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Original Article

Dose related CNS acting potential of *Tamarindus Indica*

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ABSTRACT

Mental disorders tend to co-exist together and anxiety is likely to co-morbid with depression. The aim of this study is to discover a safe and natural herbal source of remedy with minimum side effects in the form of *Tamarindus indica.* As rodents are neuro-anatomically and neuro-pharmacologically parallel to human, so *Tamarindus indica* was administered orally in 3 different doses of 50mg/kg, 75mg/kg and 100mg/kg to 3 different groups of mice having equal sex distribution and n=7 for 21 days. The CNS acting potential evaluated by behavioral responses, head dip method, cage crossings and force induced swimming test. The results revealed that *Tamarindus indica* is a potent anxiolytic agent even at low dose of 50mg/kg without sedative effects but showing low mood and week muscle tone whereas dose dependent antidepressant activity was also seen which is prominent at the high dose of 100mg/kg showing CNS alertness, elevation of mood, increase in learning behavior and overall improved performance of mice.

Keywords: Mental disorders; CNS; anxiolytic; antidepressant; herbal medicine; *Tamarindus indica*; mice; head dip method; forced induced swimming method.

Abbreviations: WHO: (world health organization), OECD: (organization for economic and cultural development), TI: (*Tamarindus indica*), TIPE: (Tamarindus indica pulp extract), CNS: (central nervous system), GAD: (Generalized anxiety disorders), BDZs: (benzodiazepines), PCSIR: (Pakistan Council for Scientific and Industrial Research)

INTRODUCTION

The prevalence rate of mental disorder since 1990s is reported as the fastest prevalence rate than any other disease. Although the prevalence of mental disorders is high in developed countries as compare to developing countries but the treatment rate is far better there, than developing countries where the treatment has been strongly neglected. The developed countries have 35.5 % to 50.3% of seriously untreated cases. This situation is even worse in less developed courtiers, where 76.3% to 85.4% of serious cases receive no treatment. The evidence-based practices of developed world are not realistic to the questions of developing world's constraints due to socio-cultural factors and health systems variability [1, 2].

Anxiety is a normal human body phenomenon provided to adjust in uncomfortable condition. The autonomic reflexes of anxiety improve routine life but if anxiety is causing high levels of autonomic arousal, wrong cognitions and fear perceptions, it results in multiple distresses and problems in social and/or routine life and hence anxiety becomes extremely vivid and weakening disorder at the same time [3]. Anxiety affects one eight of the total population and became a very important area of research interest in psychopharmacology [4]. Worldwide epidemiological studies suggested that this is a silent epidemic of the 21st century [5]. Anxiety could appear as a symptom of any other psychiatric disorder or co-exist with them and this results as an increase in help seeking behavior. For example, GAD increases the chances of depression by an odds ratio of 28.9 [6]. Evidence based research has proven that environment has a significant role in genetic outlook of anxiety and depression. As a result, there is continuous state of stress at cellular level. These stressed cells have to survive for their normal function and if this left untreated this may lead to psychiatric complications [7]. Literature search reported that BDZs are the most frequently used psychoactive

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compounds [8] but certain side effects outweigh these benefits such as sedation, muscle relaxation, anterograde amnesia, ataxia and physical dependence [9]. In the same way abrupt cessation or missing doses of BDZs cause withdrawal symptoms including confusion, anxiety, agitation, restlessness, insomnia, tension and rarely seizures. These unsolved problems with synthetic anxiolytic drugs have urged people to introduce natural and herbal therapies [10]. Adverse effects of BDZs have also pulled forward many pharmaceutical companies to find plant-derived medications with more specific anxiolytic effects including *Valeriana officinalis, Matricaria recutita, Passiflora caerulea, Salvia guaranitica, Tilia tomentosa, Tilia europaea, Stachys lavandulifolia, Echium ameonum* and *Salvia reuterana* [11]. The Physicians of Europe and Asia are more attentive to herbal medications especially to find out CNS-acting alternatives to avoid unwanted side effects [4]. The herbal sources are effective add-on therapy to minimize the reported side effects and giving the desired CNS effects [12]. This entire situation supports the development of new pharmacological agents for anxiety and depression from plant sources [13]. In these circumstances, an enormous work is in process on BDZs receptors so that we can have non-sedative anxio-selective agents [14]. There are many patients who failed to respond against standard anxiety and epilepsy treatments; therefore "Drug discovery" is also essential to satisfy ethno-pharmacological approaches [4].

