5	4	4	3	3	0
4.	22	20	19	18	15	14	13	12	11	9	7	3
5.	27	24	22	20	18	17	15	14	13	10	8	6
6.	27	26	26	20	18	16	14	12	13	9	7	5
7.	19	19	18	18	16	15	13	11	7	5	4	3
8.	13	11	9	7	5	5	5	4	3	2	2	1
9.	12	11	11	6	4	0	0	0	0	0	0	0
10.	26	26	26	13	13	10	5	0	0	0	0	0
11.	22	18	14	13	7	7	3	0	0	0	0	0
12.	27	26	9	8	7	0	0	0	0	0	0	0
13.	29	29	21	22	22	15	15	15	9	7	7	3
14.	28	28	24	24	20	10	8	0	0	0	0	0
15.	30	25	25	20	18	11	3	3	3	3	3	3
16.	16	14	14	13	5	5	1	0	0	0	0	0
17.	14	14	5	5	3	1	0	0	0	0	0	0
18.	46	30	22	18	8	4	0	0	0	0	0	0
19.	44	30	22	16	10	8	6	4	2	0	0	0
20.	43	30	28	18	8	4	2	0	0	0	0	0
21.	44	20	22	22	16	14	10	8	4	2	0	0
22.	42	30	20	18	14	12	8	8	0	0	0	0
23.	38	20	16	16	12	14	12	10	8	5	3	0
24.	38	28	18	8	4	0	0	0	0	0	0	0
25.	28	26	12	10	8	2	0	0	0	0	0	0
n	25	25	25	25	25	25	25	25	25	25	25	25
Mean	28.12	22.76	18.28	15.28	11.88	10.92	6.72	5.24	4.04	3	2.24	1.16
SD	10.40	6.25	6.14	5.56	5.80	6.67	5.70	5.54	4.87	3.84	2.91	1.80
SEM	10.61	6.38	6.27	5.67	5.92	6.81	5.82	5.65	4.97	3.92	2.97	1.84

Key: NOP = No. of Patients

APPENDIX – X

Cumulative stores of Objective signs of Pre-treated Abstinence in Group-I patients treated with Nigella Sativa 250 mg for Opioid Dependence during 12 weeks of follow up

NOP	500 mg Nigella Sativa Week								DTW		PTW	
	1	2	3	4	5	6	7	8	9	10	11	12
1.	15	15	14	12	9	7	6	5	3	0	0	0
2.	14	13	13	11	10	7	4	3	2	0	0	0
3.	11	10	9	7	5	4	2	2	0	0	0	0
4.	5	5	3	2	1	1	1	0	0	0	0	0
5.	11	10	9	7	6	5	4	3	3	0	0	0
6.	12	12	12	12	10	9	8	7	5	4	3	3
7.	12	11	11	10	8	7	6	5	2	2	1	1
8.	11	10	9	9	7	5	4	3	0	0	0	0
9.	10	7	6	4	3	3	2	0	0	0	0	0
10.	9	8	5	3	2	0	0	0	0	0	0	0
11.	7	6	3	2	1	0	0	0	0	0	0	0
12.	11	10	9	7	5	4	2	1	0	0	0	0
13.	11	9	7	5	4	3	2	2	0	0	0	0
14.	13	12	10	8	6	5	4	3	2	2	0	0
15.	11	10	8	5	2	2	0	0	0	0	0	0
16.	14	11	9	8	7	4	3	3	0	0	0	0
17.	6	6	6	6	5	4	3	2	1	0	0	0
18.	9	8	6	4	4	4	2	2	2	2	2	1
19.	8	6	5	4	3	2	1	0	0	0	0	0
20.	11	9	7	6	5	4	3	2	1	0	0	0
21.	8	7	6	5	4	3	2	1	0	0	0	0
22.	6	5	4	3	2	1	0	0	0	0	0	0
23.	5	5	5	5	5	4	3	2	1	0	0	0
24.	7	6	6	5	4	3	3	3	1	0	0	0
25.	7	6	5	4	3	2	1	0	0	0	0	0
n	25	25	25	25	25	25	25	25	25	25	25	25
Mean	9.76	8.68	7.48	6.16	4.84	3.72	2.64	1.96	0.92	0.4	0.24	0.2
SD	2.81	2.70	2.91	2.85	2.52	2.18	1.97	1.82	1.29	0.97	0.70	0.63
SEM	2.87	2.76	2.97	2.91	2.57	2.22	2.01	1.85	1.32	1	0.72	0.64

Key: NOP = No. of Patients

APPENDIX – XI

Physiological Parameters of Group-II patients Treated with Nigella Sativa 500 mg before Opioid withdrawal (Day 0)

