EFFECT OF *OCIMUM SANCTUM* ON MARBLE BURYING BEHAVIOR IN MICE: IMPLICATION FOR OBSESSIVE COMPULSIVE DISORDER

Name of student: Saurabh Dhiman

Name of student: Sumit Sharma

Supervisor: Dr. G.L. Gupta

May–2012

A

Project report

Submitted in partial fulfilment of the Degree of Bachelor of Pharmacy

DEPARTMENT OF PHARMACY

JAYPEE UNIVERSITY OF INFORMATION OF TECHNOLOGY

WAKNAGHAT -173 234,DIST.SOLAN, HIMACHAL PRADESH

TABLE OF CONTENTS-

CERTIFICATE

This is to certify that the work entitled, "**Effect of *Ocimum sanctum* on marble burying behaviour in mice: implication for obsessive compulsive disorder**" submitted by Saurabh Bhiwan Sumit Sharma ..... in partial fulfilment for the award of degree of Bachelor of Pharmacy in Department of Pharmacy, Jaypee University of Information Technology has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

Signature of Supervisor .....

Supervisor: Dr. G. L. Gupta

Designation: Lecturer

Date: 18-05-12

List Of Figures

ACKNOWLEDGMENT

Saurabh Dhiman and Sumit Sharma would like to express their utmost gratitude to Dr. R. S Chauhan, HOD (Department of Biotechnology, Bioinformatics and Pharmacy) Jaypee University of Information Technology for providing us the opportunity to do project and learn various techniques.

We are very fortunate to be blessed with the guidance and encouragement of Dr. G.L Gupta (Lecturer) and other teachers.

I would like to express my special gratitude and thanks to lab assistant Mrs. Sonika Gupta for giving me such attention and time.

Saurabh

(Saurabh Dhiman)

Sumit

(Sumit Sharma)

SUMMARY

EFFECT OF Ocimum sanctum ON MARBLE BURYING BEHAVIOR IN MICE: IMPLICATION FOR OBSESSIVE COMPULSIVE DISORDER

Obsessive–compulsive disorder (OCD) is characterized by persistent thoughts (obsessions), which are ego-dystonic and associated with seemingly purposeful behaviours (compulsions) (Rasmussen and Eisen, 1992). Its co-morbidity with major depression is often evident, and it is considered as an anxiety disorder (Bartz and Hollander, 2006). Only potent serotonin reuptake inhibitors (SSRIs) are consistently effective in patients of obsessive– compulsive disorder (El Mansari and Blier, 2006). Although fluoxetine is commonly prescribed for OCD but chronic uses have fewer side effects, which include agitation, restlessness, sweating, twitching followed by convulsion (Tripathi KD, 2008). In view of these, the herbals used, alone or in combination, in the current study may provide a better alternative as anti-OCD agents. The leaves of Ocimum sanctum (OS)were extracted (family, Lamiaceae), The medicinal plants are widely used by the traditional medical practitioners for curing various diseases in their day to day practice. In traditional systems of medicine, different parts (leaves, stem, flower, root,seeds and even whole plant) of Ocimum sanctum (known as Tulsi in Hindi), a small herb seen throughout India, have been recommended for the treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery,skin diseases, arthritis, painful eye diseases, chronic fever, insect bite etc. The Ocimum sanctum has also been suggested to possess antifertility, anticancer, antidiabetic, antifungal, antimicrobial,hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic,adaptogenic and diaphoretic actions.1 This study investigated the role Ocimum sanctum extract, N-acetyl cysteine and combination of both as an anti-compulsive effect on marble-burying behaviour in mice.

***Experimental work done*:** The studies were carried out in adult albino Swiss mice (22–25 g), group housed (n=6), under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity. OS (50-500 mg/kg), N-acetyl cysteine (25-200 mg/kg), fluoxetine (5-20 mg/kg) was administered by oral route. In combination study, subeffective dose of N-acetyl cysteine (25 and 50 mg/kg, oral) was administered 30 min after OS (50-100 mg/kg, oral). In all the experiments, one hour after treatment of OS and 30 min after fluoxetine and N-acetyl cysteine, each mouse was subjected on marble-burying behaviour and actophotometer test.

Assessment of marble-burying behaviour: The anti-compulsive effect was assessed by widely used model of studying the marble-burying behaviour of mice (Njung'e and Handley, 1991). In brief, mice were individually placed in separate plastic cages (21×38×14 cm) containing 5 cm thick sawdust bedding. Twenty small marbles of glass (diameter 10 mm), were arranged on the bedding evenly spaced in four rows of five. After 30 min exposure to the marbles, mice were removed, and unburied marbles were counted. A marble was considered 'buried' if its two-third size was covered with sawdust. The total number of marbles buried was considered as an index of obsessive–compulsive behaviour.

Motor activity was also assessed in separate group of mice using Actophotometer (Techno, Lucknow). The data were analyzed by one-way ANOVA followed by Student Newman-Keul's test. Results were considered significant at $P<0.05$.

Results: OS (200 and 500 mg/kg, oral), N-acetyl cysteine (100 and 200 mg/kg, oral) and fluoxetine (10 and 20 mg/kg, oral) - a drug used in the treatment of obsessive–compulsive disorder, significantly inhibited marble-burying behaviour in mice, without any effect on motor activity. Moreover, in separate experiments, co-administration of OS (50 or 100 mg/kg, oral), 30 min prior to N-acetyl cysteine (25 and 50 mg/kg, oral), significantly protect against marble-burying behaviour at doses, which either of these alone could not protect the behaviour.

Conclusion: These finding suggest that OS and N-acetyl cysteine protects against obsessive–compulsive disorder and co-administration of N-acetyl cysteine increases the efficacy of OS in reducing compulsive disorder in mice, which may reduce side effects developed on higher dose of fluoxetine in long-term therapy

EFFECT OF *OCIMUM SANCTUM* ON MARBLE BURYING BEHAVIOR IN MICE: IMPLICATION FOR OBSESSIVE COMPULSIVE DISORDER

Abstract

This study investigated the role of *Ocimum sanctum* (OS) (Tulsi) leaaves extract, N-acetyl cysteine and combination of both as an anti-compulsive effect on marble-burying behaviour in mice. OS (200 and 500 mg/kg, oral), N-acetyl cysteine (100 and 200 mg/kg, oral) and fluoxetine (10 and 20 mg/kg, oral) - a drug used in the treatment of obsessive–compulsive disorder, significantly inhibited marble-burying behaviour in mice. Moreover, in separate experiments, co-treatment with sub-effective dose of both OS (50 and 100 mg/kg, oral) and N-acetyl cysteine (25 and 50 mg/kg, oral) was found to significantly protect against marble-burying behaviour at doses, which either of these alone could not protect the behaviour. These finding suggest that OS and N-acetyl cysteine protects against obsessive–compulsive disorder and co-administration of OS with neutraceutical N-acetyl cysteine increases the efficacy of each other in reducing compulsive disorder in mice, which may be used as an alternative drug to reduce side effects developed on higher dose of fluoxetine in long-term therapy.

Chapter -1

Introduction

What is OCD?

Obsessive compulsive disorder (OCD) is a debiliating neuropsychiatric condition characterized by persistent intrusive thoughts (obsessions) and the expression of ritulastic repetitive behaviors (compulsions).

How common is OCD?

