# EFFECT OF *STINGING NETTLE* EXTRACT ON A MICE MODEL OF CHRONIC UNPREDICTABLE STRESS INDUCED DEPRESSION


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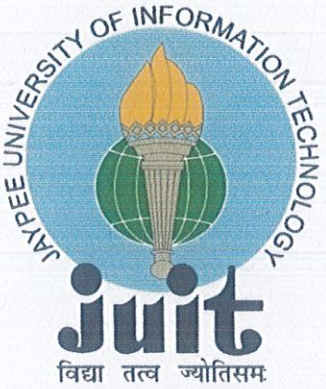



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## CERTIFICATE

This is to certify that the work entitled "**Effect of stinging nettle extract on a mice model of chronic unpredictable stress induced depression**" submitted by **Mr. Sahil Gupta** and **Ms. Kaveri Sharma,** in partial fulfillment for the award of Degree of Bachelor of Pharmacy of Jaypee University of Information Technology, Waknaghat (Solan), 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.

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**Signature of the students** .....

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**Date** .....

## SUMMARY

Depression is one of the major disorders of CNS. The effect of stinging nettle extract was studied in unpredictable chronic stress model of mice for 21 days. The animals were divided into 4 groups (gp1-control, gp2-disease=no drug, gp3-disease=standard drug (fluoxetin) gp4 disease +extract (stinging nettle) each group contain three mice. The chronic stress in mice was induced for 21days.Then, drug & extract was given from $19^{th}$ day -$21^{st}$ day by oral route Then we performed various behavioural tests –Morris water maize test, Actaphotometer test, Tail suspension test, forced swim test, Passive avoidance through task and beam walking test to study the effect of stinging nettle extract. The stinging nettle extract attenuated the memory impairment and depressive behaviour in mice exposed to chronic unpredictable stress.

# CHAPTER 1

## INTRODUCTION

Depression is an extremely common psychiatric condition, about which a variety of neurochemical theories exist, and for which a corresponding variety of different types of drug are used in treatment. [1]

### Status of depression in2011 [2]

A study based on the World Health Organization's World Mental Health Survey Initiative has said that India has the highest rate of major depression in the world. The average lifetime rates of depression, according to the study, were found to be 14.6 per cent in ten high income countries, and 11.1 percent in eight low- to middle-income countries. But lifetime incidents of Major Depressive Episodes (MDE), were highest among Indians at 35.9 percent, while China was at the lowest at 12 per cent. Average percentage of MDE was, however, considerably higher in high-income countries at 28.1 percent, compared to 19.8 percent in the low- to middle-income countries. Average percentage of MDE was, however, considerably higher in high-income countries at 28.1 percent, compared to 19.8 percent in the low- to middle-income countries.

## THE NATURE OF DEPRESSION [3]

Depression is the most common of the affective disorders; it may range from a very mild condition, bordering on normality, to severe (psychotic) depression accompanied by hallucinations and delusions. Worldwide, depression is a major cause of disability and premature death. In addition to the significant suicide risk, depressed individuals are more likely to die from other causes, such as heart disease or cancer.

The symptoms of depression include emotional and biological components.

**Emotional symptoms:** misery, apathy and pessimism low self-esteem: feelings of guilt, nadequacy and ugliness Indecisiveness, loss of motivation.

**Biological symptoms:** retardation of thought and action loss of libido Sleep disturbance and loss of appetite.

There are two distinct types of depressive syndrome, namely unipolar depression, in which the mood swings are always in the same direction, and bipolar affective disorder, in which depression alternates with mania. Unipolar depression is commonly (about 75% of cases) non-familial, clearly associated with stressful life events, and accompanied by symptoms of anxiety and agitation; this type is sometimes termed *reactive depression.* Bipolar depression, which usually appears in early adult life, is less common and results in oscillating depression and mania over a period of a few weeks. There is a strong hereditary tendency, but no specific susceptibility genes have been identified either by genetic linkage studies of affected families, affected individuals.

## Various forms of depression

### 1.1 Mixed depression (depressive mixed state)

Mixed depression is defined by the combination of depression and manic/hypomanic symptoms,. The most common symptoms of mixed depression are manic/hypomanic irritability, mental over activity (flight of ideas, racing thoughts, crowded thoughts), and behavioral overactivity (psychomotor agitation, over talkativeness). [4][5]

### 1.2. Atypical depression

Distinguishing features of atypical depression are the following:

It is more likely to be present in bipolar disorder (especially bipolar II disorder).It is more likely to be present in younger than in older individuals. It has a lower age at onset compared with non atypical depression. It is more common in females. It has more bipolar family history in comparison to non atypical depression.[4][5][6].

### 1. 3. Melancholic depression

Melancholic depression can be found in almost all mood disorders. Melancholic depression is more common in older age and in more severe and psychotic depressions. Symptoms are - lack of reactivity to pleasurable stimuli, depression worse in the morning, early morning awakening, marked psychomotor retardation or agitation, significant decreased eating or weight loss, and excessive guilt.[4][5][7]

### 1. 4. Minor depressive disorder

Minor depression is defined as a mood disturbance with between two and five symptoms of depression, including depressed mood, diminished interest, weight change, sleep disturbance, psychomotor changes, fatigue, feelings of worthlessness, poor concentration, and recurrent thoughts of death. Patients with this condition may have fewer vegetative symptoms (appetite, diurnal mood variation) and more subjective symptoms (self-blame, worry, irritability, lethargy. Minor depressive disorder is more prevalent in primary care than major depressive disorder.[4][5][8]

### 1.5. Recurrent brief depressive disorder

Recurrent brief depression (RBD), an affective disorder with a similarly high risk of suicidal behaviour as major depression (MD), it is characterized by depressive episodes occurring about once a month that last only a few days. The combination of RBD and MD is called combined depression (CD), which increases the risk of suicidal behaviour enormously. [4][5]

### 1.6. Seasonal affective disorder

Seasonal affective disorder (SAD) is a kind of depression that occurs at a certain time of the year, usually in the winter. People who live in places with long winter nights are at greater risk for SAD. Symptoms usually build up slowly in the late autumn and winter months. Symptoms are usually same as with other forms of depression: - Hopelessness, Increased appetite with weight gain, Increased sleep (too little sleep is more common with other forms of depression) Less energy and ability to concentrate, Loss of interest in work or other activities. [4][5] [9]

### 1.7 Dysthymic disorder

Dysthymia is a chronic type of depression in which a person's moods are regularly low. However, symptoms are not as severe as with major depression. The exact cause of dysthymia is unknown. It tends to run in families. Dysthymia occurs more often in women than in men and affects up to 5% of the general population. Many people with dysthymia have a long-term medical problem or another mental health disorder, such as anxiety, alcohol abuse, or drug addiction. About half of people with dysthymia will also have increase chances of major depression at some point in their lives. Dysthymia in elderly person is often caused by:-Difficulty in caring for themselves, Isolation, Mental decline,

Medical illnesses. The main symptoms of dysthymia are low, dark, or sad mood on most days for at least 2 years. In children and adolescents, the mood can be irritable instead of depressed and may last for at least 1 year.[4][5] [10]

**Pathophysiology of Depression**

Major depressive disorder (MDD) is a common disorder which is usually associated with severe and persistent symptoms leading to important social role impairment and increased mortality.