NOP	PR (per minute)	SBP (mmHg)	DBP (mmHg)	Temp: (°F)	RR (per minute)	BW (kg)	PD (mm)	CI (Kcal)
1.	90	100	70	96	18	46	2	1.8
2.	80	130	70	97	18	40	2	2
3.	82	100	70	97	17	50	2	2
4.	82	90	60	98	20	60	1.5	1.8
5.	72	90	70	97.4	20	55	1.6	1.9
6.	100	110	70	98.4	20	53.2	1.7	2.9
7.	90	120	80	97	22	64	1.6	2.8
8.	64	120	80	97	22	69	1.5	2.4
9.	80	120	80	96	22	70.6	2	2
10.	80	130	60	98	20	80.2	1.5	2.2
11.	90	140	85	98	22	44	2	2.6
12.	64	130	60	96	20	45	2	3
13.	64	100	60	96	20	48	1.5	2.5
14.	74	100	80	98	20	61.2	1.8	2.5
15.	92	100	80	97	20	64	1.6	2.6
16.	86	90	60	96	16	48	1.3	2.9
17.	76	90	60	97	18	58	1.3	2.1
18.	70	90	60	98.4	18	61	2	2
19.	80	120	70	98.4	18	42	2	2.5
20.	84	120	70	98	20	56.7	2	2.6
21.	84	110	70	98	20	58	1.5	2.8
22.	100	110	70	98	20	48	1.5	2
23.	70	100	70	97	18	48	1.3	1.8
24.	70	116	60	96	18	50	1.3	2.7
25.	80	120	80	96	18	50	2	2
n	25	25	25	25	25	25	25	25
Mean	80.16	109.84	69.80	97.18	19.40	54.79	1.70	2.33
SD	10.00	14.60	8.05	0.87	1.60	9.64	0.26	0.38
SEM	10.21	14.90	8.22	0.89	1.63	9.84	0.27	0.39

Key:

PR	=	Pulse Rate	Temp	=	Temperature
SBP	=	Systolic blood pressure	BW	=	Body weight
DBP	=	Diastolic blood pressure	CI	=	Caloric Intake
Kcal	=	Kilo Calories	PD	=	Pulse diameter
RR	=	Respiratory rate	°F	=	Degree Fahrenheit

APPENDIX – XII

Physiological Parameters of Group-II patients on Day-3 of Admission before starting Treatment with Nigella Sativa 500 mg

NOP	PR (per minute)	SBP (mmHg)	DBP (mmHg)	Temp: (°F)	RR (per minute)	BW (kg)	PD (mm)	CI (Kcal)
1.	90	110	70	98	22	46	3	1.5
2.	100	120	80	99	24	40	3.5	1.6
3.	109	114	82	99.4	20	50	3.5	1.9
4.	70	120	86	98	20	60	3	1.6
5.	108	120	70	99.6	22	55	3	1.5
6.	82	100	70	98	20	53.2	3	2.4
7.	110	130	100	101	24	64	3	2.3
8.	80	110	70	98	22	60	3.5	1.8
9.	80	90	60	99	26	50	3.5	1.9
10.	70	130	90	98	26	64	3.5	1.7
11.	110	90	60	100	26	60.6	3	2.3
12.	80	100	70	98	22	70.6	3.5	2.4
13.	80	100	70	98	26	40.2	3.5	2.5
14.	70	110	80	97	26	62.2	4	2.5
15.	100	140	90	99.8	22	80	3	2.3
16.	110	110	90	99.8	22	44	3.5	2.5
17.	70	130	90	98	26	48.6	4	2.8
18.	100	120	80	99.8	22	48	3	1.4
19.	82	130	90	99	22	49	3	1.4
20.	110	100	70	100	22	50	3	2.4
21.	106	130	90	100	22	61	3	2.5
22.	72	130	80	98	24	64	3	2.6
23.	80	116	80	98.4	20	48	4	2.5
24.	110	110	80	100	20	60	3.5	1.6
25.	80	100	80	97	22	42	4	1.9
n	25	25	25	25	25	25	25	25
Mean	90.36	114.40	79.12	98.83	22.80	54.81	3.34	2.07
SD	15.34	13.51	10.04	1.04	2.11	9.74	0.36	0.43
SEM	15.66	13.79	10.24	1.06	2.16	9.94	0.37	0.44

Key:

PR	=	Pulse Rate	Temp	=	Temperature
SBP	=	Systolic blood pressure	BW	=	Body weight
DBP	=	Diastolic blood pressure	CI	=	Caloric Intake
Kcal	=	Kilo Calories	PD	=	Pulse diameter
RR	=	Respiratory rate	°F	=	Degree Fahrenheit

APPENDIX – XIII

Physiological Parameters of Group-II patients Treated with Nigella Sativa 500 mg on Day-12 of Admission

