

**EFFECT OF *Urtica dioica* ON STRESS INDUCED CHANGES IN SOCIAL
BEHAVIOR**

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WAKNAGHAT.**

Table of Content

CHAPTER NO	TOPICS	PAGE NO
	Certificate from Supervisor	I
	Acknowledgement	II
	Summary	III
	List of Figures	IV
1	Introduction	9-11
2	Literature review	12-17
2.1	Need for Stress Related Studies	13-14
2.2	Problems with Stress	14
2.3	Mice Models	15
2.4	<i>Urtica dioica</i>	16-17
3	Design of study	18-21
3.1	Chronic Stress	19
3.2	CUMS Protocol Design	20
3.3	Drug Treatment	21

4	Materials and method	22-25
4.1	Three Chambered Apparatus	23-25
4.1.1	Equipment Setup	24
4.1.2	Animal Preparation	24
4.1.3	Habituation	25
4.2	Sociability	25
4.3	Preference for Social Novelty	25
5	Results	26-32
6	Conclusion	33-34
7	References	35-37

(I)

CERTIFICATE

This is to certify that the work titled “**Effect of *Urtica dioica* on Stress induced changes in Social Behavior**” Submitted by **Akshay Bhandari** in partial fulfillment for the award of degree of **B.Tech Biotechnology** of Jaypee University of Information Technology, Waknaghat 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 Student

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(III)

SUMMARY:

Stress significantly alters mood and behavior of people. People in day to day life suffer from different type of stress which makes them vulnerable to several psychological and mental problems. A person who suffers daily stress have more violent attitude and frequently suffers from chronic vascular diseases. [1] Stress not only affects health but also decreases the performance of an individual. Stress is linked to different section of the life. [2] Lack of social support is the biggest risk factor for stress induced depression. Several studies have shown that short term stress enhances the performance of an individual. Normally if stress starts interfering with your performance then it becomes even more dangerous. Several diseases are linked to stress (asthma, bronchitis, CVD's). Individual performance is directly linked to the stress levels. It restricts the constructive thinking of the particular individual. Stress imbalances quality of work life as well as personal life. People suffering from stress induced depression have decreased immunity. They have decreased natural killer cells, gamma interferon and T-cells which are important for boosting immune system. Studies also proved that people suffering from depression have lower T-cell response. Social isolation and feeling of loneliness severely affects immune system. Therefore stress management in both personal and professional life is critical. We basically focused on stress studies because (a) Preclinical research studies in chronically stressed animals show multiple changes that mimic those observed in depressed humans. (b) Therapeutic effects of the currently available antidepressant drugs are suboptimal and their mechanisms of action remain obscure. Therefore our aim is to dissect neurobiological changes associated with stress and anti- stress drug treatment. We divided our work on 4 experimental groups (control, stress, control +*Urtica dioica*, and stress + *Urtica dioica*). Mice were subjected to chronic unpredictable mild stress (CUMS) for 21 days. Further, social behavioral studies were done using three chambered apparatus. The

administration of drug *Urtica dioica* (50mg/kg) was started from day 1-21. Our behavioral analysis concludes that stress significantly impairs social behavior and that *Urtica dioica* treatment significantly restores social behavior in mice.

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(V)

List of Figures

FIGURE NO	TITLE	PAGE NO.
3.1	Flow Chart of chronic stress based study	19
3.2	CUMS protocol design	20
5.1	Animal handling and Dosing.	27
5.2.1	Three Chambered apparatus	27
5.3	Sociability Chart	28
5.4	Sociability (<i>Urtica dioica</i> treatment) Chart	29
5.5	Preference for Social Novelty Chart	30
5.6	Preference for Social Novelty (<i>Urtica dioica</i> treatment) Chart	31

CHAPTER 1
INTRODUCTION

1.1 WHAT IS STRESS?

It is a state of mental tension and worry caused by problems in your life. It is something that causes strong feelings of worry or anxiety. Stress is caused by Stressors. It affects every section of society (poor, rich, old and young). Stress is multi-dimensional. It can arise through section of your life. Mild form of Stress is important for motivating and energizing a person. Long term stress is called chronic stress. This type of stress leads to severe health and psychological problems. People interpret and respond to stress differently. Stress is present in all aspects of our life. For example, speaking in front of audience can be stressful for some people, and not for others. During stress people suffers from loss of emotional intelligence, Behavioral changes (speech difficulties, laughing suddenly in a high-pitch), Physiology problems (Sleeping problems, Loss of appetite) and severe psychological problems. [3]