- Estimated UK prevalence 1-2% of adult population - fourth most common mental disorder after depression, alcohol and sub-stance abuse, and social phobia .
- 1% of young people – adults often report experiencing first symptoms in childhood
- Onset can be at any age. Mean age is late adolescence for men, early twenties for women
- Age of onset in Males (17-30) & Females (20-24).
- Financial cost associated with health care in the USA estimated to be around $10.6 billion per annum [3].
- Life time prevalence in USA has been estimated 2.3% [4].

❑ Obsessions as defined by[2]

1. Recurrent and persistent thoughts, impulses images that are experienced,at some time during the disturbances as intrusive and inappropriate (causes marked anxiety or distress.
2. Contamination

3. Repeated Doubting
4. Ordering
5. Religious
6. Aggressive

❑ Compulsions are defined by[2]

1. Repetitive behaviors (hand washing, ordering, checking) or mental acts (praying, counting, repeating words silently).
2. The compulsive actions are done to reduce distress or to prevent something bad from happening, even though there is no realistic connection with preventing such an occurrence

3. OCD Facts and figures .2

- Approximately 2.3% of the population between ages 18- 54 suffers from OCD, more than any other mental and emotional based challenges such as schizophrenia, bipolar disorder or panic disorders. Approximately 1 in 50 people are affected.
- In the US approximately 3.3 million people have OCD, though some estimates have been as high as 6 million.
- The age of is typically reported from ages 17-30 for males and 20 - 24 females. New cases of OCD after the age of 40 are rare.

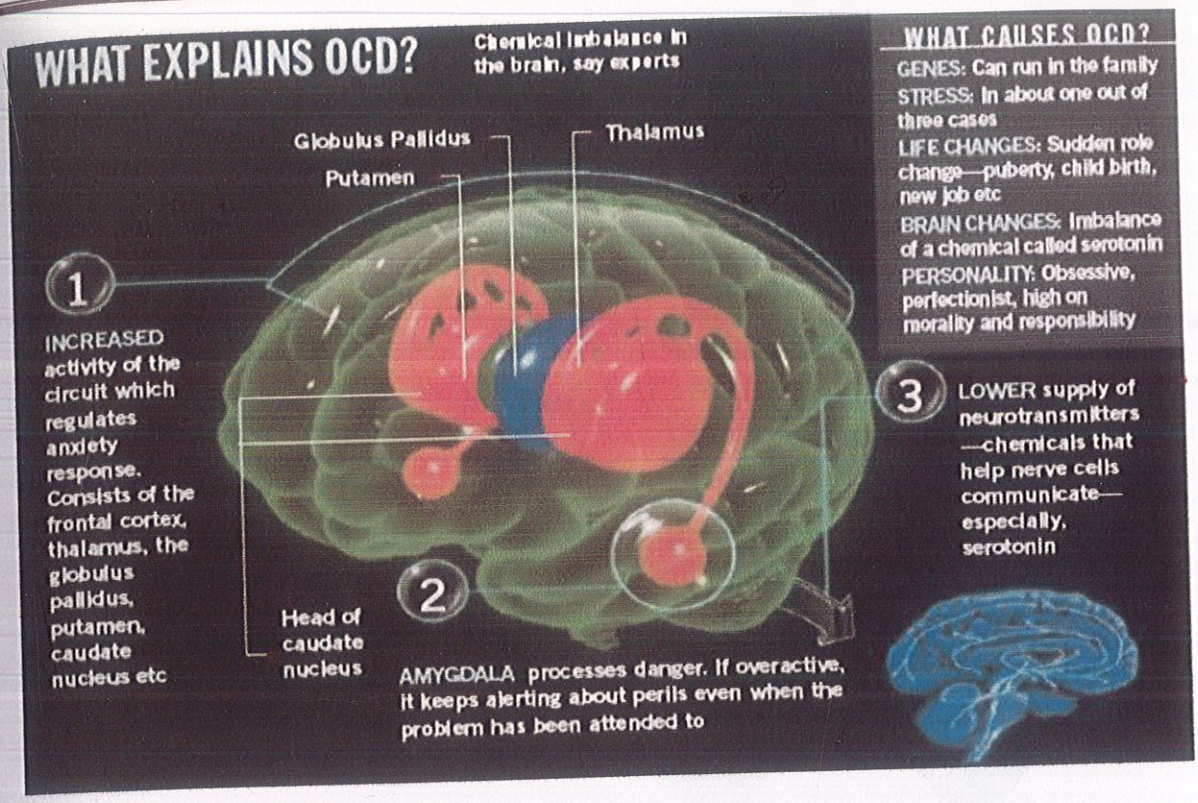
Epidemiology3

- OCD is quite common with one month and lifetime prevalence of 1.3% and 2.5%
- Early onset is common with mean ages in males and females of 18 and 21 years.
- OCD is a familial disorder; slightly more than 20% of an index patient's family members also have OCD.

- The most recent National Comorbidity Survey Replication found a consonant 12 month prevelance of 1.0% with 52% patients with serious severity.

Pathophysiology4

- Different imaging techniques and biochemical assays and probes generally support the following finding:
- Orbitofrontal cortex, anterior cingulate cortex, and caudate nuclei of patients with OCD exhibit increased metabolism compared.
- Effective short term treatment with a selective serotonin reuptake inhibitor (fluoxetine) reduces hypermetabolism in the right caudate nucleus.
- Functional neuroimaging studies have shown a hypermetabolic brain circuit involving the orbital-frontal cortex, anterior cingulate, thalamus and striatum.
- OCD symptoms can be caused or exacerbated by an autoimmune reaction in which antibodies to beta-haemolytic streptococci cross-react with proteins in the basal ganglia, this is called PANDAS (paediatric autoimmune neuropsychiatric disorders associated with streptococcal infection.
- OCD is a biological disease. Functional brain imaging studies have produced a model for pathophysiology of OCD (hyperactivity in certain subcortical and cortical regions).
- Increased activity in the head of the caudate nucleus inhibits globus pallidus fibers that ordinarily dampen thalamic activity.
- Increase in thalamic activity produces increased activity in orbitofrontal cortex, which, via the cingulated gyrus, completes the circuit to the caudate.
- Increased activity in the head of the caudate.