NOP	PR (per minute)	SBP (mmHg)	DBP (mmHg)	Temp: (°F)	RR (per minute)	BW (kg)	PD (mm)	CI (Kcal)
1.	100	100	70	98	22	46.6	3	1.7
2.	90	104	70	98.4	22	40.6	3	1.8
3.	92	90	70	98.2	24	50.6	3	1.9
4.	89	112	74	98.4	20	60.8	3	1.9
5.	72	106	74	98.4	20	55.6	2	2
6.	72	90	72	98	20	64.4	2.5	2.5
7.	82	120	80	99	20	53.6	2.5	2.5
8.	72	100	70	98.4	22	69.2	2.5	2
9.	70	120	80	98	22	50.2	2.5	2
10.	70	120	85	98	24	64	1	2
11.	72	90	60	97.4	20	60.8	2	2.5
12.	68	100	70	98	20	70.8	1	2.5
13.	70	110	85	98	24	40.2	1	2.5
14.	72	100	70	97.4	24	62.6	2	2.5
15.	82	130	90	98	22	80.6	1	2.5
16.	82	110	75	98.6	22	44.8	3	2.5
17.	90	120	80	98.2	22	48.8	2	2.8
18.	80	100	70	99	22	48.2	2	1.4
19.	82	120	80	98.4	22	49.2	3	2.2
20.	72	100	70	99	22	56.2	1	2.5
21.	82	120	80	99.2	22	61.8	2	2.5
22.	100	120	70	98	22	64.2	1	2.6
23.	70	100	70	97.6	20	48.2	3	2
24.	80	100	70	98	18	58.2	2	2
25.	70	90	60	97.4	22	61.2	2	2.5
n	25	25	25	25	25	25	25	25
Mean	79.24	106.88	73.80	98.20	21.60	56.45	2.12	2.23
SD	9.50	11.51	7.07	0.48	1.49	9.67	0.73	0.34
SEM	9.70	11.74	7.21	0.49	1.52	9.87	0.75	0.35

Key:

PR	=	Pulse Rate	Temp	=	Temperature
SBP	=	Systolic blood pressure	BW	=	Body weight
DBP	=	Diastolic blood pressure	CI	=	Caloric Intake
Kcal	=	Kilo Calories	PD	=	Pulse diameter
RR	=	Respiratory rate	°F	=	Degree Fahrenheit

APPENDIX – XIV

Physiological Parameters of Group-II patients Treated with Nigella Sativa 500 mg on Week-12 of Follow up Period

NOP	PR (per minute)	SBP (mmHg)	DBP (mmHg)	Temp: (°F)	RR (per minute)	BW (kg)	PD (mm)	CI (Kcal)
1.	72	110	70	98	18	50	1.5	2.6
2.	72	110	70	98.4	18	42	1	2.7
3.	80	100	70	98	18	52	2	2.8
4.	80	110	70	98	18	60	1	2.6
5.	70	100	60	98	18	50	2	2.6
6.	70	90	60	97	18	55	2	2.8
7.	80	120	80	98	18	60	1	2.6
8.	70	110	85	98	20	65	2	2.8
9.	80	90	60	98	18	51	1.5	2.8
10.	80	120	80	98	20	70	1	3
11.	72	100	70	98.4	18	42	2	2.8
12.	72	90	60	98	18	61	1	2.6
13.	72	110	85	80	98	70	1	2.9
14.	70	100	70	98	18	46	2	2.7
15.	80	130	90	98	18	50	1	2.7
16.	80	110	80	98	18	50	2	2.5
17.	70	120	80	98.4	18	52	2	2.5
18.	80	110	80	98.4	20	58	2	2.8
19.	80	130	90	98.4	18	60	2	2.8
20.	80	100	70	99	20	45	1	3
21.	80	120	80	98	18	45	2	2.5
22.	72	130	70	98.4	20	55	1	2.5
23.	72	90	60	97	18	60	2	2.7
24.	70	110	80	97	18	43	2	2.6
25.	60	100	70	97	18	56	2	2.4
n	25	25	25	25	25	25	25	25
Mean	74.56	109.60	73.60	97.25	21.60	53.92	1.60	2.69
SD	5.32	12.80	9.22	3.55	15.61	7.90	0.46	0.15
SEM	5.43	13.06	9.41	3.62	15.93	8.07	0.47	0.15

Key:

PR	=	Pulse Rate	Temp	=	Temperature
SBP	=	Systolic blood pressure	BW	=	Body weight
DBP	=	Diastolic blood pressure	CI	=	Caloric Intake
Kcal	=	Kilo Calories	PD	=	Pulse diameter
RR	=	Respiratory rate	°F	=	Degree Fahrenheit