There are different perspectives regarding stress; environmental stress, psychological (emotional) stress and biological stress. The environmental stress perspective basically emphasizes on environmental situations which is related to substantial adaptive demands. The psychological stress perspective basically emphasizes on people's ability to cope in different situations and experiences and the biological stress perspective emphasizes on the function of physiological systems in the body. Mild form of stress also called acute stress. Acute stress is the most common form of stress and is characterized by its small duration. It is treatable and manageable. Common symptoms include emotional distress, muscular problems. Episodic acute stress is linked to the people who suffer acute stress frequently and their lives are very disordered. People with acute stress are short tempered, irritable, anxious and tense. They always tend to be abrupt and are always in a hurry. They have difficulty in establishing interpersonal relationships. [4]

People who face the traumatic events (murder, physical assault) are likely to suffer from acute stress disorder (ASD). These events cause the development of fear, horror, and helplessness. Stress increases (catecholamine and T suppressor cells) levels, which annihilate the activity of the immune in turn raises the risk against viral infections. It raises the risk for diabetes mellitus especially in individuals having more BMI (body mass index). Stressed peoples have high acid levels in stomach which leads to the formation of ulcers in stomach (peptic ulcers). It also leads to increase in plaque levels in the arteries causing a condition called atherosclerosis. Social interaction can be defined as the person's ability to interact with other individuals or group of the individuals. Competition and Cooperation are two different phases of people which are socially more interactive. The major response of body to the stress is the increase in the heart rate and elevated blood glucose levels. Exposure to the stress signals activates two biological systems i.e. autonomic sympathetic nervous system (ANS) and the slower hypothalamic-pituitary-adrenal (HPA) axis. [5]

CHAPTER 2
LITERATURE REVIEW

2.1 NEED FOR STRESS RELATED STUDIES

Stress is not localized to a particular section of a society. It has been seen countries are growing very fast and due to rise in competition employees are working in an atmosphere of anxiety and stress. Due to industrialization competitive environment is changing drastically. So people work hard in order to get a competitive edge. In order to achieve goals they sometimes undergo severe stress. Stress is linked not only to psychological conditions (anxiety and depression) but also with physical conditions (heart attack, ulcers). Stress (depression) is one of the leading cases mental illnesses in the world. Today more people are suffering from depression than any other diseases. Stress not only links to depression but to various lifestyle diseases (diabetes). People who are older are more prone to stress related immune disorders. Nature of work is changing constantly. So in order to be in hunt person needs to work hard constantly which can cause severe stress to him. Researchers have shown that job stress is the major source of stress in many countries and have been progressively increased in past decade. The relationship between the stress induced immune changes and the development of cancer has not been scientifically proved. There is a great concern about the increasing cost of the stress-related studies especially in relation to work place. Mortality and morbidity due to stress has reached at alarming levels.

Asthma is an acute stress linked diseases. Family therapy (minimum interaction with parents) is widely used in the management of asthmatic people. Coronary Heart disease (CHD) has long been linked to a variety of psychosocial factors. The people with high risk of myocardial infarction have higher prevalence of certain stress factors (stress at different places, events in their life). Studies proved that increased levels of serum cholesterol are also linked to the stress. Emotional stress is major

contributing factor for the hypertension. Many hypotheses concluded that Rheumatoid arthritis is also linked to stress. Migraine headaches are caused due to the emotional stress and tension (high anxiety levels, nervous nature). There is also evidence that stress is associated with disorders like diabetes mellitus. Several studies had proved that psychological stress and susceptibility to cold viruses are linked. Now we can see that stress is not linked to particular disorder. It touches all aspects and affects all corners of our life.

2.2 PROBLEMS WITH THE STRESS

Stress and illness relationship is highly complex. Wide range of events interacts with a broad variety of background factors to cause an illness. Factors that influence the susceptibility to the stress are very common and are linked to our daily routine (coping ability). Everything depends on our perception of a problem. If we perceive that the problem is serious, then we will likely to face ourselves as stressed. Reaction to situations basically defines our susceptibility to illness and our well-being. All stresses do not have negative effect. If the body adjusts to the stress and uses it to enhance one's performance, then that stress is positive and good for health.

Stress is positive and beneficial to us when it encourages us to adapt in any stress environment. Positive stress increases the strength of the mechanisms of adaptation and optimal stress levels. Stress is unhealthy when it exceeds our ability to cope with behavioral, psychological and environmental stress. Immune system is the one that is most susceptible to stress. Long term stress causes wear and tear of the immune system. Most of the studies showed that stress has immunosuppressive response on the body which decreases the body's ability to cope with the infections and the responses.