The ***Tamarind indica*** is a tree-type plant. It belongs to the Fabaceae or Leguminosae family and the sub family is Caesalpinioideae. *Tamarind indica* plant was cultivated in 400 B.C in Egypt. The Ayurvedic system claimed its medicinal value around 600 AD. During 16th Century Tamarind was introduced into the America but now it has been familiar all over the world [15]. From this plant, mostly the pulp or the fruit is used called, Pulpa tamarindorum. It contains many organic acids, amino acids, proteins and aromatic agents. With these nutrients, this plant is supported as a helper, highly important, medicinal plant for mankind [12]. This necessitates to explore the hidden pharmacological properties of this herb [16]. The acute and chronic toxicity studies of the pulp of *Tamarindus indica* at 3000mg/kg and 5000mg/kg body weight resulted in no mortality and hence classified as practically non-toxic and safe by WHO and OECD [17]. During literature search, we found lack of research on CNS effects of *Tamarindus indica.* The present study was carried out to determine the anxiolytic and anti-depressant potential of TI.

MATERIAL AND METHOD

Plant material: The ripen pods of *Tamarindus indica* were collected from University of Karachi campus during the month of April. The plant material was identified by Dr. Iqbal Azhar, department of Pharmacognosy and the voucher specimen number has been deposited there for future reference. The ethanolic extract of *Tamarindus indica* was prepared by cold maceration process. It was a pleasantly fragrant, reddish brown and gummy mass which was stored in refrigerator for further use [18].

Animals; their housing and dosing protocol: There is a good evidence for the neuro-pharmacological and neuro-anatomical parallels between rodent emotionality and human anxiety [19]. Therefore, for CNS activity thirty five albino mice with equal sex distribution, weighing about 20- 25 gm were divided in to 5 groups, each group had 7 animals. One group was controlled group; taking equal volume of saline, second group was reference group taking diazepam at anxiolytic dose of 1mg/kg [20] and the remaining three groups were experimental groups which had received the freshly prepared aqueous solution of ethanolic extract of TI in three different doses of 50mg/kg, 75mg/kg & 100mg/kg according to their body kg weight. All the solutions were administered orally with the standard laboratory feeding tube. The animals were procured form the animal house of PCSIR laboratory. All procedures and protocols were in accordance of Helsinki declaration, 1964 and were approved by our Board of Advanced Studies and Research by Resol. No. 10(91) dated: 25-10-2011. These animals were adapted to the laboratory environment and apparent health was monitored one week prior the experiment and they were maintained under constant environmental conditions with 12-h light/dark cycle and temperature of 22±3˚C, with free access to standard diet and water.

Experimental Parameters: The CNS acting potential of *Tamarindus indica* was evaluated by following parameters. Each animal from 5 groups was tested after 21 days dosing.

Behavioral responses: The behavioral responses of all animal were observed during the course of study and the cumulative results were finalized after completing the dosing at 21 days. The behavioral attitudes were observed for Righting reflex, vocalization, grip strength, and passivity. The activity profile was quantified as per table below: [21]

Table 1: Behavioral Activity profile

Nature of response	Numerical Values	Interpretation
Normal response	1	Normal behavior
Subnormal response	-1	Decrease in activity
Supernormal response	2	Increase in normal activity
Abnormal response	3	Signs which are normally absent
Stereotype response	4	Repetitive abnormal response
Absence of lack of response	0	No changes in animal behavior

Head dip test: The head dipping activity is because of fearful behavior because the initial exposure of mice to the head dip apparatus is a stressful event and the animal tries to escape from the holes [22]. The head dip/hole board method is an effective method for exploring anxious behavior in mice. It consists of perforated wooden box (35cm× 45cm× 45cm) with equally spaced holes (2.5 cm in diameter) on all four sides of box. Each animal of 5 groups was individually placed in the center of the box and the number of head-dips (the animal poked its snout) were registered during 10 minutes trial [23].

Cage crossing test: Cage crossing test is used to determine the locomotor activity [24].Transparent perpex cage (26x26x26 cm) with sawdust cover floor were used to monitor the activity in familiar environment. All mice were placed individually in these cages to get them familiarized with the environment, and number of cage crossing was counted for 10 minutes. This test was performed in separate quiet room to avoid any disturbance [21].