APPENDIX – XV

Physiological Parameters of Group-I patients Treated with Nigella Sativa 250 mg Before Opioid withdrawal (Day-0)

NOP	PR (per minute)	SBP (mmHg)	DBP (mmHg)	Temp: (°F)	RR (per minute)	BW (kg)	PD (mm)	CI (Kcal)
1.	70	80	50	98	20	40.6	2	2
2.	72	90	60	98	18	43	1.5	2.2
3.	70	85	60	97	20	52.8	2	2
4.	80	90	60	98	20	58	1.5	2.2
5.	80	110	80	98	18	49.8	2	2
6.	80	120	80	98	18	44.6	2	1.6
7.	70	90	60	97	20	56	2	2
8.	68	95	60	98	20	50	1.5	2.6
9.	70	90	60	98	18	65	1.8	2.4
10.	70	85	55	97	20	63	1.5	1.7
11.	70	80	50	98	20	55	2.5	1.8
12.	64	100	70	99	22	68	2.5	2.8
13.	70	90	60	97	20	66.6	1.5	2.6
14.	80	120	80	98.5	18	42	2	1.8
15.	80	100	70	98	18	54.4	1.5	2.5
16.	70	110	70	98	18	57	1.5	2.1
17.	100	120	80	99	17	48	1.5	2.5
18.	80	120	80	97	18	60	1.6	2.1
19.	70	90	60	98	20	45	1.5	2.3
20.	72	100	70	99	18	44	1.6	2
21.	68	95	65	98	19	51.2	1.8	1.9
22.	80	100	60	98.4	17	50.6	1.7	1.9
23.	70	90	60	97	20	43	2	1.8
24.	72	110	70	97	20	49	1.8	2
25.	70	100	65	97	20	56	1.9	2.2
n	25	25	25	25	25	25	25	25
Mean	73.84	98.40	65.40	97.83	19.08	52.50	1.78	2.12
SD	7.17	12.38	9.04	0.65	1.23	7.80	0.29	0.30
SEM	7.32	12.64	9.23	0.66	1.25	7.96	0.30	0.30

Key:

PR	=	Pulse Rate	Temp	=	Temperature
SBP	=	Systolic blood pressure	BW	=	Body weight
DBP	=	Diastolic blood pressure	CI	=	Caloric Intake
Kcal	=	Kilo Calories	PD	=	Pulse diameter
RR	=	Respiratory rate	°F	=	Degree Fahrenheit

APPENDIX – XVI

Physiological Parameters of Group-I patients on Day-3 of Admission before starting Treatment with Nigella Sativa 250 mg

NOP	PR (per minute)	SBP (mmHg)	DBP (mmHg)	Temp: (°F)	RR (per minute)	BW (kg)	PD (mm)	CI (Kcal)
1.	120	100	70	101	26	40.6	4	1
2.	100	90	60	100	20	43	3	2
3.	80	100	70	98	24	52.8	3.5	1.5
4.	90	100	70	99.2	26	58	3	2
5.	80	130	85	98.4	24	49.8	4	1.6
6.	84	140	90	99	22	44.6	3.5	1.1
7.	88	100	70	99	24	56	4	1.8
8.	80	110	75	98	22	50	3	2.5
9.	86	100	75	99	22	65	3	2.1
10.	90	100	75	99	22	63.8	3	2.1
11.	80	90	60	98	24	55.6	3.5	1.5
12.	78	120	80	98.4	26	68	3.5	2
13.	70	100	70	98	22	58	3	2.5
14.	100	130	85	100	22	66	3.2	2
15.	92	116	80	99.4	22	42.4	3	2.5
16.	82	126	80	99	24	52	3	1.5
17.	120	130	85	102	26	56	3	2.5
18.	110	120	80	101	22	47	3	1.6
19.	88	110	70	99	24	60	3.5	2
20.	90	110	75	100	26	58	2.8	1.6
21.	90	110	70	99	24	56	3	1.8
22.	84	120	80	98.4	22	58.2	3.5	2
23.	90	130	85	99.2	22	49.2	3.5	2.1
24.	86	120	80	99	24	48	3.2	2.5
25.	80	140	90	98.4	22	45	3.5	2
n	25	25	25	25	25	25	25	25
Mean	89.52	113.68	76.40	99.13	23.36	53.72	3.28	1.91
SD	12.03	14.49	7.93	1.01	1.67	7.54	0.34	0.41
SEM	12.27	14.79	8.10	1.03	1.70	7.69	0.35	0.41

Key:

PR	=	Pulse Rate	Temp	=	Temperature
SBP	=	Systolic blood pressure	BW	=	Body weight
DBP	=	Diastolic blood pressure	CI	=	Caloric Intake
Kcal	=	Kilo Calories	PD	=	Pulse diameter
RR	=	Respiratory rate	°F	=	Degree Fahrenheit