**STRESS HORMONES**

The corticotrophin-releasing hormone (CRH) is released from the hypothalamus in response to the psychological stress by cortical brain regions. This hormone induces the secretion of pituitary corticotrophin, which stimulates the adrenal gland to release cortisol into the plasma.The physiologic response to stress is partly gender-specific: women show generally greater stress responsiveness than men, which is consistent with the greater incidence of major depression in women. Altered stress hormone secretion is most prominent in depressed subjects with a history of childhood trauma. Elevated cortisol may act as a mediator between major depression and its physical long-term consequences such as coronary heart disease, type II diabetes, and osteoporosis. CRH produces a number of physiological and behavioral alterations which resemble the symptoms of major depression, including decreased appetite, disrupted sleep, decreased libido, and psychomotor alterations. [11]

**THE MEDIATING ROLE OF MONOAMINES**

Most of the serotonergic, noradrenergic and dopaminergic neurons are located in midbrain and brainstem nuclei and project to large areas of the entire brain. This anatomy suggests that monoaminergic systems are involved in the regulation of a broad range of brain functions, including mood, attention, reward processing, sleep, appetite, and cognition. Drugs which inhibit monoamine reuptake, results in increased concentration of monoamines in the synaptic cleft. By inhibiting the enzyme monoamine oxidase, which induces an increased availability of monoamines in presynaptic neurons, also show antidepressant effects. These observations led to the pharmacologically most relevant theory of depression, referred to as the monoamine-deficiency hypothesis. The monoamine-deficiency theory posits that the underlying pathophysiological basis of depression is a

depletion of the neurotransmitters serotonin, norepinephrine or dopamine in the central nervous system.

Serotonin is the most extensively studied neurotransmitter in depression. The reduced central serotonin has been associated with mood congruent memory bias, altered reward-related behaviours, and disruption of inhibitory affective processing all of which add to the clinical plausibility of the serotonin deficiency hypothesis. Increased availability of the brain monoamine oxidase, which metabolizes serotonin, may cause serotonin deficiency

Dysfunction of the central noradrenergic system has been hypothesized to play a role in the pathophysiology of MDD, based upon evidence of decreased norepinephrine metabolism, increased activity of tyrosine hydroxylase, and decreased density of norepinephrine transporter in the locus coeruleus in depressed patients.[11].

**Pharmacological evidence supporting the monoamine hypothesis of depression [12]**

| Drug(s) | Principal action | Effect in depressed patients |
|---|---|---|
| Tricyclic antidepressants | Block NA and 5-HT reuptake | Mood ↑ |
| Monoamineoxidase (MAO) inhibitors | Increase stores of NA and 5-HT | Mood ↑ |
| Reserpine | Inhibits NA and 5-HT storage | Mood ↓ |
| α-Methyltyrosine | Inhibits NA synthesis | Mood ↓ |
| Methyldopa | Inhibits NA synthesis | Mood ↓ |
| Tryptophan | ( Increases5-HTsynthesis hydroxytryptophan) | Mood ↑in some studies |

**Table-1**

**The Receptor Sensitivity Hypothesis**

The super sensitivity is a compensatory response of the postsynaptic neuron when it receives too little stimulation. The neuron tries to make up for a lack of stimulation by increasing receptor responsiveness. In case of overtime, the postsynaptic neuron may also compensate for lack of stimulation by synthesizing additional receptor sites. This process is known as up-regulation. By increasing the amount of neurotransmitter in the cleft, we can normalize responsiveness.

Increase in neurotransmitter results in increase stimulation of receptor sites, which prompts the postsynaptic neuron to compensate by decreasing receptor sensitivity, a process known as desensitization. The postsynaptic neuron is also thought to compensate for increasing stimulation by decreasing the number of receptor sites, a process known as down-regulation. As antidepressant drugs are thought to work by increasing the amount of neurotransmitter in the cleft. They do this by blocking metabolism of monoamines - the MAOIs - or by blocking reuptake - TCAs. Most TCAs are more effective in blocking nor-adrenaline reuptake than serotonin reuptake.

The chronic administration of TCAs or MAOIs is thought to alter the responsiveness and/or the number of postsynaptic receptor sites. Observation of this long-term effect of antidepressants led to the Receptor Sensitivity Hypothesis. This hypothesis proposes that depression is the result of a pathological alteration (super sensitivity and up-regulation) in receptor sites, which results from too little stimulation by monoamines, i.e., a deficiency of noradrenalin and serotonin in the cleft.

Chronic administration of TCAs or MAOIs results in increased availability of noradrenalin and serotonin which causes desensitization (the uncoupling of receptor sites) and possibly down-regulation (a decrease in the number of receptor sites). According to this hypothesis, relief from depression symptoms comes from a normalization of receptor sensitivity. According to the Receptor Sensitivity Hypothesis, antidepressant drugs achieve their clinical effect by reducing receptor super sensitivity. This theory is an important step toward understanding the long delay between administration of TCAs and MAOIs and clinical response. [13]

**The Serotonin-only Hypothesis.**

Early in the 1980s, drugs were introduced that selectively blocked serotonin reuptake, resulting in more serotonin available in the cleft. These drugs were known as selective serotonin reuptake inhibitors, or SSRIs. Unlike the TCAs, which are non-selective, the SSRIs may have fewer serious side effects and are therefore easier for patients to tolerate. This has led to the Serotonin-only Hypothesis which emphasizes the role of serotonin in depression and downplays nor-adrenaline. [13]

**The Neuroendocrine Hypothesis.**

According to this hypothesis, pathological mood states are explained or contributed to by altered endocrine function. This theory historically grew out of observations that altered mood states were associated with thyroid or Crushing's disease. [13]

**Intracellular signal transduction**

A number of studies implicate intracellular signal transduction in the pathophysiology of depression. One key set of mechanisms involve phosphorylation enzymes, including protein kinases A (PKA) and C (PKC).[14]

The binding of a transmitter with G-protein linked receptor activates the coupling of g-proteins (Gs and Gq) with second messenger enzymes such adelylate cyclase (AC) or phospholipase C (PLC).Thus, in turn, catalyze the formation of the second messenger's cyclic AMP and diacylglycerol (DAG). These, in turn, bind to PKA and PKC respectively, which facilitates phosphorylation by these enzymes. [14][15]

Protein kinases are critical elements of stimulus-response coupling [14][16]. One critical effect is the subsequent phosphorylation of the transcriptional factor cyclic AMP response element binding protein (CREB). CREB phosphorylation is linked to both nor-epinephrine- (NE) and serotonin- (5-HT) linked cascades and it may represent a common target of action of more noradrenergic and serotonergic antidepressants. [14][16]. Phosphorylated CREB binds to cyclic AMP response element (CRE) in the promoter region of genes, which regulates gene expression.[14][17]. This represents an integrative set of mechanisms in which antidepressants acting via either NE or 5-HT can target a common set of genes and their respective protein products.