2.3 MICE MODELS

Mice models are currently used to study the anti-stress activity of certain novel anti-stress agents. Mice are similar to human's w.r.t physiology, anatomy. Approximately 95% of the human genome is similar to that of mice. Also mice are a cost effective and efficient tool to aid in the development of different drug therapies. Mice have several advantages (less generation time, availability, cost effective and requires less space). Genes responsible for certain complex disease like atherosclerosis are common in mice and humans. So it provides a basic structure to study the effects on certain drugs on functioning of the gene. Also mice genome can be directly manipulated to model certain diseases whose causative genes are known. It provides us to find effective treatment strategies for diseases. There are different strains of mice models used but mainly C57BL/6. Researchers had produced genetically diseased mice models by exposing mice to mutagens. Researchers have utilized an array of innovative genetic technologies to produce custom-made mouse models for a wide array of diseases. The most advanced research has been the ability to create transgenic mice in which gene of interest is inserted genome of the mice by rDNA technology. Most advanced approaches uses gene silencing technologies in which altered gene products are obtained. [6]

Mice models in stress are very useful in mimicking the depressed human conditions in mice. Mice models are very useful in pre-clinical testing's of any novel drug. A drug can undergo human's trials only when its efficacy has been tested on different animal models. To determine the efficacy of most of the drugs, testing is done on mice models. In our study we basically have used the hydro-alcoholic extract of *Urtica dioica* in the treatment of stress. [7]

2.4 *Urtica dioica*

Urtica dioica also known as stinging nettle has a varied range of medicinal properties. This herb is mainly found in high nitrogenous and moist soil. It spreads mainly with the help of rhizomes and stolons. This plant has stinging nettle called trichomes which causes the release of histamines, acetylcholine etc in blood which increases in blood pressure and blood pumping rate. In many hilly areas *Urtica dioica* leaves are taken as a cooked vegetable. It stems and leaves are advised for the person suffering from anal infections. Oil obtained from the aerial parts of this plant is used to treat muscle aches, allergies. Its areal parts are highly used to stop bleedings, acts as diuretic. [8]

Quercetin-3-*O*-rutinoside, kaempferol-3-*O*-rutinoside and isorhamnetin-3-*O*-glucoside are the major compounds isolated from the extract of the aerial parts of *Urtica dioica*. All the isolated compounds have high chemo tactic effects in variable doses. [9]

It is used as an anti-inflammatory agent for Rheumatoid arthritis.. Several clinical studies proved that its leaf extracts are quite beneficial in decreasing arthritis pain and inflammation in humans. Its anti-inflammatory activity is based on its ability to stop the production and actions of inflammation-producing immune cells in the body (cytokines etc). Various studies on animal reported that water extracts of *Urtica dioica* lowers blood pressure, decreases heart rate. It increases the urine output (diuretic actions). Its extract also has a pain-relieving activity in mice; Clinical studies on nettle root have demonstrated an improvement of clinical symptoms in benign prostate hyperplasia (BPH) and prostatitis. Nettle's beneficence in prostatitis is mainly probably due to its anti-inflammatory properties.

Recent research on nettle indicates that it interferes with or blocks the hormone-related chemical processes in the body that results in the development of BPH. Research also demonstrated that Nettle inhibits the enzyme required for the conversion of testosterone to dihydrotestosterone which results in condition called BPH. Nettle has significant effect on people suffering from BPH. Nettle root basically inhibits the growth of prostate cells and blocks pathways leading to the proliferation of prostatic cells. Hair loss (men and women) is basically due to excessive production of hormone dihydrotestosterone (DHT). Root extract of *Urtica dioica* basically prevents the conversion of testosterone to dihydrotestosterone. Hence it also aids in patients suffering from hair loss. [10] Its aerial parts have been largely used as medicine mainly used in treatment of urinary infections, stress, kidney stones. Hydro alcoholic extract of *Urtica dioica* significantly increases antioxidants levels and Superoxide Dismutase levels in patients with diabetes (Type 2). [11] Hydro-alcoholic extract of *Urtica dioica* significantly reduces high blood glucose levels, oxidative stress and depression. [12]