APPENDIX – XVII

Physiological Parameters of Group-I patients Treated with Nigella Sativa 250 mg on Day-12 of Admission

NOP	PR (per minute)	SBP (mmHg)	DBP (mmHg)	Temp: (°F)	RR (per minute)	BW (kg)	PD (mm)	CI (Kcal)
1.	105	95	60	99	25	39.2	3	1.5
2.	90	90	60	99	20	40	2	2
3.	80	100	70	98.4	22	48	2.5	2
4.	88	95	55	98	22	42.4	2.5	1.6
5.	75	120	80	99.4	24	52.6	3	1.8
6.	80	130	90	98.4	20	55	3	2.5
7.	84	96	70	98.4	18	47.3	2	1.3
8.	80	105	70	98.2	20	48.4	2.5	1.8
9.	80	100	70	98.4	22	55	3	2.5
10.	85	95	60	98.2	21	50.2	2.5	2.2
11.	75	85	55	97.2	21	65	3	1.2
12.	70	115	75	98.4	23	64	3	1.5
13.	65	100	65	97.2	25	55.8	2.5	2.5
14.	90	120	80	99	20	65.2	3	1.8
15.	88	110	75	98.6	18	59	2.5	2.5
16.	75	120	80	97.5	20	67.1	2.5	2.5
17.	120	125	85	99	22	42.2	2.6	2
18.	95	110	70	97.6	24	54.2	3	2.4
19.	80	100	65	99.2	26	58.4	3	1.9
20.	85	95	65	99	20	45.2	2.5	1.8
21.	72	100	70	98.4	21	60.6	3	2
22.	70	95	65	97	22	54	2.5	2
23.	75	90	60	97.2	24	50	3	1.9
24.	80	95	65	97	22	49.4	2.5	2
25.	70	100	70	98	22	65	3	2.5
n	25	25	25	25	25	25	25	25
Mean	82.28	103.44	69.20	98.22	21.76	53.32	2.70	1.98
SD	11.66	11.78	8.79	0.71	2.04	8.13	0.31	0.38
SEM	11.90	12.02	8.97	0.73	2.08	8.29	0.32	0.39

Key:

PR	=	Pulse Rate	Temp	=	Temperature
SBP	=	Systolic blood pressure	BW	=	Body weight
DBP	=	Diastolic blood pressure	CI	=	Caloric Intake
Kcal	=	Kilo Calories	PD	=	Pulse diameter
RR	=	Respiratory rate	°F	=	Degree Fahrenheit

APPENDIX – XVIII

Physiological Parameters of Group-I patients Treated with Nigella Sativa 250 mg on Week-12 of Follow up Period

NOP	PR (per minute)	SBP (mmHg)	DBP (mmHg)	Temp: (°F)	RR (per minute)	BW (kg)	PD (mm)	CI (Kcal)
1.	85	100	70	99	20	42	2	2.5
2.	85	90	60	99	18	43	2	2.5
3.	75	100	70	98.4	16	50	2	2.6
4.	72	100	60	98	17	52	2	2.7
5.	74	120	80	97.4	18	58	2	2.6
6.	80	125	85	98	19	55	2	2.7
7.	74	100	72	97.4	18	51	2	2.9
8.	74	100	70	98	18	50	2	2.9
9.	74	100	70	98.4	16	52	2	2.9
10.	74	100	70	98	18	66	2	2.7
11.	76	90	60	98.4	18	57.2	2.8	2.8
12.	74	110	70	98.2	20	60	2	2.9
13.	70	100	70	98	18	58	2	2.8
14.	84	120	80	98.4	18	60	2	2.6
15.	76	110	70	98	18	44.5	2	2.6
16.	72	110	70	97	18	58	2	2.9
17.	88	110	75	98	18	59	2	2.9
18.	85	110	75	98.6	18	49	2	2.7
19.	72	95	64	98	18	60	2	2.7
20.	74	100	60	98	20	55	2	2.6
21.	80	110	70	98.4	20	48	2	2.7
22.	82	115	75	98	18	50	2	2.6
23.	80	120	75	98.4	20	45	2	2.6
24.	72	120	75	98	18	48	2	2.7
25.	80	120	80	98.4	18	50	2	2.5
N	25	25	25	25	25	25	25	25
Mean	77.28	107.00	71.04	98.13	18.24	52.82	2.03	2.70
SD	5.05	9.89	6.52	0.43	1.06	6.05	0.15	0.13
SEM	5.16	10.10	6.65	0.44	1.09	6.18	0.16	0.13

Key:

PR	=	Pulse Rate	Temp	=	Temperature
SBP	=	Systolic blood pressure	BW	=	Body weight
DBP	=	Diastolic blood pressure	CI	=	Caloric Intake
Kcal	=	Kilo Calories	PD	=	Pulse diameter
RR	=	Respiratory rate	°F	=	Degree Fahrenheit

APPENDIX - XIX

DRUG REHABILITATION CENTRE
R.H.C. MURAD MEMON GOTH
KARACHI

(CONSENT FORM)

I hereby give consent to a treatment given to me. The nature and extent of such a treatment or procedure, I leave entirely to the discretion of the Medical Officer performing the above said purpose will not hold of the Hospital staff responsible for any risks involved in or incident occurring during or after the treatment or procedure.