Certain depressed patients have deficient PKA and PKC protein levels[14][18][19], lower binding of cyclic AMP to PKA, reduced phosphorylation of CREB and altered gene expression patterns [14][20][21] .Thus , decrease in the activity of these two key enzymes would be expected to alter the expression of genes that contain CRE elements in their promoters; these would include key proteins that regulate the stress response in brain, including brain derived neurotrophic factor (BDNF), the BDNF receptor trk-b and glucocorticoid receptors (GR). [14, 22, 23, 24] Moreover, GR functions as a transcriptional factor and regulates the expression of other genes, specifically exerting an inhibitory effect on corticotrophin releasing hormone (CRH). [14][25].

Hence, reduced activity of these key enzymes could enhance stress reactivity via altered regulation of the expression of specific genes. Important elements of stress regulation could, then, be vulnerable under demand conditions.

**2.2 Mechanisms for altered protein activity**

One possible avenue for altered protein activity is via protein oxidation. The oxidation-reduction (redox) potential is altered by reactive oxygen species (ROS), which are formed by a variety of factors, including inflammatory cytokines [14][26]Altered redox potential has been shown to affect the activity of kinases. Glutathione, an intracellular antioxidant, is involved in the protection of proteins against oxidative stress, by binding to redox sensitive amino acids (particularly cysteines).[14][26][27]. Proinflammatory cytokines are involved in the genesis of ROS and thereby affect oxidation and, hence, degradation of proteins. Antidepressant drugs have been shown to regulate the expression of specific cytokines, particular, inhibiting pro-inflammatory cytokines that are involved in enhancing ROS.

**2.3 cytokine-induced depression**

Cytokines [e.g., interferon (IFN) and interleukins (IL)] are pleitropic, immunomodulatory signaling molecules that have been increasingly implicated in the development of neuropsychiatric disorders, especially major depressive disorder. Cytokines signal the brain and can serve as mediators between the immune and central nervous systems.[28] There is an increase in pro-inflammatory cytokines in patients with major depression that seems to correlate with severity of illness and measures of hypothalamic–pituitary–adrenal (HPA) axis hyperactivity. Cytokines in the central nervous system are constitutively expressed and

have functions such as neuroprotection or neurodegeneration, and can be regulated by non immune factors, such as neurotransmitters and hormones.[28]

Peripheral cytokines can also access the brain and affect function via vagal nerve activation, a leaky or compromised blood–brain barrier, and active transport across the blood–brain barrier, or binding to cell surface proteins on brain endothelial cells. Acute activation of tumor necrosis factor-alpha (TNF-α) leads to chronic increases in brain levels of proinflammatory cytokines [28][29].

Administration of IFN activates expression of several IFN-stimulated genes in brain as well as in peripheral organs[30], exposure to a psychosocial stressor, greatly augments the effects of immune activation on sickness, plasma corticosterone and hippocampal nor-epinephrine, as well as on the levels of circulating IL-6, TNF-α and IL-10 [28][31], IFN-γ participates in the death of dopaminergic neurons by regulating micro glial activity[28][32], thus, IFN-γ induced activation of microglia and consequent neuronal loss may contribute to the modulatory effects of cytokines on depressive symptoms.

Cytokines and other immune molecules impact neuropsychiatric functions such as mood and cognition in part through their modulation of neuronal anatomy and function. Cytokines and other immune factors play a key role in modulating early brain development as well as adult neuronal plasticity; however, prolonged exposure to proinflammatory cytokines can impair neuronal plasticity, thereby contributing to cognitive and mood disorders. Brain regions with the highest concentrations of proinflammatory Cytokine receptors, specifically receptors for IL-1β, IL-6, and TNF-α, include the hypothalamus, hippocampus, and cortex regions [33][34]critical for antidepressant response and cognitive functioning.

At pathophysiologically elevated levels, TNF-α and IL-1β have both been shown to impair normal neuronal plasticity and to inhibit long-term potentiation (LTP).

Cytokines are critical to the regulation of neuronal plasticity and survival, and chronic disruption of the balance of these cytokines due to stress, disease or medication (e.g., IFN therapy, substance use disorders) can lead to long-lasting changes in brain anatomy and function, and therefore long-term impairments in mood, cognition, and behavior.

## 2.4 Proinflammatory cytokines, neurotransmission and oxidative stress

Proinflammatory cytokines such as IFN affect serotonin metabolism by stimulating indoleamine-pyrrole 2,3-dioxygenase (IDO or INDO ) which leads to a peripheral reduction of tryptophan and serotonin[28][35][36]. IDO is highly inducible by proinflammatory cytokines (e.g., IFN-γ and TNF-α) and is secreted by activated macrophages and other immunoregulatory cells, which catalyzes the degradation of tryptophan (serotonin precursor) to kynurenine. Kynurenine degradation leads to the formation of 3-hydroxykynurenine (3-HK, generates free-radical species that can cause oxidative stress),quinolinic acid (QA, a glutamate receptor agonist), and kynurenic acid (KA, an NMDA receptor antagonist hypothesized to be neuroprotective). This IDO-mediated imbalance of kynurenine pathway metabolites might contribute to cytokine-induced depression.

Thus, cytokine- and IDO-mediated degradation of tryptophan through the kynurenine pathway is hypothesized to influence serotonergic biosynthesis and neurotransmission in the brain resulting in significant neuropsychiatric consequences. Proinflammatory cytokines, such as IFN-γ, stimulate not only IDO but also the biosynthesis of 5,6,7,8-tetrahydrobiopterin (BH4), which is a cofactor for several aromatic amino acid monooxygenases and thus is involved in the biosynthesis of the neurotransmitter serotonin and the catecholamines dopamine, epinephrine, and norepinephrine. In macrophages, IFN-γ also causes the generation of reactive oxygen species, which can reduce BH4 levels.

Recent studies suggest that oxidative loss of BH4 in chronic inflammatory conditions can lower the biosynthesis of catecholamines, which may relate to altered neurotransmission in patients with depression [28][37].