Force induced swimming test: The FST also called the PORSOLT TEST is used to determine the CNS depression level of mouse. The FST is a good screening tool with good reliability and predictive validity [25]. In forced swimming test immobility time is measured to determine the antidepressant activity. It was performed by using acrylic glass cylinder (20cm in height, 6cm in diameters) filled with water (25±2˚C) at specific level (12cm high) and escaping was prevented by the surrounded walls. All the animals were introduced in the cylinder separately and note the immobility time after 21 days dosing with saline, reference drug which is Imipramine 20mg/kg [26] and 3 different doses of TIPE. As mice despair to swim and attained a floating posture, this is called immobility time and we note these readings with stop watch. This behavior reflects a state of depression [27]

Comparison of brain weight: We have also done other studies for the pharmacological evaluation of *Tamrindus indica* using 4 groups of rats (n=5) taking 50mg/kg, 75mg/kg, 100mg/kg of TIPE and saline respectively. At the end of our studies, we compared the brain weight of each group with the control group after sacrificing the animals.

Statistical Analysis: Statistical analysis was performed by SPSS version 20. All values were compared with control by taking mean and standard error to the mean using one-way analysis of variance (ANOVA) followed by post hoc. Data was reported as mean ± standard error to the mean with 95% confidence interval and P-values were observed. Values of *P<0.05 were considered as significant with respect to control and **P<0.005 as highly significant with respect to control, n=07/n=05; Mean ± S.E.M.

RESULTS

Behavioral responses: The Righting Reflex of mice was neither affected by three different doses of TIPE nor by the control and standard drugs. Hence all the animals were able to regain their normal body posture after any type of forced change in their body position. The results are statistically insignificant and expressed as mean ± S.E.M (Table 2, Fig1). The vocalization of Group A (0.14±0.14) and B (0.42 ±0.20) showed significant decrease (P<0.05) as compared to the control group (1.00±0.00) but group C (0.71±0.18) has insignificantly decreased vocalization. (Table 2, Fig 02). The grip strength of standard group has significantly decreased (-1.00 ± 0.00; P<0.05) as compared to the control group. In group A, the 50mg/kg dose produced similar effects as that of standard group and significantly decreased (-0.14±0.40; P<0.05) grip strength. The group B, taking 75 mg/kg dose has no significant effect on grip strength (0.85±0.34). While group C has significantly increased (2.00±0.00; P<0.05) the grip strength (Table 2, Fig 03). There is no passivity found in the control group (0.00 ± 0.00). The results of Group A & B showed insignificant increase as compared with control (0.57±0.36). The animals of group C showed significant decrease in passivity (-1.00±0.00; P<0.05). The standard group showed highly significant increase (2.00 ±0.00; P<0.005) in passivity (Table 2, Fig 04).

TABLE2: BEHAVIORAL RESPONSES (No. of scores)				
GROUPS	**RIGHTING REFLEX**	**VOCALIZATION**	**GRIP STRENGTH**	**PASSIVITY**
Control group (normal saline)	1.00±0.00	1.00±0.00	1.00±0.00	0.00±0.00
Standard group (Diazepam;1mg/kg)	1.00±0.00	0.00±0.00**	-1.00±0.00*	2.00 ±0.00**
Group A 50mg/kg TIPE	1.00±0.00	0.14±0.14*	-0.14±0.40*	0.57±0.36
Group B 75mg/kg TIPE	1.00±0.00	0.42±0.20*	00.85±0.34	0.57±0.36
Group C 100mg/kg TIPE	1.00±0.00	0.71±0.18	02.00±0.00**	-1.00±0.00*

Values are expressed as mean±S.E.M; n=07; *P<0.05 significant with respect to control; **P<0.005 highly significant with respect to control.

Head Dip Test: All the results of head dip test were highly significant (P<0.005) as compared to the control group. There is a significant decrease in head dips showing the anxiolytic effect of *Tamarindus indica* pulp extract. However the group C showed greater number of head dips (26.28±1.71) as compared with other experimental groups B (22.28±1.28) and A (21.14±1.18) (table3, Fig 5)

TABLE 3: HEAD DIP TEST	
Groups	(No. of head dips)
Control group (normal saline)	39.85±2.22
Standard group (Diazepam; 1mg/kg)	16.00 ±1.64**
Group A 50mg/kg TIPE	21.14±1.18**
Group B 75mg/kg TIPE	22.28±1.28**
Group C 100mg/kg TIPE	26.28±1.71**

Values are expressed as mean±S.E.M; n=07; *P<0.05 significant with respect to control; **P<0.005 highly significant with respect to control.