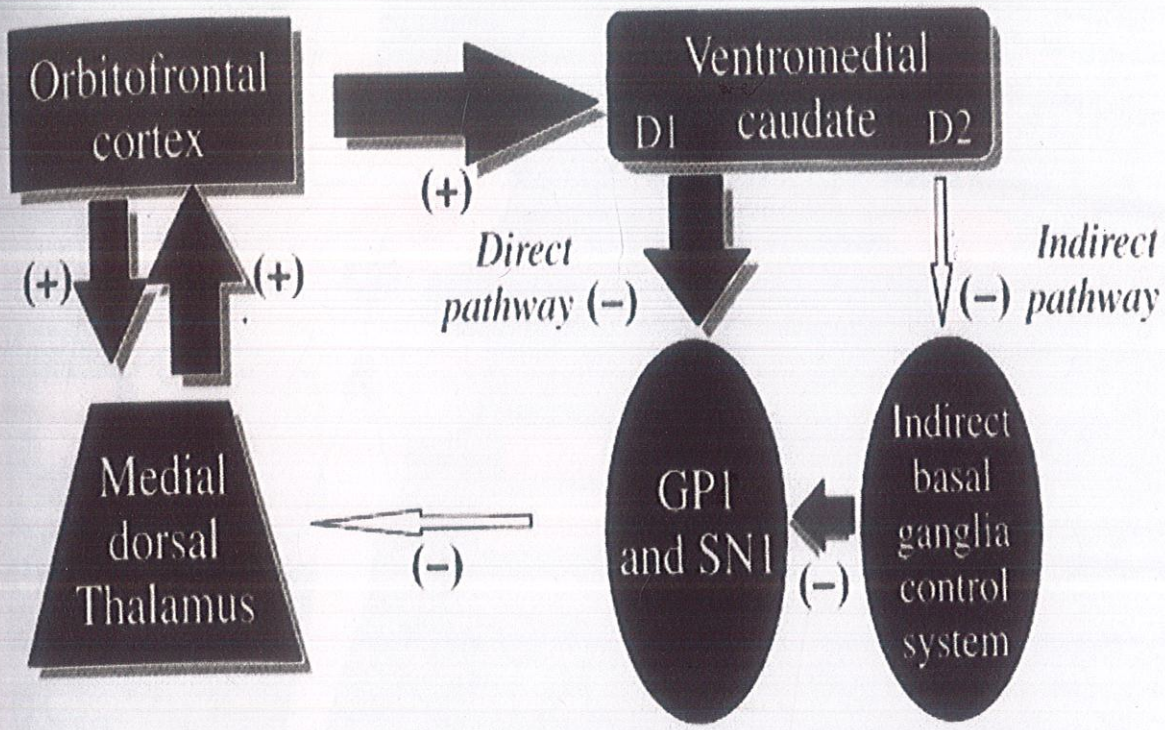


Reference to pics

. John H, Greist MD, James W, Jefferson MD. Obsessive-compulsive disorder focus. Pchiatryonlineorg

Figure 1 what explains OCD [5]

Figure 2


Orbitofrontal cortex
(+)
Ventromedial
D1 caudate D2
(+)
(+)
Direct pathway (–)
Indirect pathway
(–)
Medial dorsal Thalamus
(–)
GPI and SNI
(–)
Indirect basal ganglia control system

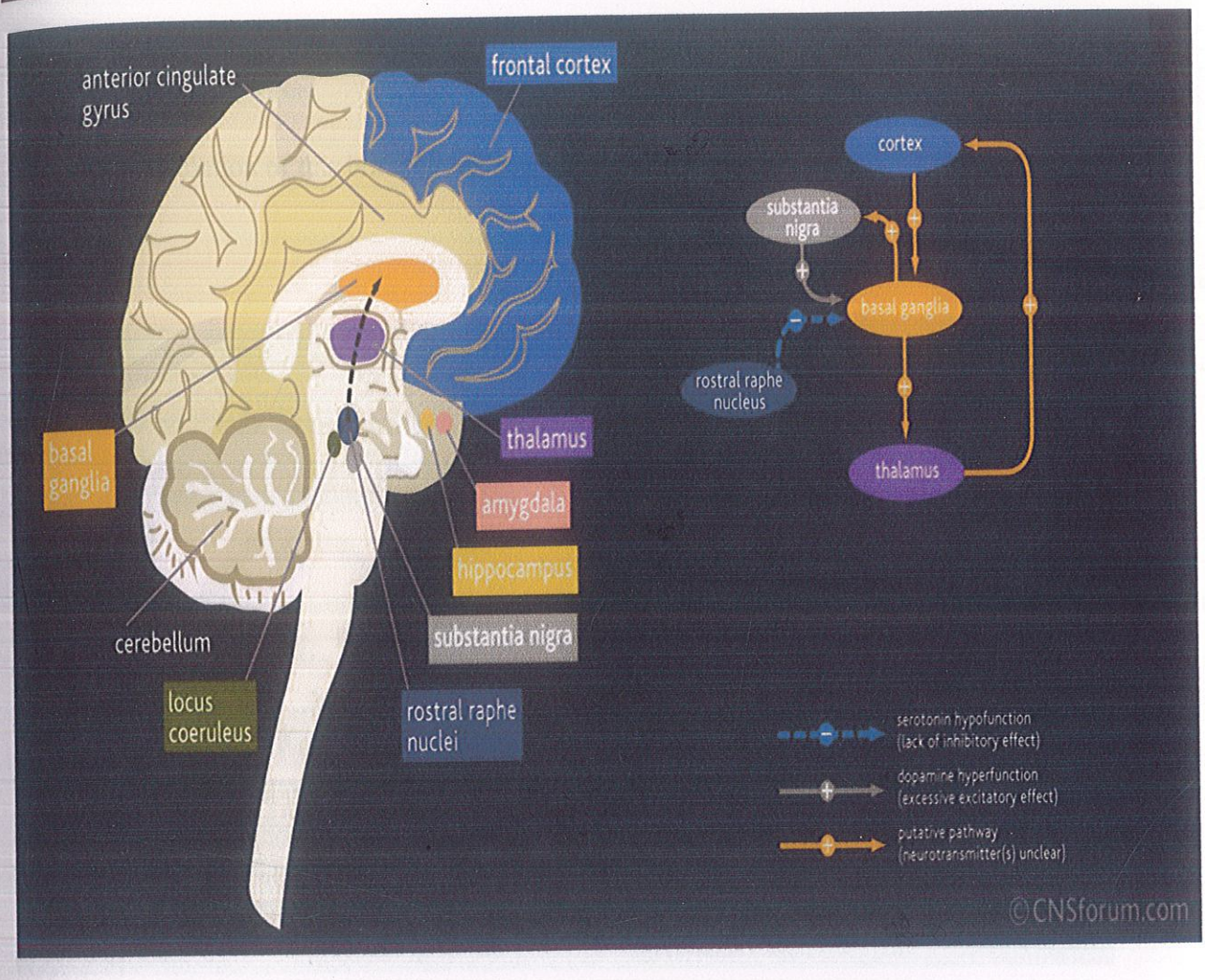


Figure 3: The areas of the brain affected in obsessive compulsive disorder

Graybiel, A. M. & Rauch, S. L. (*2000*) *Neuron 28 , 343-347*

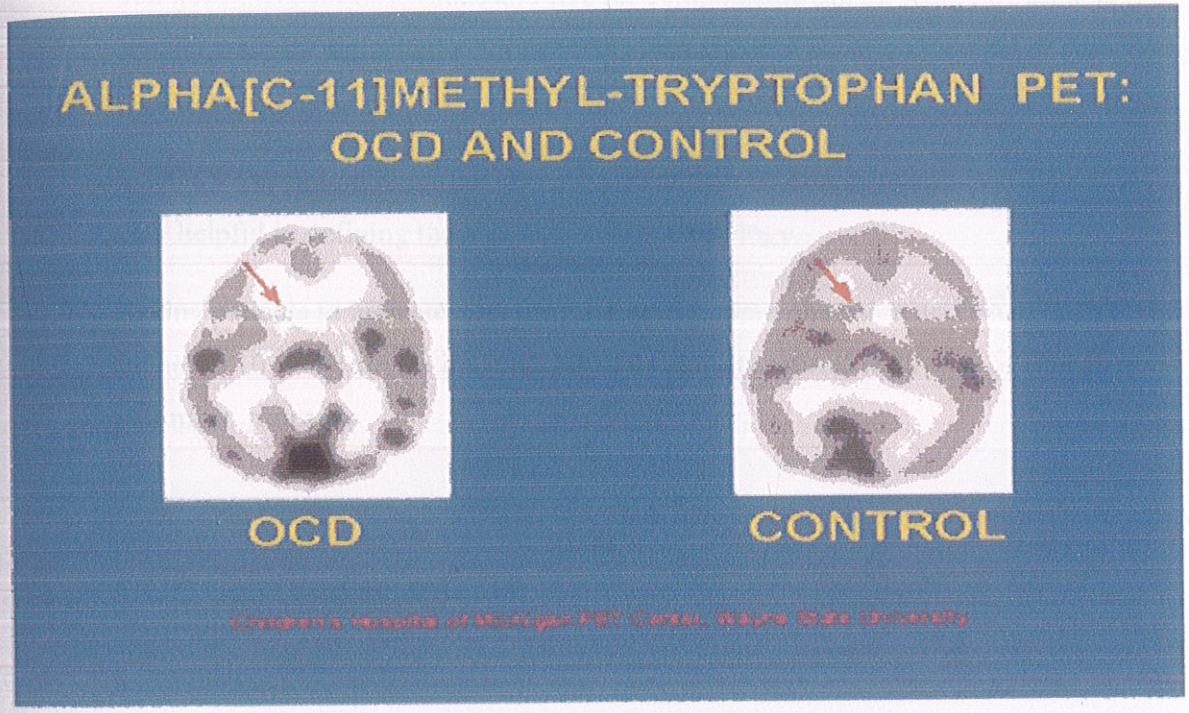
Biological causes of OCD have focused on a circuit in the brain which regu lates primitive aspects of our behavior such as aggression, sexuality, and bodily excretions.