## 2.5Neurotrophins and depression

Recent studies in humans have shown decreased plasma levels of BDNF in schizophrenia, bipolar disorder, manic and depressed patients which can be reversed by antidepressant treatment.[38] In addition, electroconvulsive shock therapy (ECT) increases the levels of BDNF in the serum of treatment resistant depressed patients[39][40][41].

Antidepressant treatment, including SSRIs and electroconvulsive shock (ECS) increase the expression of BDNF and TrkB in the hippocampus in animal models. These effects are dependent on chronic administration of antidepressant therapy, consistent with the time course of antidepressant treatments [42][43][44]. There is a positive, reciprocal interaction between BDNF expression and 5-HT, the major neurotransmitter implicated in the monoamine hypothesis.

Serotonin receptor activation induces BDNF expression in hippocampal cells [45][46][47]and conversely BDNF treatment increases the serotonergic phenotype of raphe nucleus neurons, the source of serotonergic input into the hippocampus.

BDNF and its receptor TrkB are also co-expressed in serotonergic neurons within the raphe nucleus and BDNF is retrogradely transported from 5-HT terminals in the striatum and hippocampus to cell bodies in the raphe nuclei.

**2.6 Other neuropeptides in depression**

Other neuropeptides induced by BDNF have been implicated in a wide variety of psychiatric disorders accompanied by negative affective states such as panic, anxiety and depression including NPY [38]. Substance P, CCK, ß-endorphin, OFQ and more recently galanin[38][48][49][50]. Some of the neuropeptides have opposing effects on mood. Chronic mild stress, a model of depression, results in altered levels of several neuropeptides including a decrease in NPY in the hippocampus, a decrease in galanin in the hypothalamus, but an increase in Substance P in the hypothalamus[38][51]. These neuropeptides are localized to the limbic system including the hippocampus and the prefrontal cortex, and may also participate in learning and memory

In addition, some of these peptides are co-localized with traditional neurotransmitters, such as nor epinephrine and 5-HT, as well as dopamine all of which are implicated in mood disorders [38]. Modulation of the serotonergic system within the dorsal raphe by Substance P and galanin, indicating the importance of the neuropeptide/5-HT/dorsal raphe system interactions.[38][52][53].

NPY is implicated in seizure control, food intake, anxiety-related behaviors and circadian rhythms [53][54]. NPY is released by inter neurons in the dentate gyrus[55]. NPY is dysregulated in human patients with mood disorders. NPY is reduced in the plasma of depressed patients and is upregulated in the CSF following ECT or pharmacological treatment in humans [56][57][58][59][60]. NPY mRNA is reduced the prefrontal cortex of bipolar patients.

### 3.1Metabotropic Glutamate Receptor-Mediated Long-Term Depression

The vast majority of excitatory neurotransmission is mediated by the amino acid glutamate, which acts on ionotropic and metabotropic receptors throughout the central nervous system [61]. Metabotropic glutamate receptors (mGluRs) are coupled to GTP-binding proteins that link the receptors to downstream signaling pathways [61][62][63] .The family of mGluRs comprises eight different subtypes[61][64]. Synaptic plasticity is the strengthening or weakening of synapses in response to different activity patterns. This involves specific changes in cellular activity within complex neural networks; together, these encode distinct memory traces [61][65]. The two main types of synaptic plasticity involve either a long-lasting decrease [long-term depression (LTD)] or increase [long-term potentiation (LTP)] in synaptic efficiency [61][66]. MGluR-mediated LTD was first characterized at parallel fiber-Purkinje cell synapses of the cerebellum [61][67][68][69].

It is dependent on an increase in intracellular $Ca^{2+}$ and activation of postsynaptic group I mGluRs, specifically mGlu1 receptors [70][71]. mGluRs were first shown to mediate hippocampal LTD induction when depotentiation at CA1 synapses was blocked by the group I/II antagonist _-methyl-4-carboxyphenylglycine (MCPG)[61][72][73][74]. A key property of mGluR-LTD in the hippocampus is that it is NMDAR-independent. LTD induction by either mGluR or NMDAR activation is not mutually exclusive, meaning that one form of LTD does not occlude the other [61][75][76][77][78].

## 3.2Induction and Expression Mechanisms of Metabotropic Glutamate Receptor-Mediated Long-Term Depression

### $Ca2^{+}$Independence and Activation of Kinases

mGluRs are members of the family C G-protein-coupled receptors, which includes GABAB receptors, the calcium-sensing receptor and a selection of taste, pheromone ,and olfactory receptors based on structural similarities[61][79]. mGluRs function as G-protein-coupled receptors in that agonist-induced or constitutive receptor activity leads to G-protein activation by promoting the exchange of GTP to GDP[61][79]. This results in modulation of receptor-protein interactions and activation of distinct second messenger cascades. MGluRs preferentially mediate PLC activation, which leads to diacylglycerol production via an increase in inositol triphosphate(IP3)[61][80][81][82]. Protein kinase C (PKC) activation and Ca2+ release from intracellular stores is stimulated by diacylglycerol and IP3, respectively [71][83].

$Ca^{2+}$ release from intracellular stores was not necessary for DHPG-LTD induction because intracellular $Ca^{2+}$ depletion had no effect. $Ca^{2+}$calmodulin-dependent protein kinases II (CaMKII) has an important role in LTP induction, hence it may be down-regulated during LTD. DHPG-LTD induction does not require PKC or protein kinase A activation.[61].

| Type and examples | Action(s) | Risk of overdose |
|---|---|---|
| **TCA group**(Imipramine,desipramine, clomipramine.) | Inhibition of NA/5-HT reuptake | Ventricular High risk in combination with CNS depressants |
| **Other non-selective uptake inhibitors** | | |
| Venlafaxine | Weak non-selective NA/5-HT uptake inhibitor<br>Also non-selective receptor-blocking effects | Safe in overdose |
| Duloxetine | Potent non-selective NA/5-HT uptake inhibitor<br>No action on monoamine receptors | |
| Bupropion | All highly selective for 5-HT | Low risk in overdose but must not be used in combination with MAO inhibitors. |

| **NA selective uptake inhibitors** |
|---|

| | | |
|---|---|---|
| Maprotiline | Selective NA uptake inhibitor | As TCAs |
| Phenelzine | Non-selective | Many interactions (TCAs, opioids, sympathomimetic drug. |
| **MAO INHIBITORS** | | |
| Phenelzine | Non-selective | 'Cheese reaction' to tyramine-containing foods (see text)<br>Anticholinergic side effects<br>Hypotension<br>Insomnia<br>Weight gain<br>Liver damage |

| | | |
|---|---|---|
| Moclobemide | MAO-A-selective<br>Short acting | Interactions less severe than with other MAO |
| | | |
| **MISCELLANEOUS ANTIDEPRESSANTS** | | |
| Trazodone | Weak 5-HT uptake inhibitor<br>Also blocks 5-$HT_2$and $H_1$ receptors (enhances NA/5-HT release) | Safe in overdose |