CHAPTER 3
DESIGN OF THE STUDY

3.1 CHRONIC STRESS

CHRONIC UNPREDICTABLE MILD STRESS

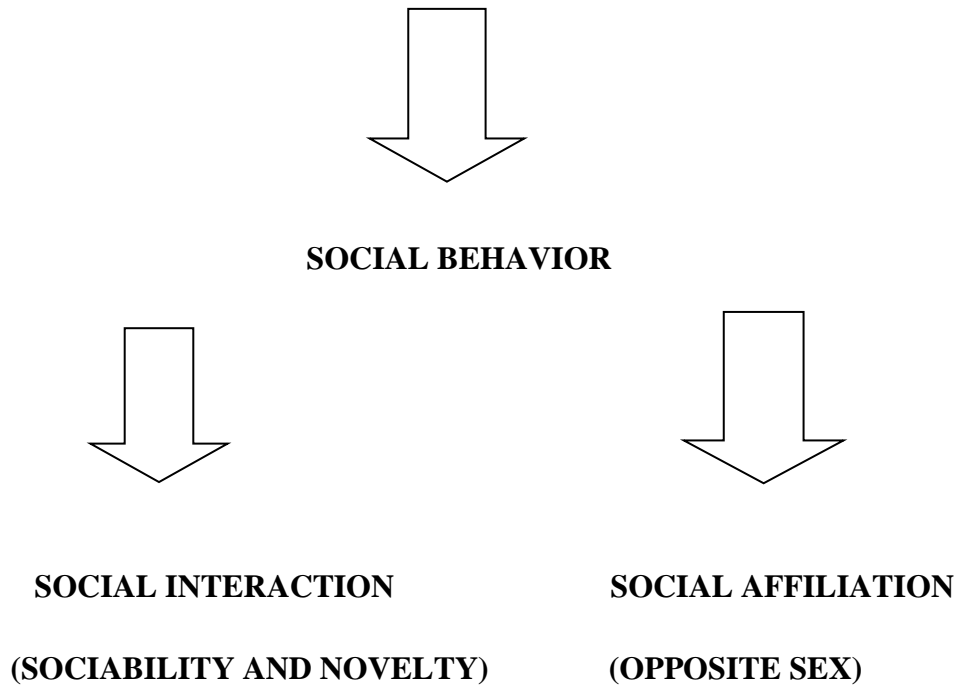


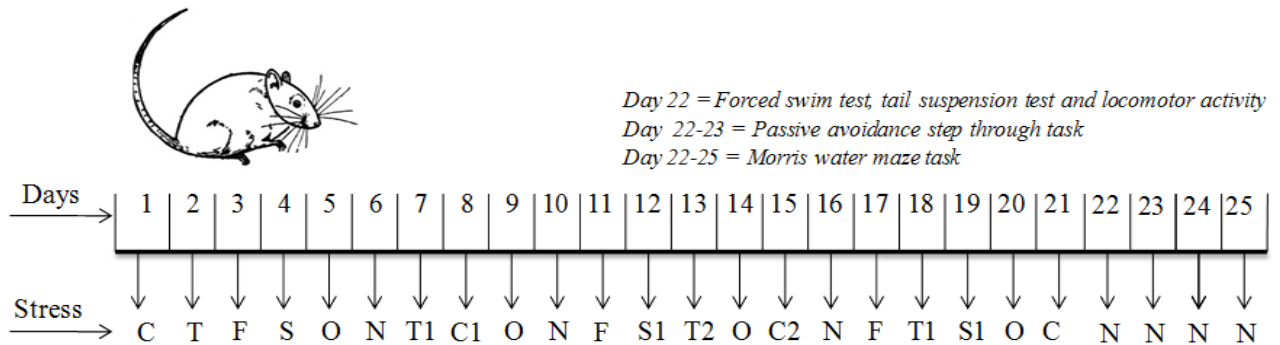
Figure 3.1 flow chart of chronic stress based study

EXPERIMENTAL DESIGN:

Acute stress is mild form of stress. Its duration is less. In Acute stress, the rodents are exposed to mild form of stress for few hours (1hr). Each animal is kept in restrainer for 1 hr. in this process the mice is not allowed to move. At the end, behavioral studies are conducted.

3.2 CUMS PROTOCOL DESIGN:

Exposure to the repetitive stress signals triggers the development of psychopathologies. Therefore CUMS model in mice is devised to study the effects of stress on social interaction. In CUMS the rodents are exposed to mild repetitive stress for 14 days such that individual stresses do not have long lasting effects on behavior of the rodents. Each animal faces the stress for 1 day and procedure is followed for full 14 days. The process was carried out in isolated rooms without any alterations from the environment. After each stress the animals were kept in recovery room followed by cleaning of the cages with fresh bedding. [13]



C — Cold swim (8 °C, 5 min); T — Tail pinch (1 min); F — Food and water deprivation (24 h); S — Swimming at room temperature (24±2 °C, 20 min); O — Overnight illumination; N — No stress; T1 — Tail pinch (1.5 min); C1 — Cold swim (10 °C, 5 min); S1 — Swimming at room temperature (24±2 °C, 15 min); T2 — Tail pinch (2 min); C2 — Cold swim (6 °C, 5 min). Starting from day 21 (60 min after drug administration), animals will be subjected to different behavioral, biochemical and histological tests. For biochemical investigation, mice will be sacrificed by decapitation and brains will be isolated and stored in phosphate buffer (0.1 M, pH 7.4) at -80 °C till further investigations.