This circuit relays information from a part of the brain called the orbitofrontal cortex to another area called the thalamus and includes other regions such as the caudate nucleus of the basal ganglia.

When this circuit is activated, these impulses are brought to your attention and cause you to perform a particular behavior that appropriately addresses the impulse .

- It has been suggested that if you have OCD, your brain has difficulty turning off or ignoring impulses from this circuit.

- This, in turn, causes repetitive behaviors called compulsions and/or uncontrollable thoughts called obsessions.
- Dysregulation of this brain circuit may be related to a problem with the serotonin system.



OCD patients have lower serotonin levels than control subjects. Source: http://neuron.med.wayne.edu/OCD/slide.htm

Figure 4

Assessment

- To assess obsessive compulsive disorder a through psychiatric history and examination should be taken to investigate symptoms.
- Ten items, five for obsessions and five for compulsions can be scored by clinicians or equally well by almost all adult patients using either a paper and pencil or computer interview.
- Y-BOCS symptom checklist identifies more than 60 obsessions and rituals and is very helpful in defining the past and present pathology.
- Asking patients to indicate a severity for each present obsession and ritual on a 0 (none) to 10 (most severe imaginable) scale permits refined follow up of change as treatment proceeds .

OCD signs and symptoms:

Compulsive behaviours

Common compulsive behaviors in obsessive-compulsive disorder (OCD) include:

- Excessive double-checking of things, such as locks, appliances and switches.
- Repeatedly checking in on loved ones to make sure they're safe.
- Counting, tapping, repeating certain words, or doing other senseless things to reduce anxiety.
- Spending a lot of time washing or cleaning.
- Ordering or arranging things "just so right"
- Praying excessively or engaging in rituals triggered by religious fear.
- Accumulating "junk" such as old newspapers or empty food containers.

Obsessions

- Contamination
- Repeated Doubting
- Ordering
- Religious
- Aggressive
- Sexual

Treatment[6]

The first medication usually considered is a type of antidepressant called a selective serotonin reuptake inhibitor (SSRI). These drugs include:

Citalopram .
Fluoxetine .
Fluvoxamine.
Paroxetine .
Sertraline .

Selective serotonin reuptake inhibitors (SSRIs) are the medications that are most commonly used to treat OCD.

- These medications increase the amount of the neurotransmter serotonin in the brain. (Remember that brain serotonin levels are thought to be low in OCD.)
- As their name implies, the SSRIs work by selectively inhibiting (blocking) serotonin reuptake in the brain..
- SSRIs do not cause orthostatic hypotension (a sudden drop in blood pressure when sitting up or standing) and heart-rhythm disturbances.

Effective Potent drugs[20]

Clomipramine	Tricyclics (apart from clomipramine)
• Monoamine oxidase inhibitors	Fluvoxamine
• Lithium	Sertraline
• Fluoxetine	Paroxetine
• Citalopram	Buspirone
• Escitropram	Electroconvulsivetherapy

Chapter2

N-Acetyl-L-Cysteine (NAC)[7]

N-Acetyl-L-Cysteine is the amino acid L-Cysteine plus an acetyl (-CO-CH_3) group attached to the amino (NH_2) group. Amino acids which contain a sulphur group have antioxidant properties. The acetyl group makes cysteine more water-soluble, and functions to speed absorption and distribution on orally ingested cysteine. The acetyl group also reduces the reactivity of the thiol (−SH), making NAC less toxic and less susceptible to oxidation than cysteine. NAC is safe, even in large doses, and is a better source of cysteine than cysteine itself.

The hydrogen atom in the sulfhydryl (−SH) group of many sulphur-containing anti-oxidant molecules (thiols) can act as an electron for neutralizing free-radicals. Lipoic acid, the tripeptide glutathione, the amino acids cysteine & methionine and the oregano sulphur compounds of garlic oil are all thiol containing antioxidants. Even albumin can have an antioxidant function in plasma as a result of its cysteine-34 residue.

L-cysteine is not very water soluble, nor is it absorbed well by the intestine. Dietary cysteine comes mainly as the breakdown product of ingested proteins & peptides. Whey protein is a particularly rich food source of cysteine. Because cysteine is so unstable, the main extracellular source of intracellular cysteine is the dipeptide cystine (two conjugated cysteine). Cystine competes with glutamate for transport into cells such that conditions of elevated extracellular glutamate can lead to glutathione depletion, worsened oxidative stress and cell death.

Oral supplementation with NAC provides an alternate means of boosting intracellular glutathione via elevated intracellular cysteine. NAC is rapidly absorbed after oral administration and reaches a maximum plasma level in 2-3 hours, with a half-life of NAC readily enters cells and is hydrolysed to cysteine.

NAC has been used in clinical toxicology for the treatment of acetaminophen poisoning (although the extremely high doses used for this purpose causes allergic reactions in some in some people — anaphylactic shock, in extreme cases).

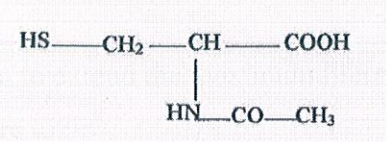


Fig 5. Chemical structure of N-acetyl cysteine[7]

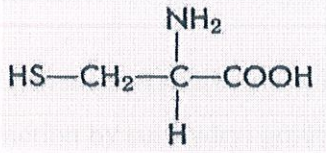


Fig 6. Chemical structure of Cysteine[7]

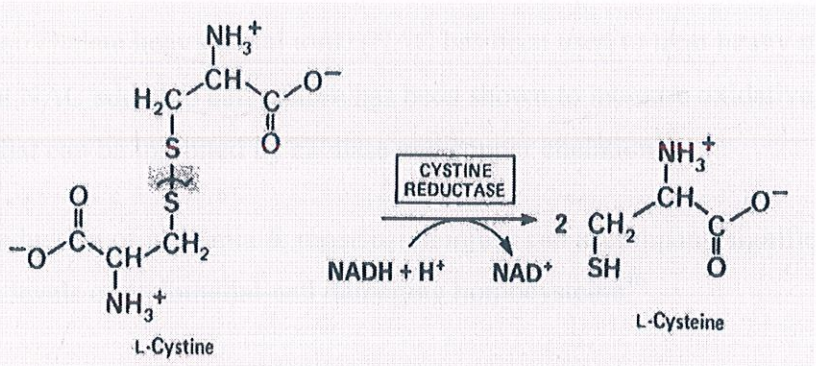


Fig7. Synthesis of L-Cystine to L Cysteine[7]

