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Investigation Of Rhinacanthus Nasutus For Cns Activity Using Albino Mice

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Abstract: *The aim of this present study is to investigate central nervous system activity of the ethyl acetate and ethanol extract of leaves of Rhinacanthus Nasutus in dose variation. The work reached the acute toxicity studies plant and its action on the central nervous system, because no data in the literature have been found of pharmacological activity of this plant in the central nervous system. The leaves were extracted with ethyl acetate and ethanol and investigated for its central nervous system activity of Albino mice in Rota-rod and Actophotometer at the dose level of 200 and 400 mg/kg. The extract exhibited significant central nervous system activity. This study established central nervous system activity in plant leaves.*
Key words: *Rhinacanthus Nasutus, CNS Activity, Irwin's Test, actophotometer, Rota-rod test*

INTRODUCTION: Medicinal plants have enjoyed use in virtually all cultures as sources of medicines. The history of the use of medicinal plants for their therapeutic purposes probably dates back to the origin of man. Neurodegenerative diseases including Alzheimer (AD) and Parkinson (PD), represent a major threat to human health and have a huge impact on society and economy [1]. Worldwide, it is estimated that nearly 45 million people have AD and 8 million live with PD. These age-related disorders are becoming increasingly prevalent, in part because the elderly population has increased in recent years, but also because of the evolution of lifestyles. Indeed, lifestyle greatly affects vascular functioning, and BBB dysfunction or loss of integrity have been reported in several of these NDs [2]. Other neurodegenerative diseases are the lipid storage diseases (LSDs) regrouping more than 50 types of inherited metabolic disorders in which harmful amounts of lipids accumulate in various tissues. Because the brain is the most cholesterol-rich organ of the body, some LSDs but not all, have devastating effects on neuron survival, development, and functionalit) [3]. According to the National Organization for Rare Disorders (NORD), LSDs are believed to have an estimated frequency of about one in every 5000 live births. Currently, there is no cure for any of these neurodegenerative diseases. Memantine and a combination of memantine and donepezil are approved for treatment of moderate to severe AD whereas L-DOPA is given to PD patients, but these treatments only affect the neurotransmitter levels and do not slow down or reverse the neurodegenerative process [4]. The difficulty to find a cure for these NDs is at least partly due to the presence of the BBB that impedes the distribution of promising drug candidates within the central nervous system [5].

The nervous system is a complex, sophisticated system that regulates and coordinates body activities. According to WHO report, mental or behavioral disorders amounts to 12.3% of global burden of disease and will rise to 15% by 2020. Depressive and anxiety disorders are among the most common mental health conditions around the world with a lifetime prevalence of 16% and 10% respectively, which are also associated with substantial comorbidity and mortality. Recent studies have reported that anxiety and depression may occur together as co-morbid conditions, which are often referred as common cold of mental disorders. Anxiety may also predispose depression or vice versa or symptoms of both these disorders may be external manifestations of any underlying cause [6].

The central nervous system (CNS) is part of the nervous system (NS) made up of mostly the spinal cord and brain. The central nervous system incorporates received information, then influences and coordinates the activities of all body parts. The central nervous system also includes the optic nerve, the retina, as well as olfactory epithelium and the olfactory nerves as part of the central nervous system, directly synapsing on the tissues of brain with no intermediate ganglia [7]. The discovery of drugs for the treatment of CNS disorders has faced some setbacks. Nervous system (NS) is vital to human body. In spite of the remarkable progress in knowledge of the CNS functions and structure, discovery of novel drugs along with their development for several central nervous system disorders have been challenging. Consequently, drug discoveries for CNS treatment and related development program have been

experienced eliminations and cutbacks for decades. Complementary and alternative medicine has played a significant role in filling these gaps. CAM has guided the development of some drugs for the treatment of central nervous system (CNS) disorders. This often results in novel or enhanced treatment for CNS disease. In general, it has been noted that CNS drugs record higher rates of failure than their counterparts, clinically and preclinically; in certain areas like neurodegenerative diseases, there has been 100% rate of clinical failures for disease-modifying treatment. Alternative medicine has played a major role to fill this gap in many parts of the world [8]. The R. nasutus (Nagamalle) is cultivated particularly as a medicinal plant has been used in treatments and preventions of diverse diseases as folklore medicines. Different parts of R. nasutus have used in traditional medicine for the treatment in diseases such as eczema, pulmonary tuberculosis, herpes, hepatitis, diabetes, hypertension and several skin diseases. Studies on phytochemical of R. nasutus species have demonstrated flavonoids, steroids, terpenoids, anthraquinones, lignans and especially naphthoquinone analogues as major constituents Naphthoquinones