Cage crossings: The standard group showed highly significant decrease (18.14±0.85; P<0.005) as compared to the control group (29.00±1.17). The experimental group A with dose of 50mg/kg showed highly significant decrease (23.42±0.84; P<0.005).Group B with 75mg/kg dose showed significant decrease (25.28±0.35; P<0.05) in cage crossing. Whereas the group C animals taking 100 mg/kg dose showed insignificant decrease (26.85±1.05) in cage crossings with respect to control (Table 4, Fig 06).

TABLE 4: CAGE CROSSINGS	
Groups	(No. of crossings)
Control group (normal saline)	29.00±1.17
Standard group (Diazepam;1mg/kg)	18.14±0.85**
Group A 50mg/kg TIPE	23.42±0.84**
Group B 75mg/kg TIPE	25.28±0.35*
Group C 100mg/kg TIPE	26.85±1.05

Values are expressed as mean±S.E.M; n=07; *P<0.05 significant with respect to control; **P<0.005 highly significant with respect to control.

Forced swimming test: Table 5 and Fig 7 show the comparison of immobility time. The group A which took 50mg/kg dose showed significant decrease (78.57±2.10; P<0.05) in immobility time as compared to the control group. While the group B and group C showed highly significant decrease (P<0.005) in the immobility time with values (73.28 ± 2.24) and (52.57±1.06) respectively, showing that TIPE has anti-depressant effects.

TABLE 5: FORCED SWIMMING TEST

Groups	Immobility Time (Sec)
Control group (normal saline)	90.14±1.29
Standard group (Imipramine; 20mg/kg)	60.16±8.32**
Group A 50mg/kg TIPE	78.57±2.10*
Group B 75mg/kg TIPE	73.28±2.24**
Group C 100mg/kg TIPE	52.57±1.06**

Values are expressed as mean±S.E.M; n=07; *P<0.05 significant with respect to control; **P<0.005 highly significant with respect to control.

Brain weight of rats: The experimental group A and B receiving 50mg/kg and 75mg/kg doses of TIPE has insignificant decrease (1.49±0.06) and insignificant increase (1.68±0.39) on brain weight of rats as compare to control. Group C with 100mg/kg dose has highly significant increase (1.84±0.03; P<0.005) in brain weight as compare to control group (Table 6, Fig 8).

TABLE 6: COMPARISON OF BRAIN WEIGHT OF RATS

Parameter (gm)	Control group (normal saline)	Experimental groups		
		Group A 50 mg/kg	Group B 75 mg/kg	Group C 100 mg/kg
weight of brain	1.52±0.04	1.49±0.06	1.68±0.39	1.84±0.03**

Values are expressed as mean±S.E.M; n=05; *P<0.05 significant with respect to control; **P<0.005 highly significant with respect to control.

DISCUSSION

The righting reflex, also known as the Labyrinthine righting reflex, is a reflex that corrects the orientation of the body when the animal is placed on the surface of its back. If the animal fails to regain its normal standing position, it is called loss of righting reflex [28]. There were no such losses of reflexes by any dose of TIPE. Hence there were no signs of CNS depression. The interesting observation is that diazepam at anxiolytic doses of 1mg/kg even for 21 days remain capable to maintain righting reflex, without depressing CNS completely [29]. The vocalization of animals taking 50mg/kg dose and 75mg/kg dose was decreased and they remain introverted similar to those of having inhibitory effects of diazepam [30]. While 100mg/kg dose showed insignificant decrease in vocalization and these animals remain extroverted with elevated mood which is clearly different from the control group having aggressive behavior for each other and even injured each other in overall duration of study. There was partial increase in the passivity of animals taking 50mg/kg and 75mg/kg doses due to anxiolytic potential without sedation which is contradictory to complete passive condition of reference group showing sedative effects of Diazepam [31]. Conversely 100mg/kg dose showed significant decrease in passivity hence there was improved CNS excitation along with anxiolytic potential to improve the actions and overall performance of mice [21]. Grip strength of animals taking 50mg/kg dose of TIPE decreased markedly similar to that of reference group taking Diazepam. This is in accordance with anxiolytics' reported poor motor co-ordination and increased muscle relaxation which can even result in falls [32] and cause sedative effects [33]. This is also supported by previous findings that long term administration of anxiolytic dose of diazepam cause poor grip strength [34]. 75mg/kg dose has no effect on grip strength. 100mg/kg dose has improved grip strength and muscle tone, which may be related to its antidepressants activity in which increased 5HT levels are increasing grip strength [35]. There was highly significant decrease in head dipping which revealed that all the experimental doses have significant anxiolytic effects. The reduction in exploratory activity is associated with relieving anxiety and producing calming action [36]. However 100mg/kg dose showed less decrease in number of head dips as