Other uses of NAC[7]:

NAC has been shown to extend the maximum lifespan of fruit flies by nearly a quarter, but the highest doses were toxic[8]

Twice daily 600 mg doses of NAC given to volunteers who smoked tobacco inhibited the formation of lipophilic DNA adducts [9]

NAC can prevent DNA mutation and protect against cancer [10]

NAC has been used to regenerate oxidative phosphorylation complexes in mitochondria from age-related decline in function by sulfhydryl group action, rather than antioxidant effect[11]

NAC has been shown to inhibit the growth of *Helicobacter pylori* (the bacterium responsible for the great majority of ulcers) in both mice12 and human [13]

NAC protects against radiation damage by a direct radical scavenger action rather than by conversion to glutathione [14]

NAC can chelate heavy metal ions. (NAC has been used to treat heavy metal poisoning.) By contrast NAC added to cell culture has been shown to increase oxidative damage to DNA, an effect that can be inhibited by catalase and copper chelation[15]

NAC reduction of ischemia & reperfusion injury can accompany significantly reduced plasma levels of endothelial-cell damaging homocysteine[16]

NAC also inhibits the expression of endothelial adhesion molecules and peroxynitite free radical damage associated with ischemia/reperfusion [17]

NMDA receptor antagonist[8]

The NMDA receptor is an inotropic receptor that allows for the transfer of electrical signals between neurons in the brain and in the spinal column. For electrical signals to pass, the NMDA receptor must be open. To remain open, glutamate and glycine must bind to the NMDA receptor. An NMDA receptor that has glycine and glutamate bound to it and has an open ion channel is called "activated.

Chemicals that deactivate the NMDA receptor are called antagonists. NMDAR antagonists fall into four categories: Competitive antagonists ,which bind to and block the binding site of the neurotransmitter glutamate; glycine antagonists, which bind to and block the glycine site non-competitive antagonists, which inhibit NMDARs by binding to allosteric sites and uncompetitive antagonists, which block the ion channel by binding to a site within it

NMDA receptor antagonists are a class of anaesthetic that work to antagonize or inhibit the action of, the N-methyl d-aspartate receptor (NMDAR). They are used as anaesthesia for animals and less commonly for humans. The state of anaesthesia they induce is referred to as dissociative anaesthesia.

Proposed mechanism of NAC as NMDA receptor antagonist in OCD[18, 19]

NAC also inhibits marble burying behaviour without affecting the locomotors activity. NAC is converted to cystine, a substrate, for the glutamate /cystine antiporter located on glial cells. The uptake of cystine by glial cells causes the reverse transport of the glutamate into the extracellular space where it appears to stimulate inhibitory metabotropic glutamate receptor on glutamatergic nerve terminals and reduce the synaptic release of glutamate[18]

Systemic administration of NAC prevents cocaine –primed drug seeking in rats by increasing activity of the glutamate cystine antiporter and restoring extracellular glutamate concentrations in the nucleus accumbens[19.]

Thus NAC modulate brain glutamate neurotransmission. The antioxidant alpha tocopherol had no effect on marble burying behaviour, suggesting that the anti OCD effect of NAC is not due to antioxidative action[19]

Table 1

List of other NMDA receptor antagonist[20]

Drug Name	FDA	Mechanism(s) of Action	Effectiveness

	Approval		
Riluzole	ALS	Reduction of synaptic glutamate release via multiple presynaptic mechanism :stimulation of glutamate uptake by astrocytes	Slightly more than half of the 32 total OCD patients treated open label with riluzole have shown significant improvements
Topiramate	Epilepsy	block of voltage-dependent Na^+ and K^+ channels; reduction of AMPA/kainate receptor channel conductance; positive modulatory actions on GABAA receptors	12/17 cases showed improvements; topiramate induced OCD symptoms in one isolated case report
Lamotrigine	Epilepsy Biopolar disorder	Inhibition of voltage dependent Na+ channels to reduce synaptic glutamate release from neurons	1/8 OCD patients Shows benefits ,lamotrigine induced OCD symptoms in 7 cases and 3 from other studies
N-acetyl cysteine	acetaminophen toxicity	stimulation of the glial cystine/glutamate exchanger resulting in activation of mGluR2 receptors which dampens presynaptic glutamate release from neurons during bouts of excessive neuronal activity	a single case report indicates potential benefit as augmenting agent
LY354740	N/A	Agonist of type2	N/A

		metabrotropic receptors, dampens presynaptic glutamate release from neurons during bouts of excessive neuronal activity	
Amantadine	Influenza A virus infection, Parkinson's disease	NMDA type glutamate receptor open channel blocker	N/A

Psychotherapy2

- **Cognitive-behavioral therapy** for obsessive-compulsive disorder (OCD)
- Exposure and response prevention involves repeated exposure to the source of your obsession.
- Cognitive therapy focuses on the catastrophic thoughts and exaggerated sense of responsibility you feel.
- Cognitive therapist seek to change thoughts, feelings and behaviors.
- Faulty cognitions permit and then maintain unpleasant affects and dysfunctional behaviors.
- A big part of cognitive therapy for OCD is teaching you healthy and effective ways of responding to obsessive thoughts, without resorting to compulsive behaviour.
- For example, if you are a compulsive hand washer, you might be asked to touch the door handle in a public restroom and then be prevented from washing.

Behaviour Therapy for OCD: Facing Your Worst Fears

- This exposure provides you with an opportunity to gain new information in hopes of disconfirming your worst fears.
- One of the most popular and effective forms of behavior therapy for OCD is *exposure and response prevention* or ERP. ERP involves exposing you to the anxiety that is provoked by your obsessions and then preventing the use of rituals to reduce your anxiety.
- This cycle of exposure and response prevention is repeated until you are no longer troubled by your obsessions and/or compulsions.

Ocimum sanctum:

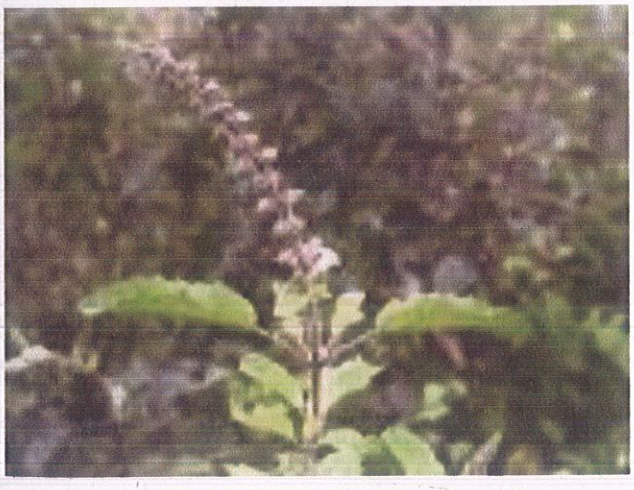


Figure 8: Picture of *Ocimum sanctum*

MORPHOLOGICAL FEATURES[21]

Kingdom: Plantae

Order: Lamiales

Family: Lamiaceae

Genus: Ocimum

Species: O. sanctum

Common name is sacred basil, holy basil.