Figure 3.4 CUMS protocol design

3.3 DRUG TREATMENT:

The administration of drug *Urtica dioica* (50mg/kg) was started from day 1-21. The behavioral analysis is done after 20 hr. of the last dosage so that acute effects of the drugs can be avoided. The drug treatment is followed till 21st day.

CHAPTER 4
MATERIALS AND METHOD

MATERIALS:

Stop watch, animal cages, data sheet, video camera, ethanol, tissue papers, mice models, animal feed, water bottles, and husk.

4.1 THREE CHAMBERED APPARATUS:

This is the apparatus which is used to study the sociability and preference of social novelty. The main principle of this test is based on the time spent by mouse in 3 compartments in less than 3 set of experimental sessions which includes the contact of subject mice to familiar followed by novel mice.

Sociability is determined by the time spent by the mice with conspecific compared to that with time in the empty compartment by itself. Preference for social novelty is determined by the time spent by the subject mice with the novel mice rather than the familiar mice.

METHOD:

We used Three chambered apparatus to study social ability and preference for social novelty. We have designed protocol as per guidelines. Whole set of protocol is divided under 3 set of sessions:

- Habituation
- Sociability
- Preference for social novelty

4.1.1 EQUIPMENT SETUP:

- The apparatus used is mainly Three chambered. Chamber is a rectangular box with sides made of Plexiglas having an open small section in the middle which allows easy movement of mice.
- The two identical wires like containment cups with metal wires are used to hold the mice and also prevent direct contact of the subject mice with that of object and novel mice.
- Reading should be taken twice a day.
- Before starting everything should be wiped with 70% ethanol.
- The person who is recording the data should be 2 meters away from the apparatus.
- The apparatus used is mainly Three chambered. Chamber is a rectangular box with sides made of
- Plexiglas having an open small section in the middle which allows easy movement of mice. The two identical wires like containment cups with metal wires are used to hold the mice and also prevent direct contact of the subject mice with that of object and novel mice. Readings should be taken twice a day.
- Before starting everything should be wiped with 70% ethanol. The person who is recording the data should be 2 meters away from the apparatus.

4.1.2 ANIMAL PREPARATION:

- Prior to the test, all the mice should be placed in separate cages so that any biasness in the results can be avoided.
- Cages should be kept in animal house with taking care of the appropriate room conditions like temperature etc.
- Females should not be kept with the males.

4.1.3 HABITUATION:

- Close the openings connecting the right and left chambers from the central chamber using Plexiglas.
- Now keep the subject mice in the middle compartment for 5 minutes without disturbing.
- Keep 2 meters away from the compartment.

4.2 SOCIABILITY:

- Now after the habituation, place the object mice in right compartment covered with containment cups.
- Place any novel object for e.g. empty containment cup as a novel object in the left compartment.
- Open all the walls connecting the sides of the chamber.
- Immediately began recording the following parameters (Duration, time spent by the subject mice in which the object mice compartment as well as the novel object compartment, direct contact of subject mice with the object mice, duration of the contact, Number of entries in the each compartment).
- Duration of the whole process is 10 min.

4.3 PREFERENCE FOR SOCIAL NOVELTY:

- Remove the novel object and place the novel mice in containment cups in other chamber and monitor the parameters described above.

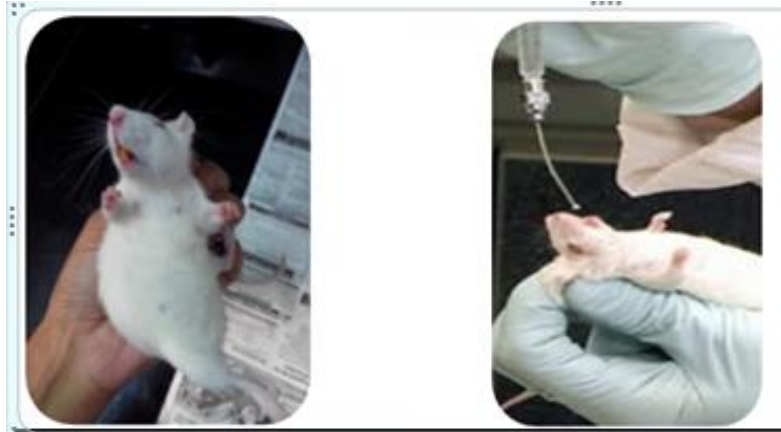
Duration is 10 min. [14]