MATERIAL AND METHODS

CNS activity: Ethyl acetate and ethanol extract of both plants were found to contain more phytochemicals than petroleum ether and aqueous extract. Ethyl acetate and ethanol extract of both plants were found to possess antioxidant activity. Hence Ethyl acetate and ethanol extract of both plants were selected for further in vivo CNS activity.

Animals: Adult albino mice (25–35 g) were used for in vivo activity. The animals were housed in cages at 25 ± 2°C, and relative humidity (50 ± 5%) with 12 h light, and 12 h dark cycle. All the animals were acclimatized to laboratory environment for a week before the experiment. They were provided with free access to food and water ad libitum. The animals were cared and used in accordance with the CPCSEA guidelines and experimental protocols approved by institutional animal ethics committee.

Acute Toxicity Studies: The objective of this Acute toxicity Study of Rhinacanthus Nasutus leaves leaves extract to assess the toxicological profile of the test drug when given intraperitoneally once (single dose) to the test system and monitor the vital signs for 14 days. Plants extract administered in the dose of 10mg/kg, 100mg/kg, 500mg/kg and 2000 mg/kg and observed for 14 days. Animals were observed for autonomic or behavioural response during this period. The body weight was also observed. Mortality was observed up to 14 days. In acute oral toxicity study, extracts at the dose of 2000 mg/kg neither showed visible signs of toxicity nor mortality during the study and observations and measurements did not indicate any evidences of substance-related toxicity.

Experimental protocol: The extracts and standard drugs were suspended in distilled water using tween 20 as suspending agent for experimental purpose. The animals either sex were dividing into eleven groups for extracts and ten groups for fractions for biological evaluation of CNS activity. Each group consists of four animals. The following dosage regimens with respective groups were given for extracts and fractions throughout the study

Table 4.1: Experimental protocol for CNS activity of *Rhinacanthus Nasutus leaves*

Normal Control	received 1% tween solution (5ml/kg)
Positive Control	Standard drugs vary according to the model
EARN 200	Ethyl acetate extract of Rhinacanthus Nasutus leaves at dose of 200 mg/kg
EARN 400	Ethyl acetate extract of Rhinacanthus Nasutus leaves at dose of 400 mg/kg
EORN 200	Ethanol extract of Rhinacanthus Nasutus leaves at dose of 200 mg/kg
EORN 400	Ethanol extract of Rhinacanthus Nasutus leaves at dose of 400 mg/kg

EARN: Ethyl acetate extract of Rhinacanthus Nasutus leaves
EORN: Ethanol extract of Rhinacanthus Nasutus leaves

Evaluation of psychotropic and neurotropic activities

Irwin's Test: Effects of Rhinacanthus Nasutus leaves extract on gross behaviour and physiological function were investigated using the original procedure described by Irwin (Irwin 1968). The animals were subsequently observed for changes in behaviour and physiological function.

Effect on locomotor activity on actophotometer: The spontaneous locomotor activity of each mouse was recorded individually for 10 min using actophotometer, which enables movement of the animal across a light beam to be recorded as a locomotion count. This test can demonstrate a CNS depressant or stimulant activity profile. The animals were allowed to adapt to the new environment for at least 5 min and then the locomotor activity was counted. The test drugs and standard drugs were administered 30 min before the assessment of locomotor activity. Counts were then taken after 30, 60, 90 and 120 min.