**Table-2**

### 4.1 Extract of stinging nettle

Stinging nettle or common nettle, *Urtica dioica*, is an herbaceous perennial flowering plant, native to Europe, Asia, northern Africa, and North America, and is the best-known member of the nettle genus Urtica. The plant has many hollow stinging hairs called trichomes on its leaves and stems, which act like hypodermic needles that inject histamine and other chemicals that produce a stinging sensation when contacted by humans and other animals. [85]

### 4.2 SCIENTIFIC CLASSIFICATION[86]

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Rosales
- Family: Urticaceae
- Genus: Urtica
- Species: U. dioica
- Binomial Name : *Urtica dioica*

### 4.3 CONSTITUENTS

It contains on average 22% protein, 4% fats, 37% non- nitrogen extracts, 9-21% fiber, and 19-29% ash. The leaves contain about 4.8 mg chlorophyll per gram of dry leaves, depending on whether the plant was grown in the sun or shade. The dried leaf of nettle contains 40% protein. They are one of the highest known sources of protein in a leafy green, and of superior quality than many other green leafy vegetables The leaves are also noted for their particularly high content of the metals selenium, zinc, iron, and magnesium. The fresh leaves contain vitamins A, C, D, E, F, K, P, and b-complexes as well as thiamin, riboflavin, niacin, and vitamin B-6, all of which were found in high levels, and act as antioxidants [87].

### 4.3 Phytochemicals:

Histamine, acetylcholine, serotonin, flavonol glycosides, sitosterol, lectin, coumarins, hydroxysitosterol, tannins, lignans, scopoletin [88]

## 4.4 DISTRIBUTION:

Stinging nettles are abundant in northern Europe and much of Asia.In North America it is widely distributed in Canada and the United States also can be found in northernmost Mexico. It grows in abundance in the Pacific Northwest, especially in places where annual rainfall is high.[89]

## 4.5 USES:

The stinging nettle has stimulating action on the kidneys and bladder. Nettle shoots, eating during spring, helps to clean the body of toxins. Stinging nettle is used to treat inflammation of the urinary tract. Stinging nettle improves the excretion of uric acid thereby reducing the symptoms of gout and arthritis. Stinging nettle leaves have diuretic properties. Nettle root is also used for the treatment of urinary retention caused by prostate enlargement [88][90].

## 4.6 APPLICATIONS:

**Aerial part: INFUSION** – This herbal form of the nettle remedy can be used to stimulate the circulatory system in people suffering from impairment in the flow of blood and it can also be used as a detoxification agent to cleanse the system of toxins in individuals afflicted by disorders such as arthritis, it can be used to treat rheumatism, to treat symptoms of gout, and to treat symptoms of eczema. The herbal infusion made from the nettle also helps in increasing the flow of milk in nursing mothers with lactation issues. A revitalizing spring tonic can be produced from the fresh shoots of the nettle.

**TINCTURE** - The herbal tincture form of the nettle is utilized in combination with other beneficial herbs in the treatment of various disorders such as arthritic conditions, to treat various skin problems, and in the treatment of heavy uterine bleeding in women suffering from menstrual diseases.

**WASH** – The herbal remedies made from the nettle can also be used as a healing salve and herbal wash and applied to burns, to insect bites, and to wounds.

**JUICE** – The herbal nettle remedy can also be used in the form of a nettle juice and this can be prepared by liquefying the whole fresh plant to make a good herbal tonic for the treatment of debilitating conditions and cases of anemia, this same tonic can be used to soothe the stings of the nettle hairs.

The nettle based tonics are also often prescribed for the treatment of cardiac insufficiency coming along with disorders such as edema.

**POWDER** – Herbal remedies made from the powdered leaves of the nettle can be inhaled as a snuff for the treatment of nosebleeds.

**Root: HAIR RINSE** – The nettle roots can also be used to make an herbal decoction, which can be used as a rinse for the treatment of dandruff, to stem the causes of falling hair, and as a general conditioner for a healthy scalp.

**OINTMENT** – As an herbal nettle ointment, the nettle is used to topically treat cases of hemorrhoids, the ointment is directly applied to the affected region of the body.

**COMPRESS** – The herbal remedies made from the nettle can be used to make a herbal compress by soaking a pad in the herbal tincture of the nettle[91]

## Objectives

To induced depression in an experimental animal model of chronic unpredictable stress .

To evaluated the animal model on behavioural tests to assess depression

To study the alteration in the memory during stress induced depression

To study the effect of depression on motor activity and muscle coordination

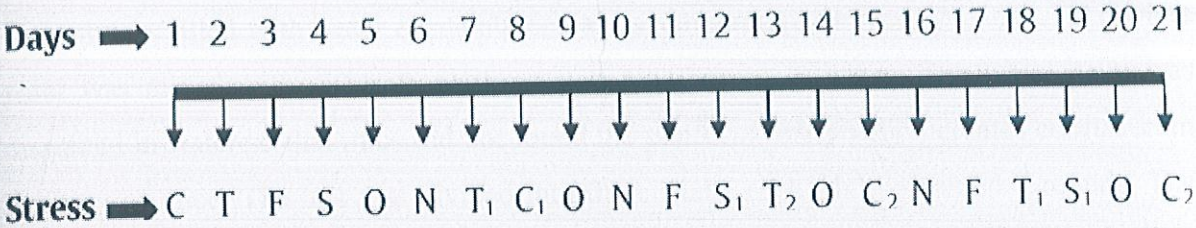
To investigate the effect of stinging nettle extract on depression mediated behavioural alterations.

# CHAPTER 2

## MATERIALS AND METHODS

### PROCEDURE TO INDUCE CHRONIC STRESS IN MICE

We perform following procedure to induce chronic stress in mice for 21 days:-




C - COLD SWIM (8 °C, 2 min). T- Tail pinch (1 min). F - Food and water deprivation (24h).S - Swimming at room temperature (24±2 °C, 3 min).O - Overnight illumination.N - No stress.T1- Tail pinch (1.5 min).C1 - cold swim (10 °C, 2 min). S1- Swimming at room temperature (24±2 °C, 3min). T2 -Tail pinch (2 min). C2- Cold swim (6 °C, 3min).[92][93][94].

After 21 days mice were subjected to different behavioural test.

We divide the animals into three groups G1-control, G2-disease but no drug, G3-disease and drug (fluxotein), G4 – extract and disease.