CHAPTER 5

RESULTS

RESULTS

5.1 ANIMAL HANDLING AND DOSING



TAGGING

DOSING

Figure 5.1 Animal handling and dosing

5.2 THREE CHAMBERED APPARATUS (Fig 5.2.1)



Figure 5.2.1 Three Chambered Apparatus

Three chambered apparatus as explained earlier is used for behavioral testing's. It has 3 chambers (1 in the middle and 2 adjacent to it). There are separate openings for all compartments. The subject mice are kept in the middle compartment and proper procedure is followed. At a single point of time, only subject mice are allowed to move. Rest all mice are kept in cages in their respective compartments. This compartment is kept under closed system.

5.3 SOCIABILITY (Fig 5.3)

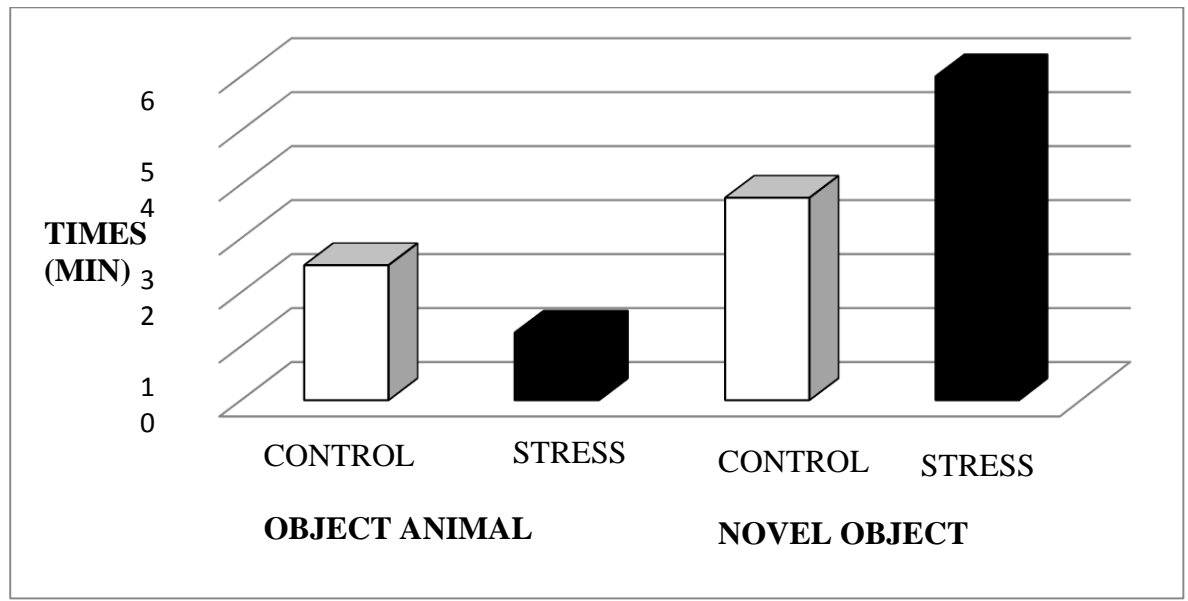


Figure 5.3 Sociability Chart.

In (Fig 5.3) we can see that chronic stress has significantly altered the social behavior of the mice. Subject (stressed) mice spends more time with the novel object as compared to control (normal) mice.