Rotarod Test (Effect of extract of on motor coordination): The rotarod consisted of a rotating rod (diameter: 3 cm) rotating at a constant speed of 25 revs/s with individual compartments for each mouse such that each mouse is physically separated from the other mice. Mice were trained for three days to stay on the rotating rod for 180 s. On the test day (24 h after the last training session), the animals received intraperitoneally extracts, diazepam (8 mg kg−1), d-tubocurarine (0.01 mg kg−1), or distilled water (10 ml kg−1 p.o.) and placed on the rotating rod to walk. Latency to fall off the rotating rod within a maximum cut-off time of 180 s was determined at 0, 1, 1.5, 2 h, and 3 h after drug administration. Animals remaining on the rod for 3 minutes or more in three successive trials were selected for the experiment. The drugs both standard and test along with control groups were administered before 30 minutes of the experiment. Then the animals were placed on the rod. The time taken for the mice to fall from the rotating rod was noted

Pentobarbitone-Induced Sleeping Time: The effect of ethyl acetate and ethanol extracts of Rhinacanthus Nasutus leaves extract (EARN and EORN); on pentobarbitone-induced sleeping time was investigated in the pentobarbitone interaction test. Animals received extracts (200 and 400 mg kg−1, p.o.), diazepam (8 mg kg−1), caffeine (16 mg kg−1), or distilled water (10 ml kg−1, p.o.) intraperitoneally. Sodium pentobarbitone (50 mg kg−1) was administered intraperitoneally one hour after the respective drug treatments. Latency to sleep (time between pentobarbitone injection and loss of righting reflex) and duration of sleep (time between loss of and regaining of righting reflex) were recorded.

Effect of plants extract on exploratory behaviour (head dip test): The first two groups (Group-I and Group-II) receive 1% tween solution (5ml/kg) as vehicle control and diazepam (4mg/kg) as standard drug respectively. The animals were placed individually on a wooden board with 16 evenly spaced holes. The number of times they dipped their heads in to the holes during 3 minutes was counted. The animals were divided in different groups for the test. The animals were first administered after thirty minutes the test was performed.

Y-maze test: Y-maze task used to measure the spatial working through the spontaneous alternation of behaviour. The maze is made of black painted wood. The first two groups (Group-I and Group-II) receive 1% tween solution (5ml/kg) as vehicle control and diazepam (4mg/kg) as standard drug respectively. The remaining groups of animals were administered the extracts in different dose levels, intraperitoneally. The rats were placed individually in a symmetrical Y-shaped runway (33 × 38 × 13cm) for 3 min and the number of times an animal entered in the arm of the maze with all 4ft (an 'entry') were counted. The series of arm entries, including possible returns into the same arm, are recorded visually. This test was performed in the groups of animals at 30, 60, 90 and 120 min after administration of either Solvent or test and standard compounds.

RESULT AND DISCUSSION

Procurement and Authentication of Plant material: A systematic approach is necessary in pharmacognostic study, which helps in confirmation and determination of identity, purity and quality of a crude drug. The plant specimen Rhinacanthus Nasutus were collected and authenticated by botanist.

CNS activity: Ethyl acetate and ethanol extract of were found to contain more phytochemicals than petroleum etherand aqueous extract. Ethyl acetate and ethanol extract of both plants were found to possess antioxidant activity. Hence Ethyl acetate and ethanol extract of both plants were selected for further in vivo CNS activity.

Acute Toxicity Studies: Plants extract administered in the dose of 10mg/kg, 100mg/kg, 500mg/kg and 2000 mg/kg and observed for 14 days. Animals did not produce any significant changes in the autonomic

or behavioural response during the observation period. The body weight was not significantly altered. No mortality was observed up to 14 days of monitoring. In acute oral toxicity study, extracts at the dose of 2000 mg/kg neither showed visible signs of toxicity nor mortality during the study and observations and measurements did not indicate any evidences of substance-related toxicity. The no-observed adverse effect level was detected at the dose of 2000 mg/kg. So, the extracts were safe for administered up to the dose of 2000 mg/kg.

Gross behavioural study (Irwin's Test): Effects of Rhinacanthus Nasutus extract on gross behaviour and physiological function were investigated using the original procedure described by Irwin (Irwin 1968). The animals were under observation for behavioural changes if any, at 30 minutes interval in the first hour and at one-hour intervals for next 4 h for different parameters. The awareness and alertness were recorded by visual measure of the animal's response when placed in different positions and its ability to orient itself without bumps or falls. The normal behaviour at resting position was scored as 0. Similarly little activity (+), moderate flexibility (++), strong response (+++) and abnormal restlessness (++++) were recorded. The spontaneous activity of mice was recorded by placing the animal in a bell jar. It usually shows a moderate degree of inquisitive behaviour. Less or moderate activity was scored as ++ and strong activity as +++. If there is slight or little motion, the score was + while the animal sleeps, the score was -. Excessive or very strong inquisitive activity like constant walking or running was scored as ++++. The touch response was recorded by touching the mice with a pencil or forceps at a various part of the body (i.e. on the side of the neck, abdomen and groin). The pain response was graded when a small artery clamp was attached to the base of the tail, and response was noted. Albino mice normally utter no sound, so that vocalization may indicate noxious stimulus.