We have given drug to mice continuously for three days.

After that we performed different behavioural tests.

## BEHAVIORAL TESTS

**Morris water maze test**- it is the behavioural test which is performed to analyze memory test in mice. This test was carried out for 3 days continuously.

**Materials required**- stopwatch, white paint, circular tub, paper, pen, any heating equipment to maintained temperature of water (25-30c).

**Methodology:-**

The tub was filled with water & maintained at its temperature between 25-30°C.Then white paint was mixed with water & platform was put (bottle) in water. The white paint was dissolved in water so that mice did not found the platform easily which creates confusion in memory of mice. The test was divided into three trials, each trial contained 3 rounds. The mice was put in water for 60 seconds ,if the mice could not able to found the platform before 60 sec, then the mice was helped to found the platform & remain to sit on that for 10 seconds in every round , this test was performed continuously until the mice was able to found the platform by himself. Similar procedure was performed for the rest of groups. The time required by mice to found the platform was recorded. This test was continued for 3 days and each day 3 trials (9rounds) were performed. This behavioural test tell us that mice with diseased state(G2) take more time to found the platform as compare to controlled group of mice (G1) ,drug treated mice(G3).and G4(extract is given).[95]

**Actaphotometer test**- Animal locomotors behaviour was monitored using actaphotometer. Actaphotometer is provided with digital counter photocell & a light source was used to measure locomotor activity of animals. Each animal was placed in actaphotometer for 5 min & basal activity was recorded for all groups of animals. The mice in diseased condition showed less locomotor activity as compare to control, extract and drug treated mice.[96]

**Tail suspension test** – In tail suspension test, the mice is suspended from tail for 6 minutes dùring which mice showed periods of agitation & immobility. The duration of immobility was measured. The mice in disease condition showed more immobility as compare to controled, extract and drug treated mice.[97]

**Forced-swim test (FST)** - The animals were individually forced to swim in a 60×30×45 cm(L×B×H) filled with water (23–25 °C) up to a height of 25 cm..After the initial 2–3 min of vigorous activity the animals showed period of immobility by floating with minimum movements. An animal is considered to be immobile whenever it remained floating

passively in the water in a slightly hunched but upright position, its nose above the water surface. The total immobility period during the 6 min test was recorded with the help of stop watch. The mice in disease condition show more immobility as compare to extract, drug treated & control mice.[92][98]

**Passive Avoidance Step through Task:-**

The inhibitory avoidance test, also called passive avoidance, has been used as a screening test to evaluate drug effects on the memory in mice. The test is based on the natural photophobia of mice or rats, and evaluates the long-term memory of animals The apparatus consists of a box divided into two compartments of equal sizes. One compartment was illuminated with a torch placed on the top of the chamber while the second compartment was kept dark. The two compartments are separated by a guillotine door. In a trial, the animal was placed in the bright compartment and readily enters the dark compartment. Simultaneously, at that moment, the door separating the two compartments automatically closes, and the animal receives a brief mild electric shock. During a subsequent trial, the latency to enter the dark compartment was recorded as an index of memory consolidation. The longer the latency to enter the dark compartment, the better the animal is supposed to remember it received an electric shock during a previous trial. G1 (control group) G3 (drug treated),G4(extract was given) has longer latency to enter the dark compartment while G2 has less latency to enter the compartment[99].

**Beam Walking Test:-**

To assess active balance, through the ability to balance while walking along a elevated beam. The mice will start at the one end, step on to the beam, and walk the entire length of the beam to the other end. The time interval for 1min is noted down; if the mice were able to fall off before the 1 min then it showed the negative results.

G1(control group) ,G3 (drug treated) and G4(extract given) will be able to walk on the beam for approx 1min while G2 has less tendency to walk on beam and fall immediately before 1min.[100]

# CHAPTER 3

## RESULTS

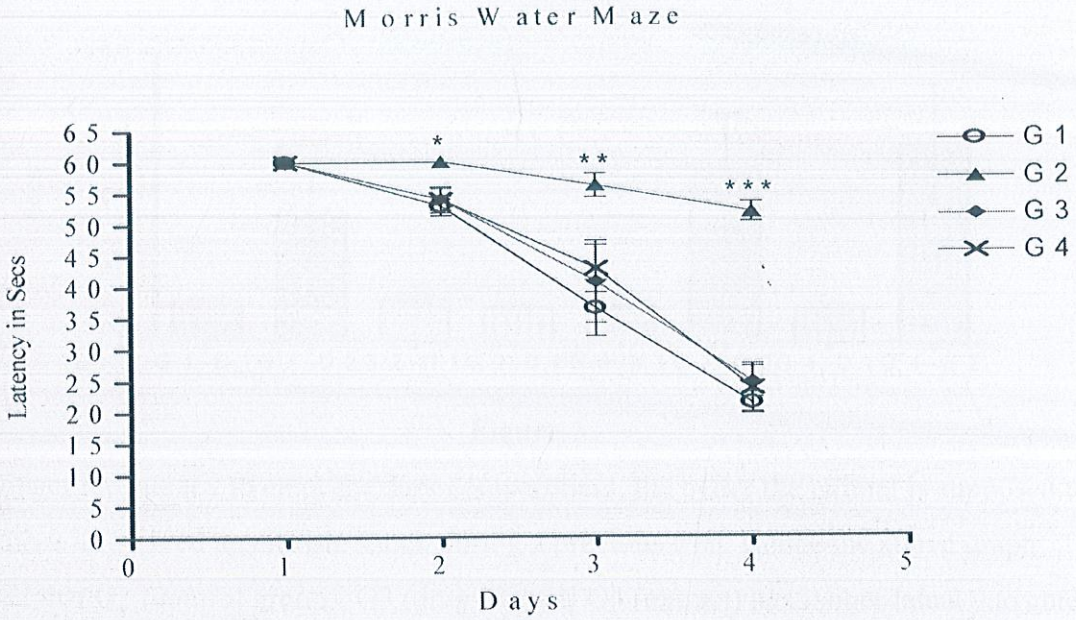



**Figure-1**

From above the graph, groups G1 (control) , G3 (drug treated) and G4(extract) showed as the experiment proceeds, their latency time decreases which shows their spatial memory increases while for G2 group which is in disease state which does not depict variation in latency time and their spatial memory.