5.4 SOCIABILITY (*Urtica dioica* Treatment) (Fig 5.4)

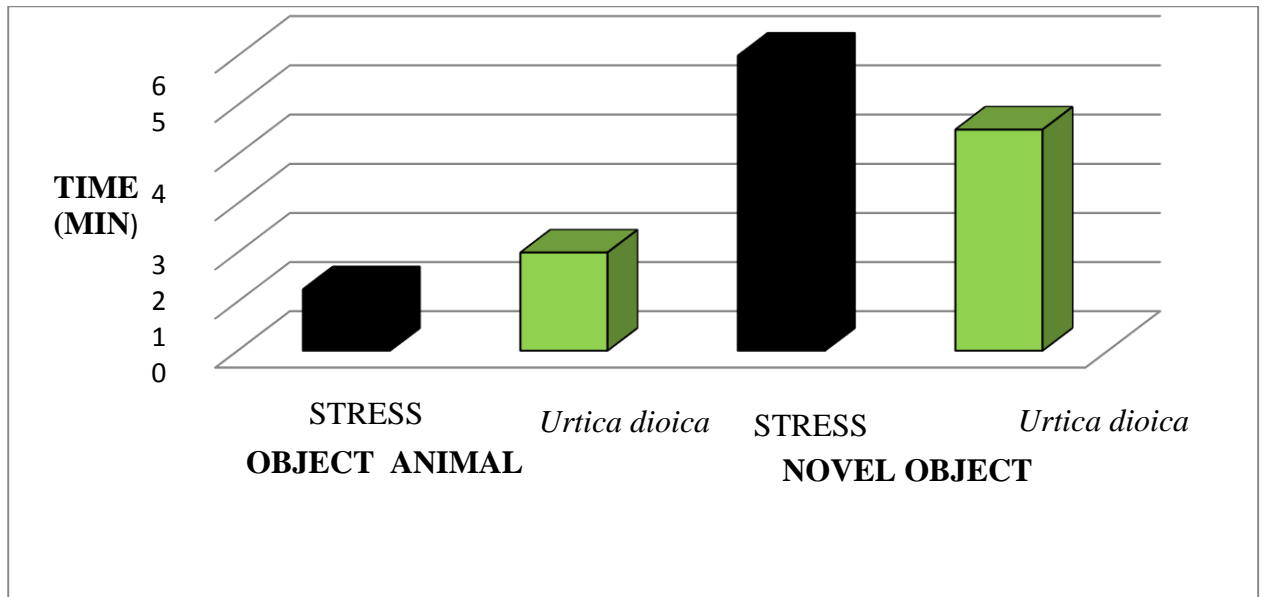


Figure 5.4 Sociability (U.D. treatment) chart.

In (Fig 5.4) we can see that after *Urtica dioica* treatment, the subject (stressed) mice has increased its activity with the object mice as compared to that of novel object.

5.5 PREFERENCE FOR SOCIAL NOVELTY (Fig 5.5)

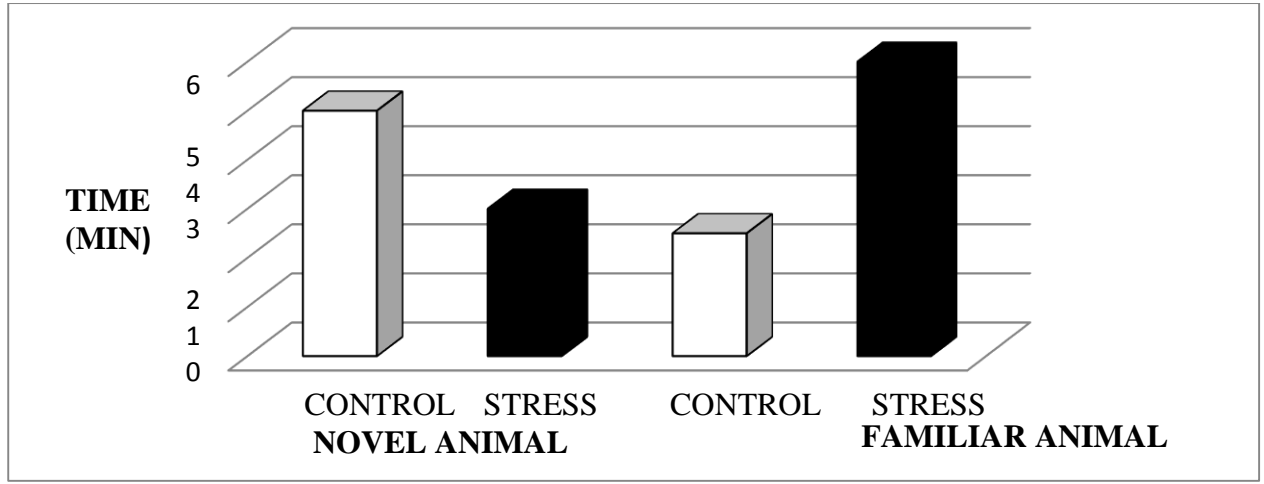


Figure 5.5 Preference for social novelty chart.

In (Fig 5.5) we can see that subject mice spend more time with the familiar mice as compared to the novel mice. This concludes that subject mice is stressed.