Table 1: Effect of *Rhinacanthus Nasutus* leaves on general behavioural profiles

Behaviour type	Normal Control	chlorpromazine	EARN 200 mg/kg	EARN 400 mg/kg	EORN 200 mg/kg	EORN 400 mg/kg
Spontaneous activity	-	++++	++	+++	+++	++++
Alertness	-	+++	+	+	++	++
Awareness	-	+++	+	++	++	+++
Sound response	-	++++	++	+++	+++	++++
Touch response	-	++++	++	+++	+++	++++
Pain response	-	++++	++	++	++	+++
Righting reflex	-	++++	++	+++	+++	++++
Pinna reflex	-	++++	++	+++	+++	++++
Grip strength	-	++++	++	+++	+++	++++

(-) no effect, (+) slight depression (++) moderate depression (+++) strong depression

Effect of the Rhinacanthus Nasutus leaves extract on general behavioural profiles were observed using the original procedure described by Irwin. The experimental on general behavioral profile indicate that the ethyl acetate and ethanol extract of Rhinacanthus Nasutus influence the general behavioral profiles. The ethyl acetate and ethanol extracts affect spontaneous activity, righting reflex, pinna reflex, grip strength and pain responses at the dose of 200 and 400 mg/kg and produced moderate to strong depression relating to awareness and alertness. Ethanol extract of Rhinacanthus Nasutus causes significant depression of all these responses compared to standard. The results indicate that the Rhinacanthus Nasutus extracts influence general behavioural profile. Reduction of pinna reflex may be due to blocking synapses of the afferent pathway.

Effect on locomotor activity on actophotometer

This test can demonstrate a CNS depressant or stimulant activity profile. The result indicated that the test extracts ethyl acetate and ethanol extracts at 200 and 400 mg/ kg for Rhinacanthus Nasutus leaves and standard decreased the locomotor activity in comparison to solvent control group. Thus, the results

of the present investigation indicate that the Rhinacanthus Nasutus leaves ethanol extracts dose dependently depresses the central nervous system, induced decrease in locomotor activity.