compared to moderate doses of 75mg/kg and low doses of 50mg/kg. This showed that 50mg/kg and 75mg/kg doses are more anxiolytic than 100 mg/kg which showed more antidepressant effects. This comparative increase in number of head dips is also indicator of CNS excitation and elevated mood to explore the environment [37]. These findings are also supported by cage crossing test and forced swimming test (table 4 and 5). The 50mg/kg and 75mg/kg doses showed highly significant and significant reduction in number of cage crossings respectively due to its anxiolytic potential which is similar to diazepam because anxiolytic drugs reduce locomotor activity [38]. This could be because of lower level of serotonin is associated with locomotor activity and muscular co-ordination [35] which is not seen with 100mg/kg dose which has not reduced cage crossings. This could be because of profound antidepressant activity. It is also confirmed by Noreen S et al [39], that increased level of 5HT relieves depression. 50mg/kg dose showed significant antidepressant activity but 75mg/kg and 100mg/kg doses showed highly significant antidepressant activity. This could be because 50mg/kg dose has shown more anxiolytic response as compare to antidepressant activity while moderate and high doses have shown more antidepressant activity which was resulted in no stress to water [40]. Our results have same findings as was reported by Najam and Anser [21]. 50mg/kg and 75mg/kg doses have no effects on weight of brain but 100mg/kg dose showed increase in brain weight as compare to control group. This can be related to neuro-degeneration mechanism associated with stress and anxiety [41], which is not seen with 100mg/kg dose rather it appeared to be neuroprotective and enhances neuron strength and function as a result improves mental alertness and increased learning attitudes during behavioral studies [42]. This finding is similar to Omega 3 Fatty acids to improve performance, decrease in inflammation and psychological distress [43]. This similarity is also supported by unsaturated fatty acids of *Tamarindus indica* pulp as that of Omega 3 Fatty acids. Taken as a whole, the antidepressant and anxiolytic dose of 100 mg/kg decreases the abnormal metabolic neuro-degeneration and results in increased brain weight [44].

CONCLUSION

These CNS studies concluded that even low dose (50 mg/kg) of TIPE has anxiolytic potential as was found with the standard Diazepam group showing sedation, low mood, week muscle tone and poor reflexes. Moderate dose of 75mg/kg dose showed partial anxiolytic as well as antidepressant effects while 100mg/kg dose found to be effective for adjusting with the environment, elevating mood, CNS excitation, posture maintenance, improved muscle tone and quick reflexes. Although the exact mechanism is not well understood but it may lead to the idea that 100mg/kg dose has significantly decreased 5HT associated hyper-metabolic activity in amygdale to give its anxiolytic effects [45] but at the same time gives SSRIs like antidepressant effect by working at synaptic cleft [39]. This showed prominent indication as antidepressant and anxiolytic agent. The overall behavioral results are supported by a previous study in which the anxiolytic constituents improve overall performance [21]. However there is need to further investigate the involved molecular mechanism and critically re-evaluate the results on larger sample size of different animal models or human beings. In order to make TIPE as an effective remedy in future, isolation studies should be done to explore which phyto-constituent is responsible for these therapeutic effects.