5.6 PREFERENCE FOR SOCIAL NOVELTY (*Urtica dioica* Treatment) (Fig 5.6)

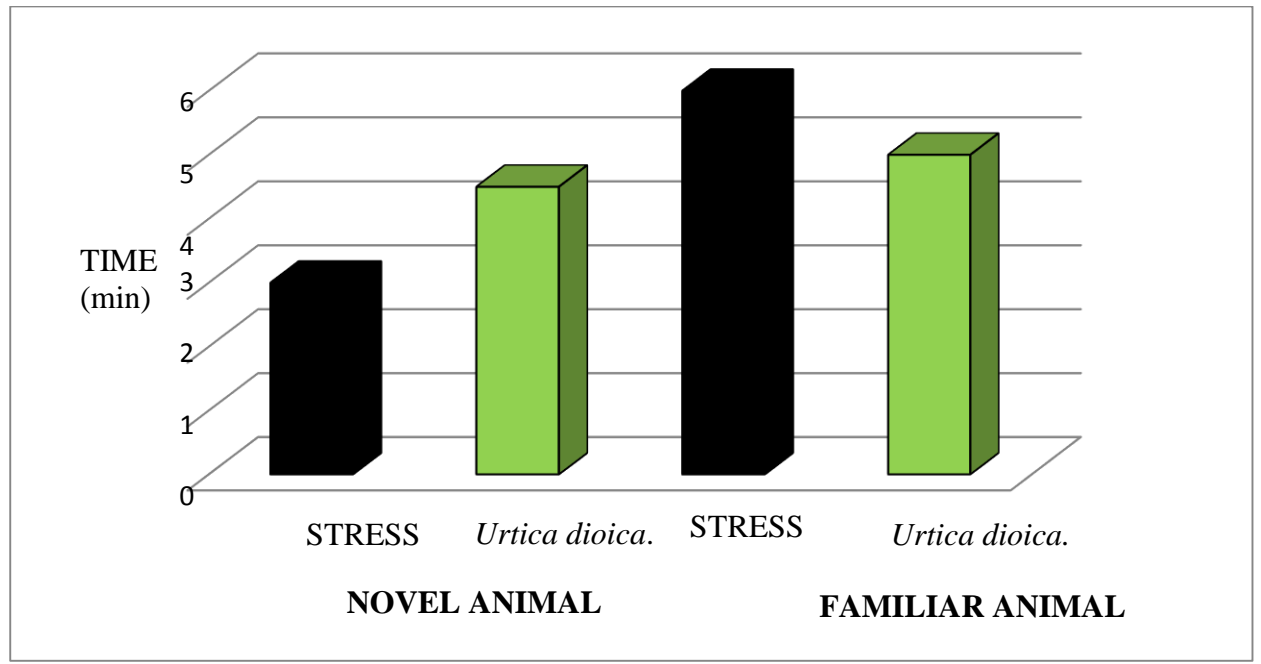


Figure 5.6 Preference for social novelty (*Urtica dioica* treatment) chart.

In (Fig 5.6) we can see that *Urtica dioica* treatment has decreased the activity of subject (stressed) mice with familiar mice. Its activity towards novel mice has been increased.

So finally we can say that chronic stress significantly alters the behavior of the mice. Our studies basically proved that chronic stress affects both sociability as well as preference for social novelty. Time spent by the subject mice with novel object (in sociability testing) and familiar mice (in testing for preference for social novelty) has been increased which indicates that the mice is stressed. So extract of *Urtica dioica* is used to see the

changes in social behavior due to chronic stress. After the treatment time spent by subject mice with novel object (in sociability testing) and familiar mice (in testing for preference for social novelty) has been decreased which indicates that *Urtica dioica* has significant affect on the social behavior of the mice.

CHAPTER 6
CONCLUSION

CONCLUSION

Stress not only deteriorates the health but also builds up psychological pressure which causes the changes in the social behavior of the person. Studies proved that acute stress is not bad in all conditions. In competitive conditions this stress builds our confidence and work performance. On the other hand chronic stress is very unhealthy for body and mind. It disturbs the body homeostasis. Stress is not only linked to particular part of life but it is found in all sections of our life. Chronic stress causes several problems like CAD, atherosclerosis. So management of both kind of stress is very important. Stress management helps a person relieving his pain and stays healthier. In our study we have used hydro-alcoholic extract of *Urtica dioica* and check efficacy in improving the stress induced changes in Social behavior results obtained from our research are motivating. It has wide applications on which research is going on. Our idea is simply based on the behavioral analysis of the mice in a Three chambered apparatus. In future the scope of our study increases as it not only has anti-stress activity but has other valuable properties and uses.

CHAPTER 7
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