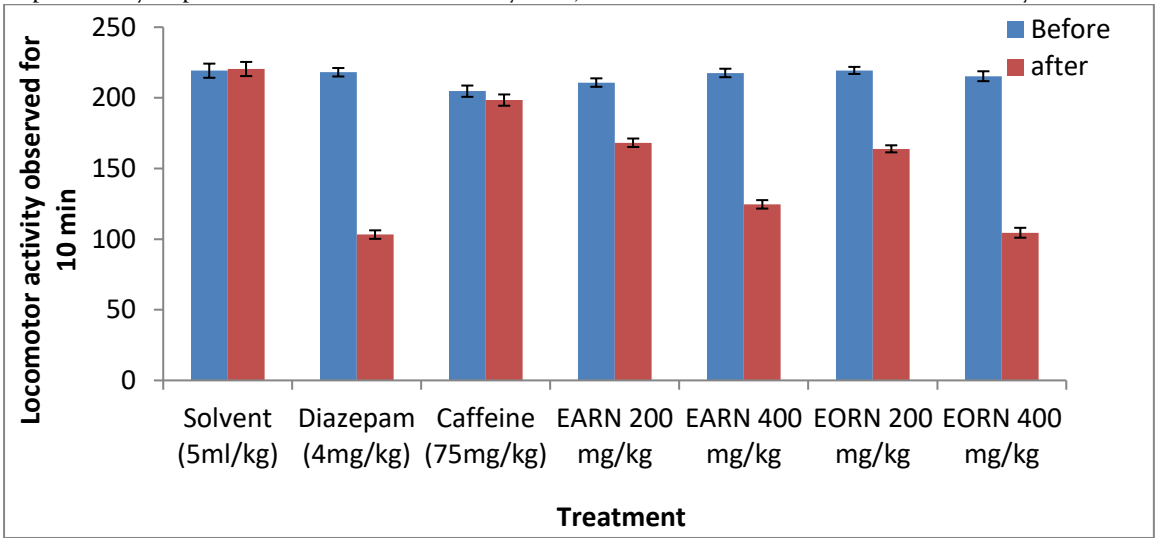

Figure 1: Effect of Rhinacanthus Nasutus leaves extracts on locomotor activity

Alertness and sedative action were considered through locomotor activity. Increase in locomotor activity is considered as an increase in alertness and decrease in locomotor activity indicated sedative activity. Gammaaminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Sedative-hypnotic drugs elucidate their action through GABA; therefore it is possible that methanol extracts of Rhinacanthus Nasutus leaves may acts by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain.

Effect of extract of on motor coordination in mice (Rotarod Test): This test was performed to elucidate the effect of ethyl acetate and ethanol extracts of Rhinacanthus Nasutus (EARN and EORN) on neuromuscular coordination. Animals remaining on the rod for 3 minutes or more in three successive trials were selected for the experiment. The drugs both standard and test along with control groups were administered before 30 minutes of the experiment. Then the animals were placed on the rod. The time taken for the mice to fall from the rotating rod was noted. This test was performed to elucidate the effect of ethyl acetate and ethanol extracts of Rhinacanthus nasutus leaves (EARN and EORN).

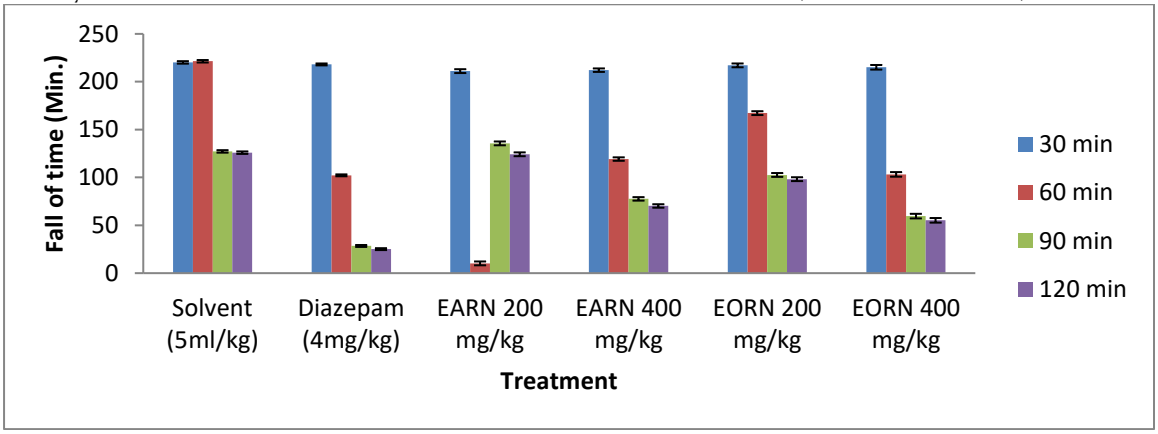

Figure 2: Effect of Rhinacanthus Nasutus leaves extracts on motor coordination in mice

The result showed that ethanol extracts of Rhinacanthus Nasutus at 200 mg/ kg and standard drug reduced the fall off time in comparison to solvent control group in different time intervals i.e. 30, 60, 90 and 120 min. The ethanol extracts of Rhinacanthus Nasutus at 400 mg/ kg also produced significant activity ($p< 0.05$). Since, ethyl acetate extract of Rhinacanthus Nasutus produced significant ($p<0.01$) effect on the reduction in the fall off time as comparable to solvent control. The study also revealed that the extract produces their effect in a dose-dependent manner for reduction in the fall off time.

Pentobarbitone-Induced Sleeping Time: The effect of extracts of Rhinacanthus Nasutus leaves (EARN and EORN) on pentobarbitone-induced sleeping time was investigated in the pentobarbitone interaction

test. Latency to sleep (time between pentobarbitone injection and loss of righting reflex) and duration of sleep (time between loss of and regaining of righting reflex) were recorded. The result showed that ethyl acetate and ethanol extracts of Rhinacanthus Nasutus (EARN and EORN) at 200 and 400 mg/ kg and standard drug increase the duration of sleeping time in comparison to solvent control group.