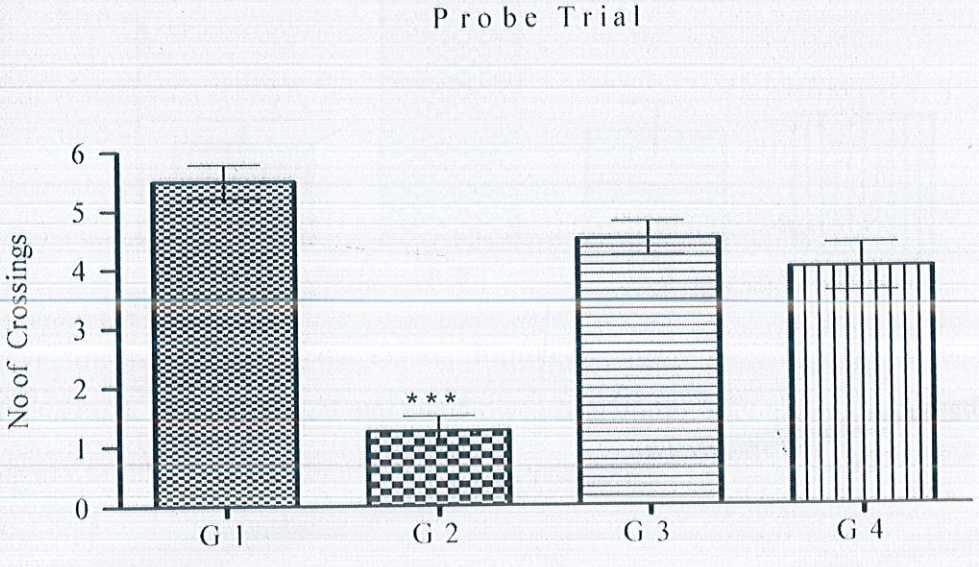



**Figure- 2**

From the above graph, groups G1 (control), G3 (drug treated, fluoxitine) and G4 (extract) showed more number of crossings, as their spatial memory.

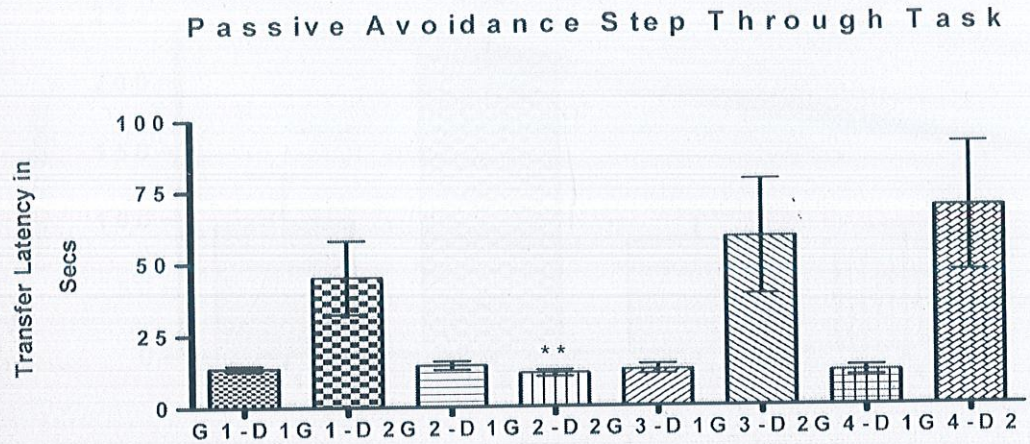



**Figure-3**

The longer the latency to enter the dark compartment, the better the animal is supposed to remember it received an electric shock during a previous trial. Hence the above graph showed that G1 (control group), G3 (drug treated) G4 (extract) has longer latency to enter the dark compartment while G2 has less latency to enter the compartment.

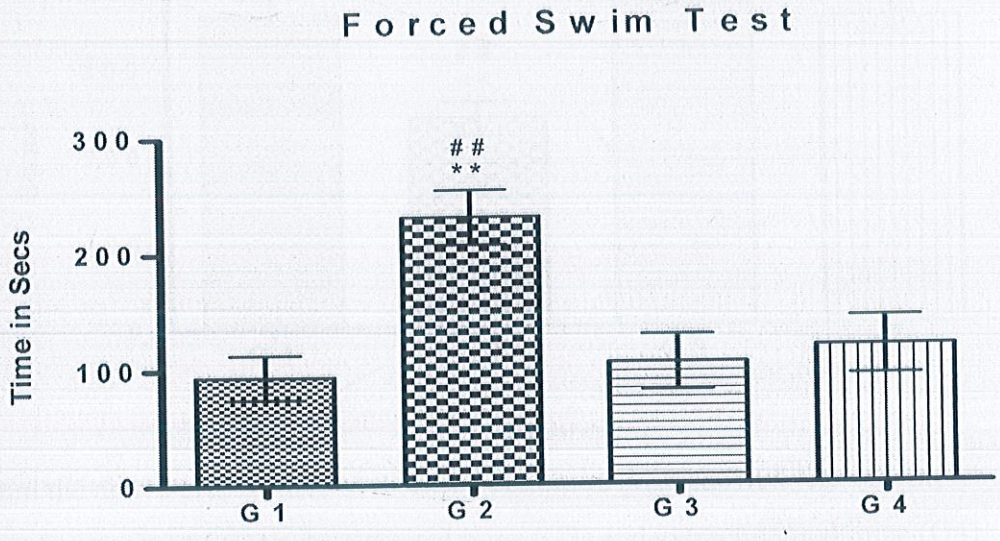



**Figure-4**

From the graph, groups G1, G3 and G4 showed less immobility during forced swim test as compare to group G2 (diseased state)

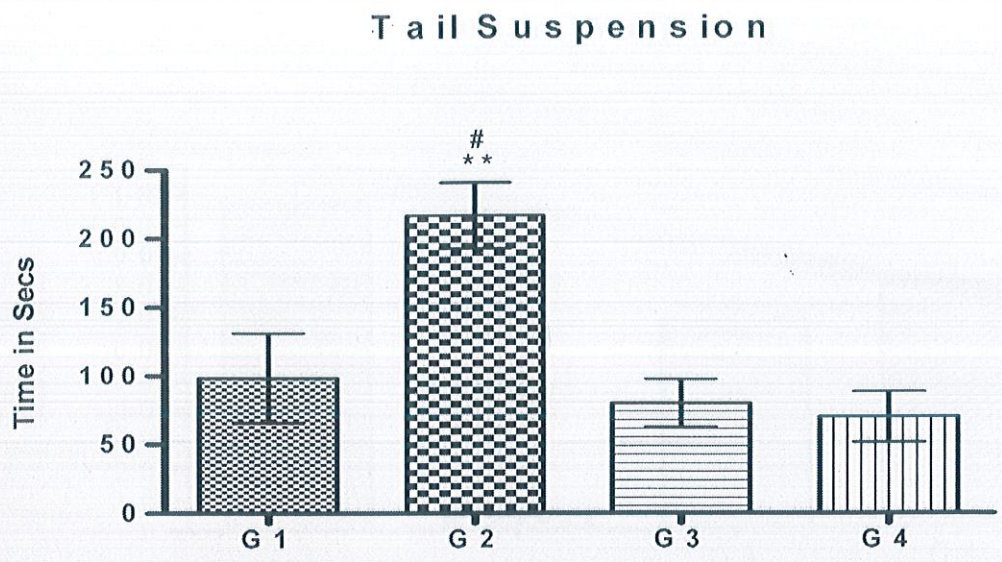



**Figure-5**

From the above graph, groupsG1, G3 and G4 showed less time of immobility while the group G2 (diseased state) is more immobile as compare to G1 and G3.