The medicinal plants are widely used by the traditional medical practitioners for curing various diseases in their day to day practice. In traditional systems of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of *Ocimum sanctum Linn* (known as Tulsi in Hindi), a small herb seen throughout India, have been recommended for the treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever, insect bite etc. The *Ocimum sanctum L.* has also been suggested to possess antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions.

Eugenol (l-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *Ocimum sanctum L.,* has been found to be largely responsible for the therapeutic potentials of Tulsi. Although because of its great therapeutic potentials and wide occurrence in India the practitioners of traditional systems of medicine have been using *Ocimum sanctum L.* for curing various ailments, a rational approach to this traditional medical practice with modern system of medicine is, however, not much available. In order to establish the therapeutic uses of *Ocimum sanctum L.* in modern medicine, in last few decades several Indian scientists and researchers have studied the pharmacological effects of steam distilled, petroleum ether & benzene extracts of various parts of Tulsi plant and eugenol

on immune system, reproductive system, central nervous system, cardiovascular system, gastric system, urinary system and blood biochemistry and have described the therapeutic significance of Tulsi in management of various ailments. These pharmacological studies have established a scientific basis for therapeutic uses of this plant. [21]

CHEMICAL CONSTITUENTS[21]

Triterpenoids

- Ursolic acid
- Oleanic acid

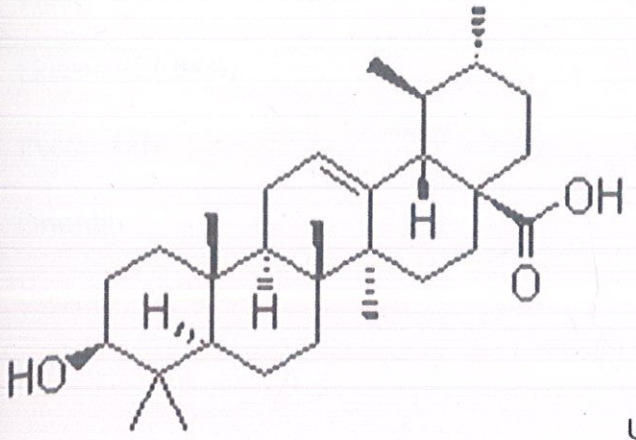
Phenolics

Eugenol.
Rosmarinic acid

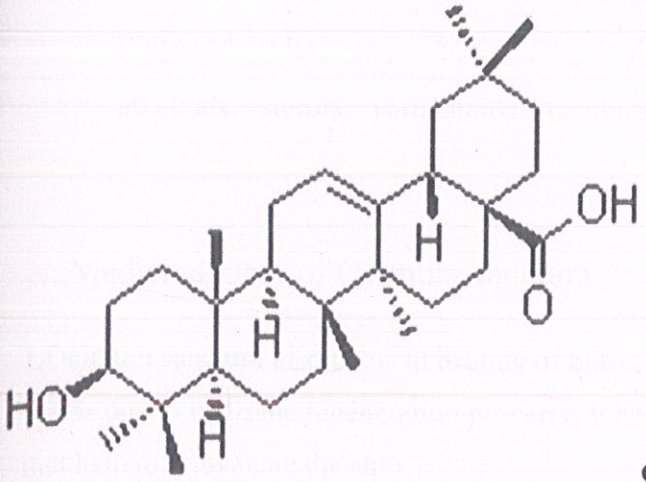
Flavonoids

Circilineol.

Chemical Structures:

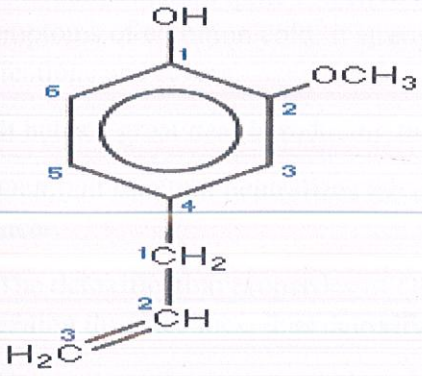


Ursolic acid



Oleanic acid

Figure 9



Eugenol

Figure 10

Essential oil

Methyl chavicol (44.63%).

Linalool (21.84%).

Flavonoids

Orientin .

Vicenin

Other components

- Polysaccharides, amino acids, fatty acids, sesquiterpenes, alkaloids, sterols, carotenoids, tannin, chlorophyl, pectin, inorganic salt etc .

Medicinal uses of Ocimum sanctum [22]:

1.Ocimum sanctum also helps in healing of burns, bruises and cuts, sprains, sore muscles and scalds due to its tissue regeneration property. It has anti bacterial and anti fungal properties that help to rejuvenate the skin.

2. Decoction of the Ocimum sanctum leaves with aloe vera gel and ginger can be used to get rid of respiratory diseases like asthma, bronchitis and influenza. You can also make a Ocimum sanctum tea for even better results.

3.Ocimum sanctum quite effective in the treatment of coughs, sore throats and other symptoms of common cold. It strengthens the body's ability to resist viral and bacterial infections and fevers.

4.It helps a great deal in reducing the negative physical and psychological effects of stress.

5.Ocimum sanctum neutralizes the dangerous bio chemicals that cause diseases like to cancer.

6.The detoxification properties of Ocimum sanctum help in aiding the digestion process and cleaning the intestines, thus detoxifying the body.

7.Ocimum sanctum can considerable bring down the high cholesterol levels. It diminishes the quantity of acids in the blood and strengthens the veins and arteries. It promotes a healthy respiratory system and can be used to treat bronchitis, asthma and allergies.

8.Headache and stress related problems can also be treated with this herb. Keep Ocimum sanctum leaves in a water container and then boil the water. The medicinal properties f Ocimum sanctum get immersed into water. Drink that water. You can use this remedy twice or thrice in a week.

9.The stomach disorders are also treated effectively with the use of Ocimum sanctum. One can prepare a juice of Ocimum sanctum leaves, which can be taken at least twice a day results in noticeable improvements.

Chapter 3

Objective

- To investigate the role of *Ocimum sanctum* as anticompulsive effect using marble burying test and actophotometer as a behavior paradigm.
- To investigate the effect of N-acetyl cysteine as anticompulsive effect using different behavior paradigm.
- To check the effect of combination of *Ocimum sanctum* & N-acetyl cysteine as anticompulsive effect using different behavior paradigm.

Plan of work

- Review of literature
- Procurement of plant
- Extraction of Plant
- TLC studies
- Phytochemical screening
- Animal toxicity studies
- Animals Models
- Experimental design
- Stastistical analysis of results

Procurement of plant

- We collected plant from Shimla district ,Himachal pradesh.

- Whole plant is used in the project.