Signature:

Date:

Address:

APPENDIX – XX

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS
BASIC MEDICAL SCIENCES INSTITUTE
JINNAH POSTGRADUATE MEDICAL CENTRE, KARACHI

DETOXIFICATION DATA

IN-PATIENTS RECORD:

Name of Hospital:_____
Indoor No. _____ Bed No. _____ Date of Admission _____

IDENTIFICATION:
Name:_____
Father's Name:_____

AGE CODE:
1. 21-30 years 2. 31-45 years

SEX CODE:
1. Male ☐ 2. Female ☐

MARITAL CODE:
1. Married ☐ 2. Unmarried ☐ 3. Previously Married ☐

Address:_____

OCCUPATION:
Present Code: Description _____
Past Code: _____
1. Unemployed ☐ 2. Unskilled ☐ 3. Semi-skilled ☐
4. Skilled ☐ 5. Managerial and Above ☐
6. Self-employed/Business ☐

SOCIOECONOMIC STATUS CODE:
1. Upper ☐ 2. Middle ☐ 3. Lower ☐

[Continued.....]

FAMILY:
Name of the Spouse:_____ Age Code:_____ Job Code:_____
Siblings No.: Male _____ Female _____
Children No.: Male _____ Female _____

ADDICTION DATA CODE:
1. Heroin ☐ 2. Others ☐

Mode of Onset:
1. Self ☐ 2. Peer Pressure ☐ 3. Any other ☐

Method of Addiction:
1. Smoking ☐ 2. Inhaling Pannee (Chasing the Dragon) ☐
3. Injections ☐ 4. Others ☐

Age at Onset: (Use Age Codes as above): _____

Previous Drug Free Period: _____

PREVIOUS TREATMENT:
1. Home ☐ 2. Govt. Hospital ☐ 3. Pvt. Hospital ☐

TREATMENT GIVEN: _____

SIDE-EFFECTS CODE: 1. No 2. Yes 3. Specify

1. Nil ☐ 2. Mild ☐ 3. Moderate ☐ 4. Severe ☐

DURATION OF TREATMENT:_____

REASON FOR RE-STARTING: _____

REASON FOR RE-STARTING:

CODE: 1. Craving ☐ 2. To avoid withdrawal ☐
3. Peer Pressure ☐ 4. Stress ☐
5. Others ☐

[Continued.....]

PSYCHIATRY HISTORY:
1. Present Code:______________________________
2. Previous Code:______________________________

MOTIVATION CODE:
1. No Motivation 2. Partial Motivation 3. Full Motivation

FORENSIC HISTORY:

Previous:	1.	No	2.	Yes	3.	Specify
Present:	1.	No	2.	Yes	3.	Specify

H/O BLOOD DONATION CODE:
1. No 2. Yes

PROGRESS IN WARD:

DATE	ADVERSE DRUG EFFECT	PULSE	B.P.	TEMP	DATE	ADVERSE DRUG EFFECT	PULSE	B.P.	TEMP
	Morning					Morning			
	Evening					Evening			
	Night					Night			
DATE	ADVERSE DRUG EFFECT	PULSE	B.P.	TEMP	DATE	ADVERSE DRUG EFFECT	PULSE	B.P.	TEMP
	Morning					Morning			
	Evening					Evening			
	Night					Night			
DATE	ADVERSE DRUG EFFECT	PULSE	B.P.	TEMP	DATE	ADVERSE DRUG EFFECT	PULSE	B.P.	TEMP
	Morning					Morning			
	Evening					Evening			
	Night					Night			
DATE	ADVERSE DRUG EFFECT	PULSE	B.P.	TEMP	DATE	ADVERSE DRUG EFFECT	PULSE	B.P.	TEMP
	Morning					Morning			
	Evening					Evening			
	Night					Night			
DATE	ADVERSE DRUG EFFECT	PULSE	B.P.	TEMP	DATE	ADVERSE DRUG EFFECT	PULSE	B.P.	TEMP
	Morning					Morning			
	Evening					Evening			
	Night					Night			

1. General Examination: ______________________________

2. Systemic Examination: (Positive Findings only)
   - G.I.T. ______________________________
   - C.V.S. ______________________________
   - Resp: ______________________________

[Continued..........]