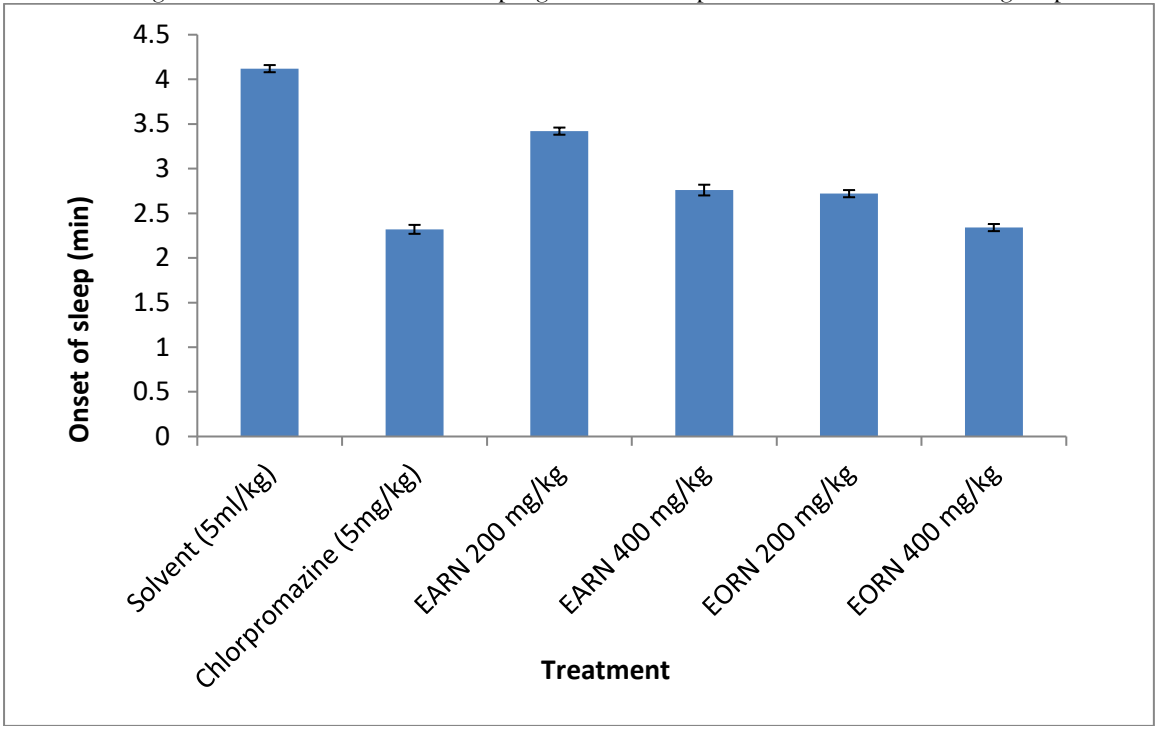

Figure 3: Effect of Rhinacanthus Nasutus leaves extracts on phenobarbitone induced sleeping time in mice