Extraction procedure

- We grinded the whole plant (*Ocimum sanctum)*
- After that we have been done the extraction through soxhlet apparatus..
-
-

Solvent-methanol

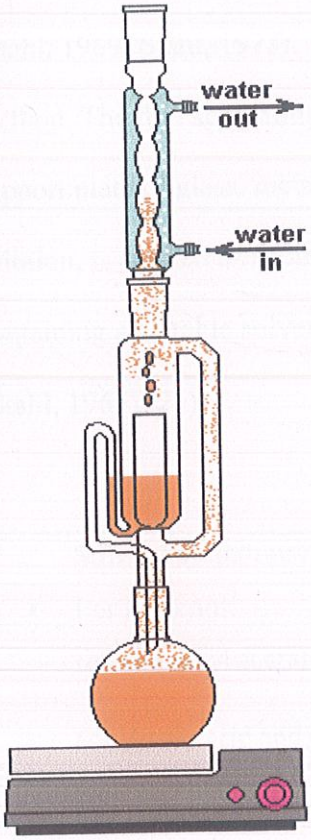


Figure 11 Soxhlet apparatus

Solvent system used-

- Fatty acids removal-petroleum ether.
- Evaporation of solvent through rotatory vaccum evaporator **(Company).**

Thin layer chromatography (TLC) studies

Chromatography is essentially a group of techniques for the separation of the compounds of mixtures by their continuous distribution between two phases, one of which is stationary and other is mobile. The main principle of separation may be either partition or adsorption. Among the various chromatographic techniques, thin layer chromatography (TLC) is the best suited for the analysis of the drugs in phytochemical laboratories. This method requires only small investment in instruments, consumes little time for the completion of analysis (15-60 min.) and require minimum amount of sample (about 0.1 gm) (Stahl, 1969; Stahl, 1973). Thin layer chromatography is a physicochemical separation method. The thin separating layer of granulating material (stationary phase) is placed on support plate of glass, metal or suitable film. The mixture to be separated, in the form of solution, is applied as spots or bands. The plate is placed into a tightly closed chamber containing a suitable solvent (mobile phase), separation take place during capillary migration (Stahl, 1965).(25)

Solvent system used-

- For Eugenol:

toluene:ethyl acetate (93:7) we have taken 1/5th of this ratio that is (18.6:1.4).

For Ursolic acid and oleanolic acid

CHCl3:CH3COOH:CH3OH:H2O

(60:32:12:8) taken 1/4th of quantity.

Rosmarnic acid:

toluene:ethyl formide:formic acid

(50:40:10)

Detection: Spraying agent

- Detecting reagent- vanillin sulphuric acid reagent for eugenol.
- Uv visible-365 nm
- Detecting reagent preparation- 0.1 ml anisaldehyde, 2 ml glacial acetic acid, 17 ml methanol, 1 ml sulphuric acid.

Animals used

- Adult mice (20-25 g) were group housed under a standard period of time for 1 hr . All the experiments were carried out in a noise free room.
- Separate group **(n=6)** were used for each set of experiment.

Animal toxicity study[23]

- The plant *Ocimum sanctum* is reported to be nontoxic at dose 5 g/kg, oral.
- All the selected doses in the study has also been found to be nontoxic.

Animal models used[24]

Marble Burying Behavior Test:

Marble burying describes the tendency to of mice to dig repetitively when placed in cage with deep bedding in the presence of inert object (typically marbles), this can be quantified as the number of objects buried in a given interval of time. It has been variably interpreted as reflecting anxiety or compulsivity.

The burying was reduced by serotonin reuptake inhibitor raised the possibility that this behavior may be related to OCD (23). Indeed careful analysis of marble burying behavior has later led to the conclusion that it does not model anxiety, but may rather related to compulsive behavior. Thus mice did not avoid the marbels when given the opportunity to do

so, suggesting that the marbles have no aversive or fear provoking properties and repeated exposure to marbles did not lead to habituation to marble burying suggesting that this behavior is not related to novelty or fear.

The number of unburied marbles was counted. A marble covered at least two-third of its size by saw dust was considered as "buried". The anti-compulsive effect was assessed by the widely used model of studying the marble-burying behavior of mice. In brief, each mouse was individually placed in plastic cage (21x38x14cm) containing 5 cm thick sawdust bedding. Twenty small glass marbles (diameter 10-12 mm) were arranged on the bedding, evenly spaced in four rows. After 30 min exposure to the marbles, mice were removed, and unburied marbles were counted. A marble was considered 'buried' if its two-third size was covered with sawdust. The total number of marbles buried was considered as an index of obsessive–compulsive behavior.

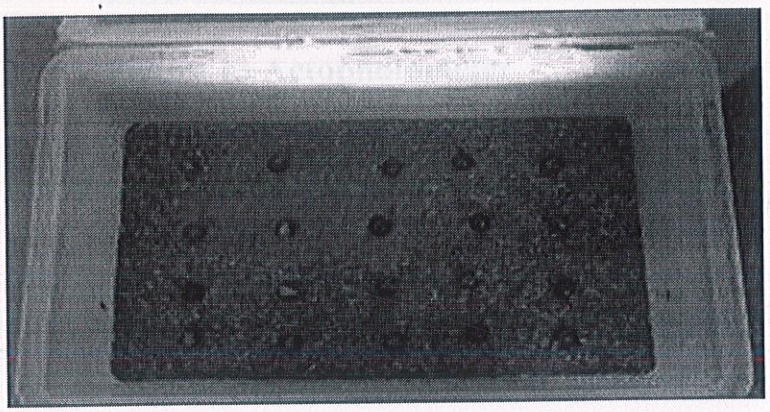


figure 12.

Actophotometer test

- Motor activity was assessed in separate group of mice using Actophotometer (Techno, Lukhnow), which had a circular arena of 40 cm, equipped with three infrared beams and photo_cells connected to digital counter. Motor activity was assessed in terms of total number of countsof light beams interruptions in 30 minutes.

Figure 13 Actophotometer

Experimental design:

Each experimental group had a separate set of mice being allotted randomly. All subjects were experimentally naïve at the beginning of each study. Animals from different groups were administered with vehicle (5% w/v gum acacia in distilled water, oral, n=6), OS (50-500 mg/kg, oral, n=6 per group), N-acetyl cysteine (25-200 mg/kg, oral, n=6 per group) and fluoxetine (5-20 mg/kg, oral, n=6 per group). One hour after OS and thirty minutes after N-acetyl cysteine and fluoxetine administration, individual mice was subjected to marble burying test for thirty minutes. Immediately after completion of test on marble burying, each mouse was also tested for locomotor activity on actophotometer.

Following groups of mice were prepared for assessing anticompulsive activity.

Group I: Control (5% w/v Gum acacia)

Group II: *Ocimum sanctum* (50 mg/kg, oral)

Group III. *Ocimum sanctum* (100 mg/kg, oral)

Group IV. *Ocimum sanctum* (200 mg/kg, oral)

Group V. *Ocimum sanctum* (500 mg/kg, oral)

Group VI. Fluoxetine (5 mg/kg, oral)

Group VII. Fluoxetine (10 mg/kg, oral)

Group VIII. Fluoxetine (20 mg/kg, oral)

Group IX. N-acetyl cysteine (25 mg/kg, oral)

Group X. N-acetyl cysteine (50 mg/kg, oral)

Group XI. N-acetyl cysteine (100 mg/kg, oral)

Group XII. N-acetyl cysteine (200 mg/kg, oral)

Group XIII. *Ocimum sanctum* (50 mg/kg, oral) + N-acetyl cysteine (25 mg/kg, oral)

Group XIV. *Ocimum sanctum* (100 mg/kg, oral) + N-acetyl cysteine (25 mg/kg, oral)

Group XV. *Ocimum sanctum* (50 mg/kg, oral) + N-acetyl cysteine (50 mg/kg, oral)

Group XVI. *Ocimum sanctum* (100 mg/kg, oral) + N-acetyl cysteine (50 mg/kg, oral)

Statistical analysis

The data is presented as Mean ± SEM. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by post hoc Student-Newman-Keuls test. Differences were considered to be significant at $P<0.05$.