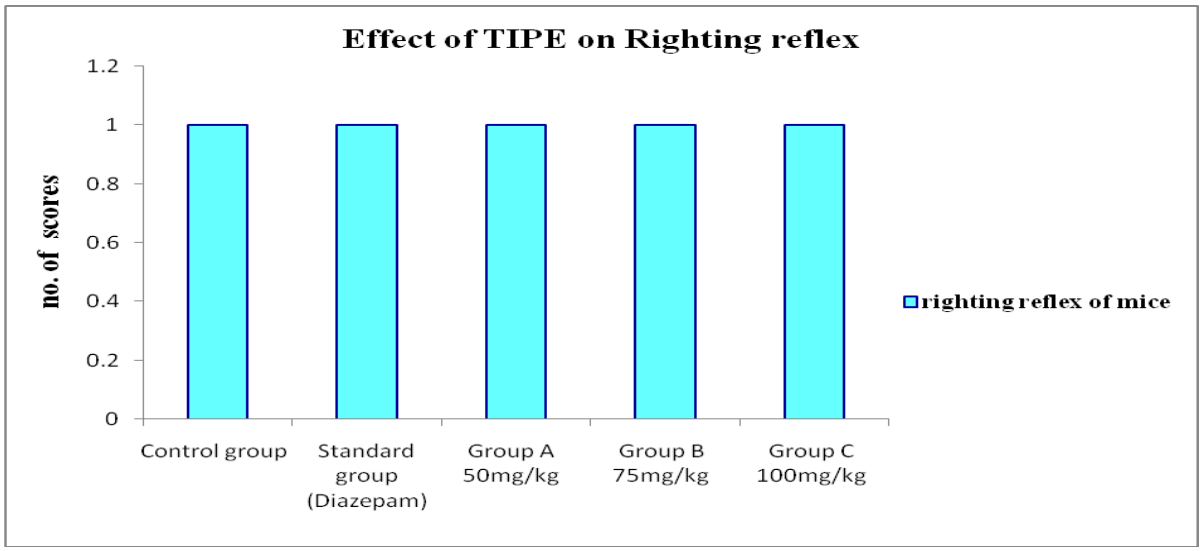

Fig 1: COMPARISON OF RIGHTING REFLEX

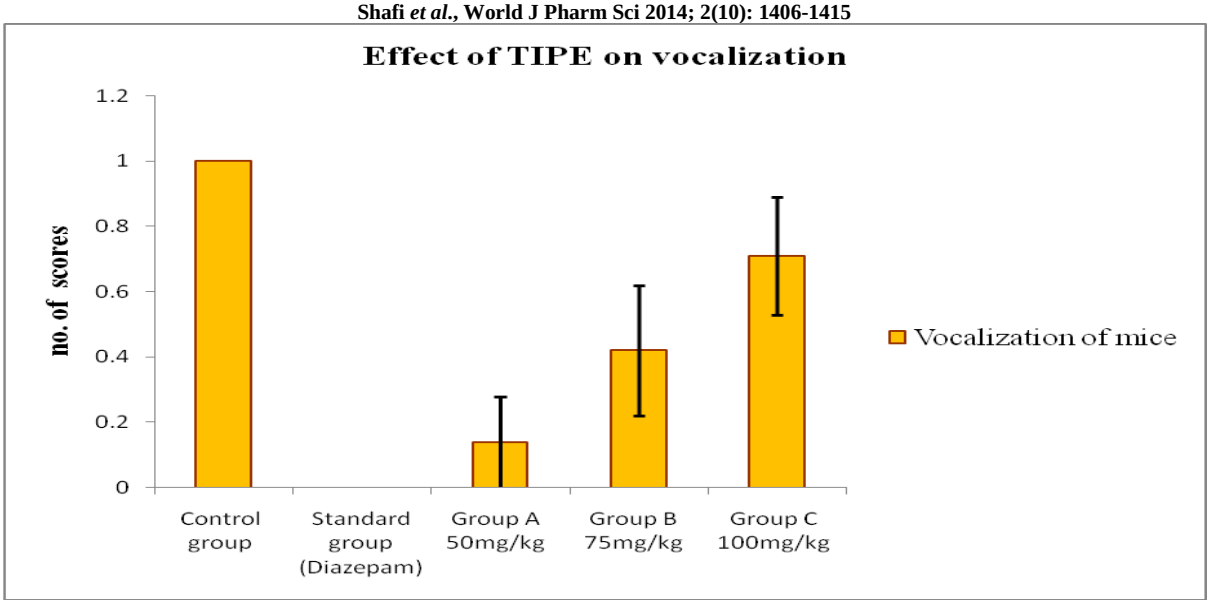

Fig 2: COMPARISON OF VOCALIZATION