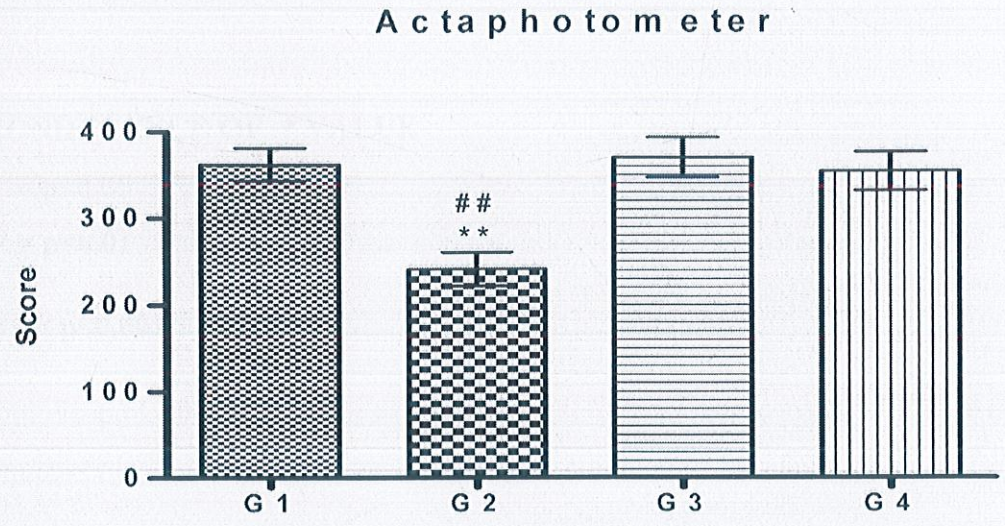



**Figure-6**

From the above graph, mice in diseased condition (G2) showed less locomotor activity as compare to control (G1), drug treated mice (G3) and extract treated mice (G4).

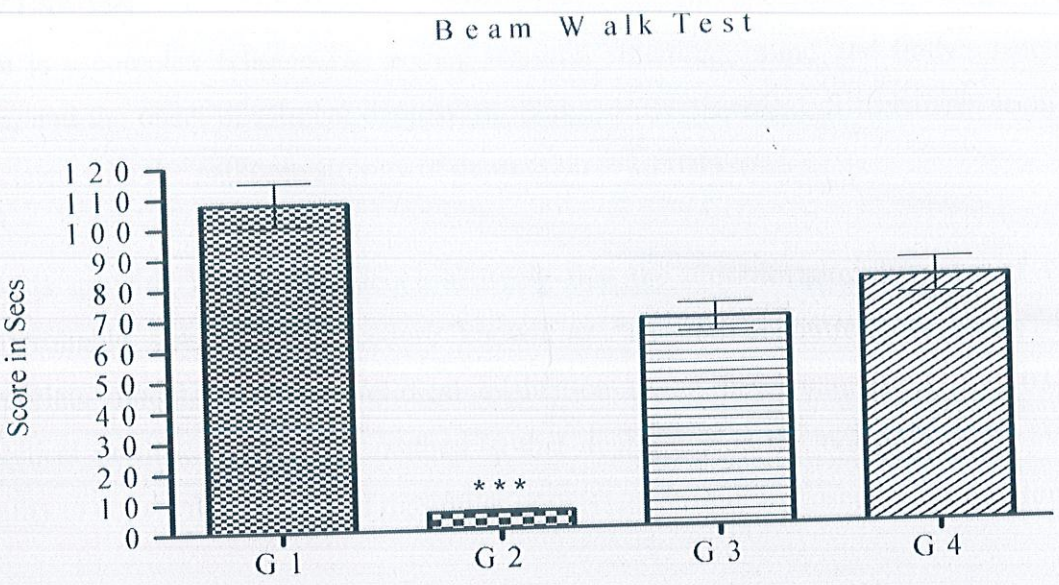



**Figure-7**

From the above graph, G1(control group) ,G3 (drug treated) , G4(extract) will be able to walk on the beam for approx 1min while G2 has less tendency to walk on beam and fall immediately before 1min.

**SIGNIFICANCE OF * VALUE**

*** = p<0.05**

**** = p<0.01**

***** = p<0.001**

## DISCUSSION

There is a complex relationship among stressful situations, mind and body's reaction to stress, and the onset of clinical depression. Some stress-provoked disturbances seem to be associated with the pathophysiology of depression.[92][101]

There is growing body of evidence showing that the chronic administration of various uncontrollable stresses, a procedure known as "chronic uncontrollable stress", is an appropriate model for the pre-clinical evaluation of antidepressants[92][102][103]. The theoretical premise behind this method is that depression is the outcome of an eventual inability to cope with a stream of dissimilar unpleasant stimuli imposed by the environment

.

To stimulate this effect in animals, stressors are used to induce behavioural deficits which can subsequently be reversed by antidepressant treatments [92][104][105]. So, we used chronic unpredictable stress model to determine whether administration of stinging nettle extract can alleviate or reverse the stress-induced depressive like behaviour in mice models

.

Chronic unpredictable stress prolongs learned helplessness behaviour and increase plasma corticosterone levels [92][106]. It also inhibits the brain monoamine oxidase (MAO-A and MAO-B) enzyme activity [92][107] which may further result in the depletion of brain monoamine levels. Various antidepressant drugs, either by inhibiting MAO enzyme or by inhibiting reuptake mechanism, increase the central monoamine levels and reverse the stress induced depressive-like behaviour.

It was observed from the results that chronic stress induce depressive like behaviour evidenced from tail suspension and forced swim test. The stressed animals showed increased immobility. Further in actaphotometer and beam walk the stressed animals showed reduced locomotion and physcial activity. In addition the passive avoidance and morris water maze test showed that chronic stress altered the memory functions. The stinging nettle extract significantly attenuated the stress induced alteration in behavioural parameters like locomotion, muscular co-ordination and memory.

**CONCLUSION** The stinging nettle extract attenuated the chronic stress induced alteration in behavioural studies in forced swim, tail suspension, actaphotometer, passive avoidance step through and morris water maze task.

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