3. Urine Toxicology for Opiates:	DAY	NEGATIVE	POSITIVE
	1	☐	☐
	5	☐	☐
	10	☐	☐

PATIENT'S DISCHARGE RECORD:

Name of Hospital: _____
Indoor No. _____ Bed No. _____
Patient's Name: _____
Address: _____
Date of Admission: _____
Date of Discharge: _____
Diagnosis: _____
Treatment Given: _____

APPENDIX – XXI

Inpatient Records of 25 Opiate Addicts (Nigella Sativa 250 mg Treatment Group)

#	NAME	FATHER'S NAME	AGE (YEARS)	ADDRESS	INDOOR NO.	BED NO.	DATE OF ADMISSION	DATE OF DISCHARGE
	Arif	Iqbal	25	52/291 Saoodabad Gulshan Gali	25/12	12	03-10-01	16-10-01
	M. Ibrahim	Haji M. Siddique	34	C-1, 5/1, Bhains Colony, Malir Khokhrapar	25/11	11	03-10-01	16-10-01
	A. Lateef	Noor Muhammad	22	H # 7/2 Main Road, Turbat	26/12	12	16-10-01	29-10-01
	Ahmed	Suleman	40	C-1, 18/10, Haji Sukhya Goth, Khokhrapar	26/11	11	16-10-01	29-10-01
	Akhtar	Ismail	20	Rexar Afghani Gate, Kalakot, Karachi	27/12	12	30-10-01	12-11-01
	Maqbool	Zahoor A.	40	G-90/8, Liaquat Market, Malir, Karachi	28/10	10	13-11-01	26-11-01
	A. Qadir	A. Ghani	25	Hassan Ali Mir Muhammad Road, Piri Jamadar, Nawa Lane, Lyari	29/12	12	27-11-01	10-12-01
	M. Haneef	Azizullah	28	H # 150, Abdul Karim Mohallah, Memon Goth	30/10	10	12-12-01	25-12-01
	Zahid	Abdul Khalique	35	Faisal Mansion, 16 Gidiwan Street Near Jubilee Cinema, Karachi	31/1	1	20-12-01	02-01-02
0.	M. Shafi	Moula Bux	25	Gul Muhammad Lane, Chakiwara, Near Jenu Bai Girls School	32/12	12	01-01-02	14-01-02
1.	A. Rehman	Hanif	22	H # A-214, Kausar Town, Malir	33/12	12	15-01-02	28-01-02
2.	Maqsood	Ayoob	33	H # 33/33, Madni Mosque, Jacob Lane	34/9	9	29-01-02	12-02-02

(Continued.....)

#	NAME	FATHER'S NAME	AGE (YEARS)	ADDRESS	INDOOR NO.	BED NO.	DATE OF ADMISSION	DATE OF DISCHARGE
1.	Wajid Ali	Faiz M.	20	Street-15, Mujahid Colony, Dalmia	35/9	9	12-02-02	25-02-02
2.	A. Razzaq	A. Rahman	22	Allahabad Town, Hub Chowki, Dakhana, Near Ibrahim Brohi Shop	36/1	1	26-02-02	10-03-02
3.	Wassayo	Ali Muhammad	30	Bhitai Chowki, Abbasi Para, Ibrahim Hydri	37/2	2	12-03-02	25-03-02
4.	Fateh M.	Faqir M.	25	Samo Goth, Malir, Karachi	38/1	1	26-03-02	08-04-02
5.	Asif Khan	Sardar Khan	35	G-10/4, Brigade Police Station, Jacob Lane, Karachi	42/1	1	21-05-02	04-06-02
6.	S. Hafeed A. Shah	S. Hassan Shah	45	Daoo Goth, Malir	38/2	2	26-03-02	08-04-02
7.	Imdad Hussain	Nek Muhammad	24	Degree Science College, Kalakot	39/1	1	09-04-02	22-05-02
8.	G. Hyder	Saleh Muhammad	45	Street-2, Bakra Piri, Lyari	40/12	12	23-04-02	06-05-02
9.	Tanveer	M. Rafique	35	Kalakot, Lyari, Karachi	45/10	10	08-07-02	21-07-02
10.	A. Aziz	A. Ghafoor	25	H # A-494, Phase-I, Gulshan-e-Hadeed	41/10	10	07-05-02	20-05-02
11.	Altaf	A. Samad	20	Near Roshan Hotel, Baloch Para Ibrahim Hydri	43/04	04	04-06-02	17-06-02
12.	M. Nadeem	M. Younis	30	B-12/26, Hijazi Mosque, Behind Jacob Lines	44/01	1	18-06-02	01-07-02
13.	Abid Hussain	Haji Khuda Bux	39	D-83, BL-7, Kehkashan, Clifton	46/8	8	24-07-02	07-08-02