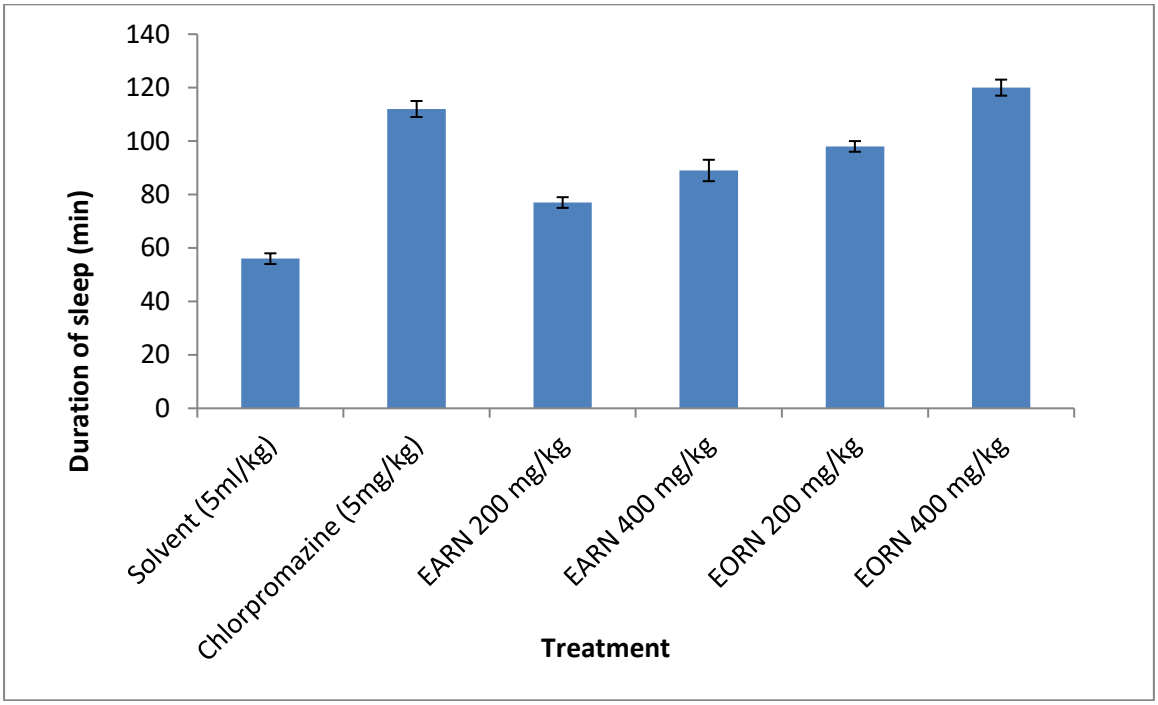

Figure 4: Effect of Rhinacanthus Nasutus leaves extracts on phenobarbitone induced sleeping time in mice

Effect of plants extract on exploratory behaviour (head dip test): The first two groups receive 1% tween solution (5ml/kg) as vehicle control and diazepam (4mg/kg) as standard drug respectively. The animals were placed individually on a wooden board with 16 evenly spaced holes. The number of times they dipped their heads in to the holes during 3 minutes was counted. The animals were divided in different groups for the test. The animals were first administered after thirty minutes the test was performed.

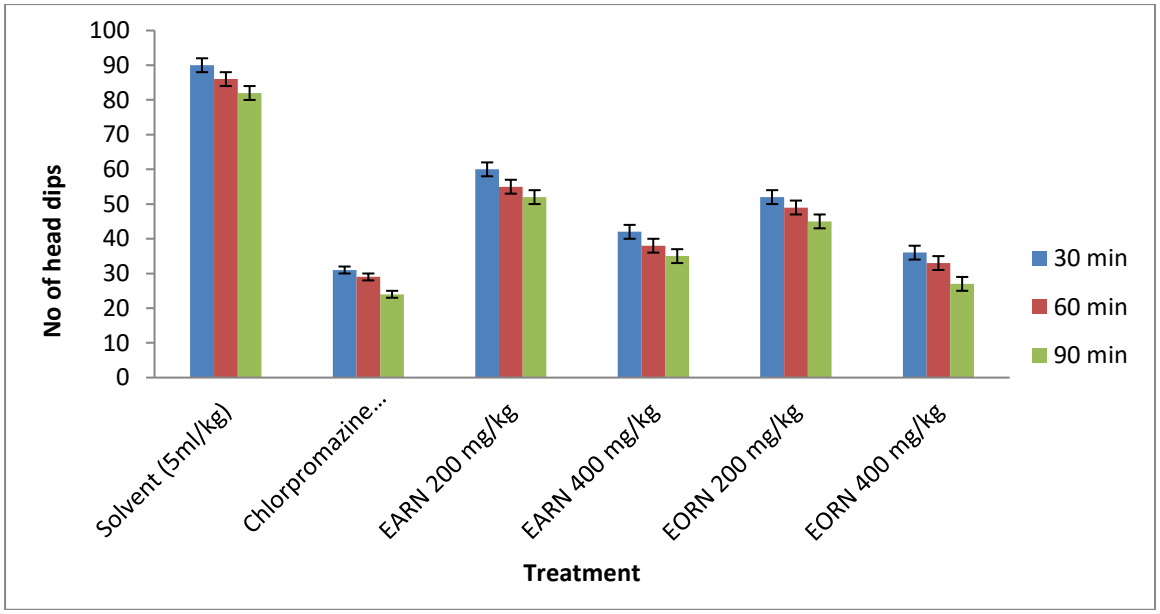

Figure 5: Effect of Rhinacanthus Nasutus leaves extracts on exploratory behavior (head dip test) in mice

The result of Head dip test for Rhinacanthus nasutus leaves extracts showed that the Rhinacanthus nasutus leaves (EARN and EORN) and standard drug decreased the number of head dips in comparison to solvent control group at 5 minutes interval. Ethanol extract of Rhinacanthus nasutus leaves produced significant effect ($p<0.05$) at 400 mg/kg.