Results

- Extraction through soxhlate - 14 % yield

Phytochemical screening of extract *Ocimum sanctum*

➢ **Class of compounds**	**Results**
1. Alkaloids	negative
2. Steroids	postive
3. Glycosides	positive
4. Fixed oils	positive
5. Proteins	positive
6. Carbohydrates	positive

Table 2.Effect of methanolic extract *Ocimum sanctum* on MBT in mice

Dose (mg/kg,oral)	No.of marbles buried M1	No.of marbles buried M2	No.of marbles buried M3
Vehicle	13	18	17
O.S (50)	5	7	13
O.S (100)	14	6	8
O.S (200)	5	4	9

Table 2. Effect *Ocimum sanctum* of on actophotometer in mice

Dose (mg/kg,oral)	Actophotometer counts M1	Actophotometer counts M2	Actophotometer counts M3
Vehicle	388	458	570
O.S(50)	465	458	359
O.S(100)	515	588	499
O.S(200)	555	630	432
O.S(500)	476	390	409

Table 3. Effect of fluoxetine on MBT in mice

Dose(mg/kg, oral)	No. of marbles buried M1	No. of marbles buried M2	No. of marbles buried M3
Vehicle	11	13	13
Fluoxetine(5)	12	6	4
Fluoxetine(10)	9	4	6
Fluoxetine(20)	4	2	5

Table 4. Effect of fluoxetine on actophotometer in mice

Dose(mg\kg,oral)	Actophotometer counts (M1)	Actophotometer counts)M2	Actophotometer counts (M3)
Vehicle	300	229	467
Fluoxetine (5)	344	290	399
Fluoxetine (10)	298	222	456
Fluoxetine(20)	367	290	467

Table 5. Effect of N-acetyl cysteine on MBT in mice

Dose(mg\kg,oral)	No. of marbles buried(MI)	No. of marbles buried(M2)	No. of marbles buried(M3)
Vehicle	16	18	13
NAC(25)	12	9	8
NAC(50)	5	4	7
NAC(100)	7	5	2
NAC(200)	12	9	6
NAC(400)	9	12	8

Table 6. Effect of N-acetyl cysteine on actophotometer in mice

Dose(mg\kg,oral)	Actophotometer	Actophotometer	Actophotometer

	counts(M1)	counts(M2)	counts(M3)
Vehicle	278	267	288
NAC(25)	289	233	223
NAC(50)	174	123	199
NAC(100)	199	256	278
NAC(200)	267	233	277
NAC(400)	223	277	244

Table 7. Effect of combination of N-acetyl cysteine and *Ocimum sanctum* on MBT in mice

Dose(mg\kg,oral)	No. of marbles buried(MI)	No. of marbles buried(M2)	No. of marbles buried(M3)
Vehicle	12	16	13
NAC(25),O.S(50)	4	8	6
NAC(25),O.S(100)	3	3	7
NAC(50),O.S(50)	6	4	7
NAC(50),O.S(100)	2	3	1

Table 8. Effect of combination of N-acetyl cysteine and *Ocimum sanctum* on actophotometer in mice

Dose(mg\kg,oral)	Actophotometer counts(M1)	Actophotometer counts(M2)	Actophotometer counts(M3)
Vehicle	497	538	475
NAC(25),O.S(50)	330	298	302
NAC(25),O.S(100)	290	337	390
NAC(50),O.S(50)	229	290	323
NAC(50),O.S(100)	214	229	170

Conclusion

These finding suggest that Ocimum sanctum and N-acetyl cysteine protects against obsessive–compulsive disorder. Co-administration of N-acetyl cysteine increases the efficacy of Ocimum sanctum in reducing compulsive disorder in mice and the effect was comparable to that shown by fluoxetine, a reference standard drug, which may reduce side effects developed on higher dose of fluoxetine in long-term therapy. In the same way, identification and isolation of compound(s) responsible for the activity could be used as prototype(s) to design new substances with anti-OCD activity. Further major active components and precise anti-compulsive mechanisms need to be identified.

Reference

1 Atal CK, Kapoor BM. Cultivation and utilization of medicinal plants (Eds. PID CSIR), 1989.

2 Nestadt G, Bienvenu OJ, Cai G, et al. Incidence of obsessive-compulsive disorder in adults. J Nerv Ment Dis 1998;186:401–6.

3 Behar D, Rapoport JL, Berg CJ, et al. (1984) Computerizedtomography and neuropsychological test measures in adolescents withobsessive-compulsive disorder. Am J Psychiatry, 141: 363-369

4 Singleton, V.L., Orthofer, R. and Lamuela-Ravento's,R.M.: Analysis of total phenols and other oxidationsubstrates and antioxidants by means of Folin-Coicalteau reagent. In: Methods in enzymology(Packer L. ed), San DiegoCA. Academic Press, 299:pp 152-177 (1999).

5 Archer T, Fredriksson A, Lewander T, Soderberg U. Marble buryingand spontaneous motor activity in mice: interactions over days and the effect of diazepam. Scand J Psychol 1987; 28:242–249.

6. Umathe SN, Vaghasiya JM, Jain NS, Dixit PV. Neurosteroids modulate compulsive and persistent behavior in rodents: implications for obsessive-compulsive disorder. Prog Neuropsychopharmacol Biol Psychiatry 2009;33:1161-6.

9. Wagner H, Bladt S. Plant drug analysis "A thin layer chromatography atlas". II nd edition, Springer verlag Berlin Hiedelberg 1996, pg 314 & 324.-9.

11. Matsushita M, Egashira N, Harada S, Okuno R, Mishima K, Iwasaki K, et al. Perospirone, a novel antipsychotic drugs, inhibits marble-burying behavior via 5-HT 1A receptor in mice: implications for obsessive-compulsive disorder. J Pharmacol Sci 2005;99:154–9.

12. Greene-Schloesser DM, Van der Zee EA, Sheppard DK, Castillo MR, Gregg KA, Burrow T, Foltz H, Slater M, BultIto A. Predictive validity of a non-induced mouse model of compulsive-like behavior. Behav Brain Res 2011;221:55-62.

13. Camfield DA, Sarris J, Berk M. Nutraceuticals in the treatment of obsessive compulsive disorder (OCD): a review of mechanistic and clinical evidence. Prog Neuropsychopharmacol Biol Psychiatry 2011;35:887-95.

14. Oikawa S, Yamada K, Yamashita N, Tada-Oikawa S, Kawanishi S. N-acetylcysteine, a cancer chemopreventive agent, causes oxidative damage to cellular and isolated DNA. Carcinogenesis 1999;20(8):1485-1490.

15. Sochman J. N-acetylcysteine in acute cardiology: 10 years later: what do we know and what would we like to know?! J Am Coll Cardiol 2002;39(9):1422-1428.

16. Cuzzocrea S, Mazzon E, Costantino G, Serraino I, De Sarro A, Caputi AP. Effects of n-acetylcysteine in a rat model of ischemia and reperfusion injury. Cardiovasc Res 2000;47(3):537-548.

17. Moran MM, McFarl K, Melendez RI, Kalivas PW and Seamans JK. Cystine/glutamate exchange regulates metabot ropic glutamate receptor presynaptic inhibition of excitatory transmission and vulnerability to cocaine seeking. 2005;25:6389-6393

Name : Saurabh Sharma

Date of Birth : 24th may, 1990

Residential Address : 34 vard no 8 hb colony Hamirpur (H.P)

Email id : saurabhdhima@gmail.com