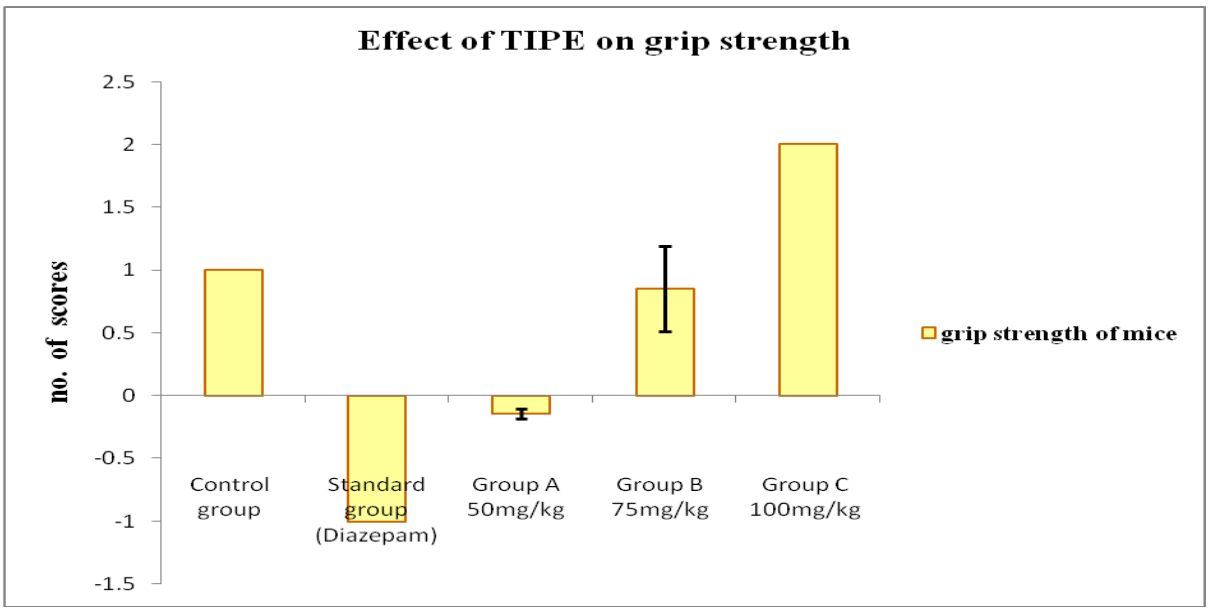

Fig 3: COMPARISON OF GRIP STRENGTH

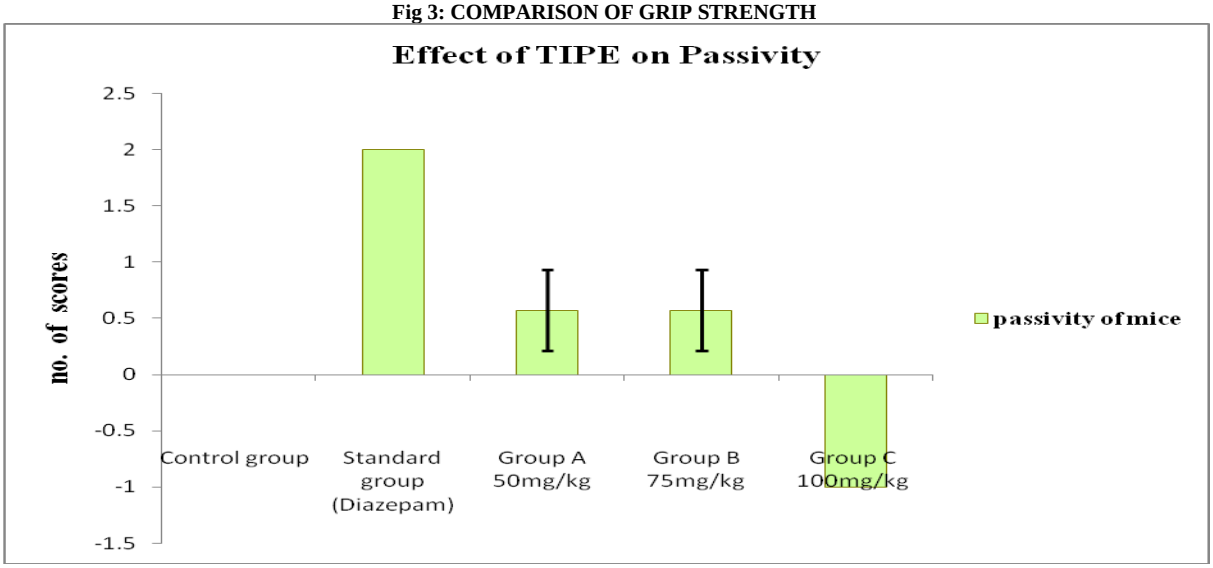

Fig 4: COMPARISON OF PASSIVITY