Y-maze test: Y-maze task used to measure the spatial working through the spontaneous alternation of behaviour.

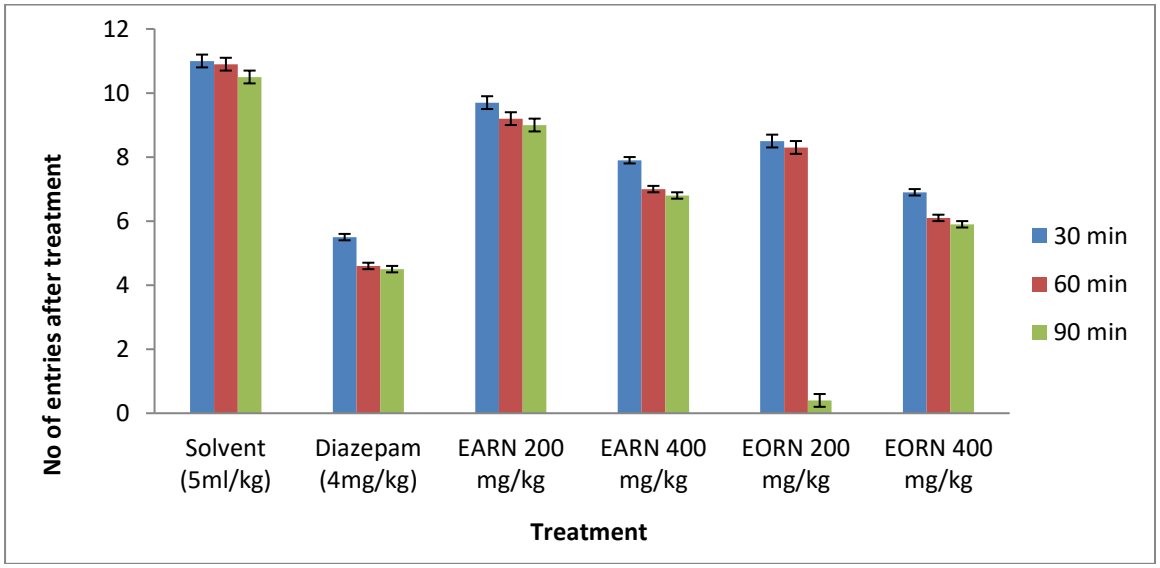

Figure 6: Effect of Rhinacanthus Nasutus leaves extracts on Y-maze test

The result showed that the ethanol extract of Rhinacanthus nasutus leaves at doses 200 and 400 mg/kg increase the number of entries in open arm and also time spent in open arm when compared to the vehicle control. The time spent and numbers of entries are also comparable with standard drug except all the doses of ethyl acetate extract of Rhinacanthus nasutus leaves. Ethanol extract, of Rhinacanthus nasutus leaves produces effect in dose dependent manner.

CONCLUSION:

Effect of the Rhinacanthus Nasutus leaves extract on general behavioural profiles were observed using the original procedure described by Irwin. The experimental on general behavioral profile indicate that the ethyl acetate and ethanol extract of Rhinacanthus Nasutus influence the general behavioral profiles. The

ethyl acetate and ethanol extracts affect spontaneous activity, righting reflex, pinna reflex, grip strength and pain responses at the dose of 200 and 400 mg/kg and produced moderate to strong depression relating to awareness and alertness. Ethanol extract of Rhinacanthus Nasutus causes significant depression of all these responses compared to standard. The results indicate that the methanol and aqueous extracts influence general behavioural profile. Reduction of pinna reflex may be due to blocking synapses of the afferent pathway. This test can demonstrate a CNS depressant or stimulant activity profile. The leaves were extracted with ethyl acetate and ethanol and investigated for its central nervous system activity of Albino mice in Rota-rod and Actophotometer at the dose level of 200 and 400 mg/kg. The extract exhibited significant central nervous system activity. This study established central nervous system activity in plant leaves.

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