All the patients were (a) male, (b) residents of Karachi, (c) addicted to opioid, and (d) admitted to Drug Rehabilitation Centre, R.H.C. Memon Goth, Malir

APPENDIX – XXII

Inpatient Records of 25 Opiate Addicts (Nigella Sativa 500 mg Treatment Group)

S #	NAME	FATHER'S NAME	AGE (YEARS)	ADDRESS	INDOOR NO.	BED NO.	DATE OF ADMISSION	DATE OF DISCHARGE
	Dad Muhammad	Imam Bux	50	PPO Jhol, Sanghar	47/12	12	06-08-02	20-0802
	Muneer A.	M. Waryal	25	Hot Chashma, Manghopir	48/8	8	20-08-02	02-09-02
	Noor Hussain	A. Hussain	24	Sahibdad Goth, Sheesh Mahal (Malir), Karachi	49/1	1	27-08-02	10-09-02
	M. Ali	M. Hashim	25	Juman Shah, Street-6, Rexar Lane, Karachi	51/2	2	24-09-02	13-10-02
	Akhtar	Ghuram Khan	20	Village Dageri Khan, Turbat	50/1	1	10-09-02	22-09-02
	M. Rafique	M. Yaqoob	39	Zargar Mohalla, Memon Goth	52/7	7	23-10-02	06-11-02
	Ishaque	M. Ibrahim	53	H # 535/107, Near Javed Clinic, Yasir Society, Phase-II	53/7	7	05-11-02	18-11-02
	M. Hussain	Miran	38	Street-69, Manghopir Road, Karachi-16	54/12	12	11-11-02	24-11-02
	Rehman Shah	Amir Bud	45	Malang Hotel, Pathan Colony, Manghopir Road	56/2	2	23-12-02	08-01-03
).	A. Ghaffar	Bashir Ahmed	39	H # N-11, Street-1, Usmanabad	57/2	2	23-12-02	08-01-03
1.	M. Aslam	Hakimdad	60	Wali Muhammad, Hassan Ali Road, Chakiwara	46/6	6	16-07-02	29-07-02
2.	Suleman	Buro	22	Ibrahim Hydri	46/5	5	16-07-02	29-07-02

(Continued.....)

#	NAME	FATHER'S NAME	AGE (YEARS)	ADDRESS	INDOOR NO.	BED NO.	DATE OF ADMISSION	DATE OF DISCHARGE
.	Aslam	A. Ghafoor	30	Jamote Mohallah, Ibrahim Hydri, Near Roshan Hotel	62/2	2	17-03-03	31-03-03
.	Intizar Ali	Faja Ali	25	Plot # 172, Bhains Colony, Landhi	62/4	4	22-03-03	05-04-03
.	Salahuddin	Sazuddin	25	H # B-61, Sector-51/B, Korangi-6	62/6	6	26-03-03	08-04-03
.	Abdul Jalil	Dad Muhammad	29	Bakra Piri, Jahanabad, Miran Shah Road	63/10	10	31-03-03	13-04-03
.	Haroon	M. Yusif	28	H # 317, Street-22, Hub Chowki, Allahabad Town	61/1	1	04-03-03	16-03-03
.	Shahid	Malik Sobah Sadiq	20	H # 32, Gabri Ground, Goshat Gali, Quaidabad	61/2	2	06-03-03	18-03-03
.	M. Hussain	Alam Khan	19	100-quarters, Street-17, Korangi	60/1	1	17-02-03	02-03-03
.	A. Samad	G. Muhammad	40	Street-14, Hassan Ali, Mir Muhammad Road, Usmanabad	60/2	2	18-02-03	02-03-03
.	Ahmad	M. Afzal	19	Street-1, Usmanabad, Lyari	60/3	3	21-02-03	05-03-03
.	M. Haroon	A. Karim	55	Street-4, Saleem Manzil, Ranchore Lane	60/4	4	23-02-03	10-03-03
.	Ali Muhammad	Usman	40	Street-3, Usmanabad, Karachi	60/5	5	23-02-03	08-03-03
.	Liaquat Ali	Noor Muhammad	22	Aminabad, Hub Chowki	59/4	4	04-02-03	16-02-03
.	Shaukat Ali	Noor Muhammad	25	Sadran Road, Allahabad Town, Hub Chowki	59/5	5	04-02-03	16-02-03

All the patients were (a) male, (b) residents of Karachi, (c) addicted to opioid, and (d) admitted to Drug Rehabilitation Centre, R.H.C. Memon Goth, Malir