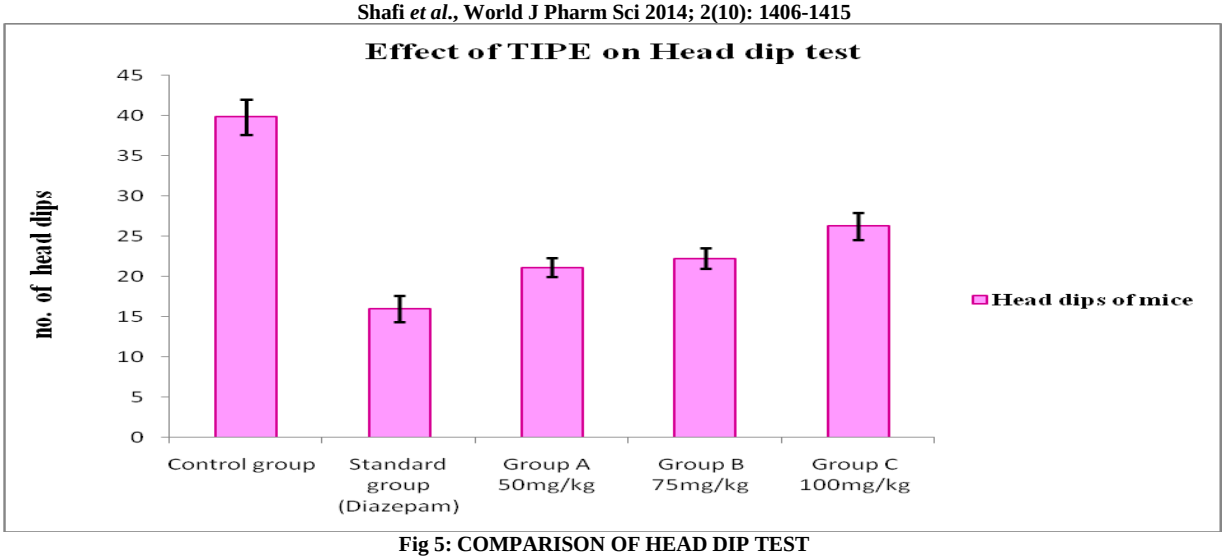

Fig 5: COMPARISON OF HEAD DIP TEST

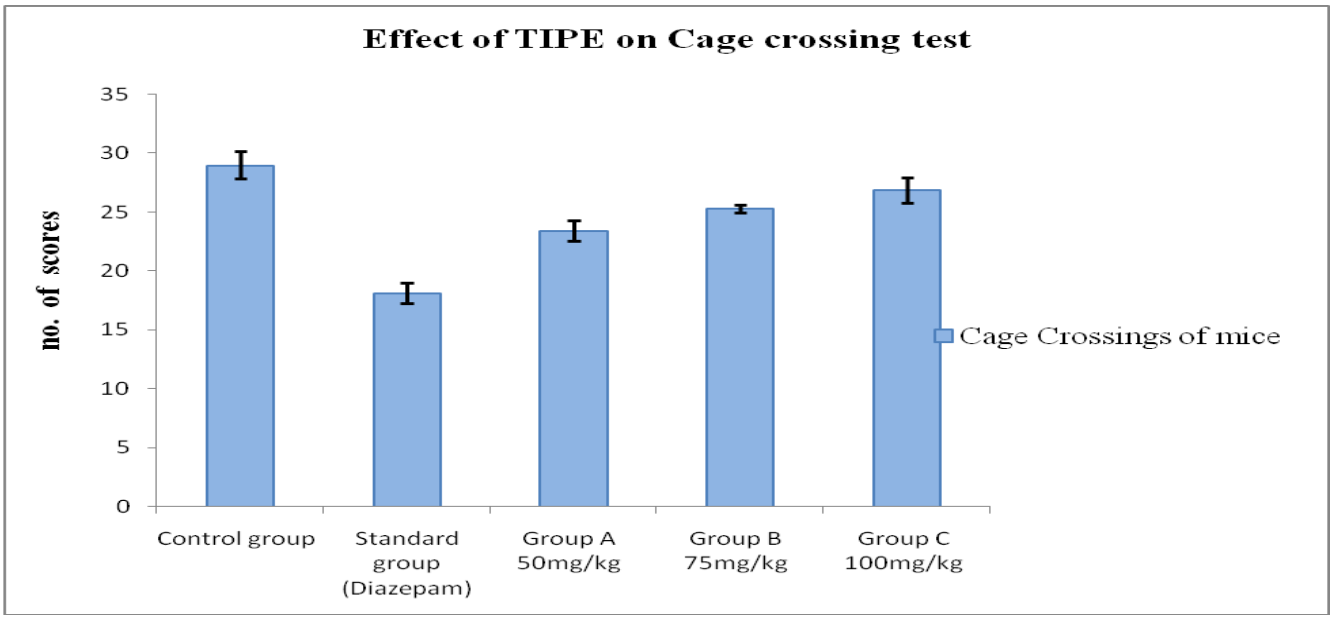

Fig 6: COMPARISON OF CAGE CROSSINGS

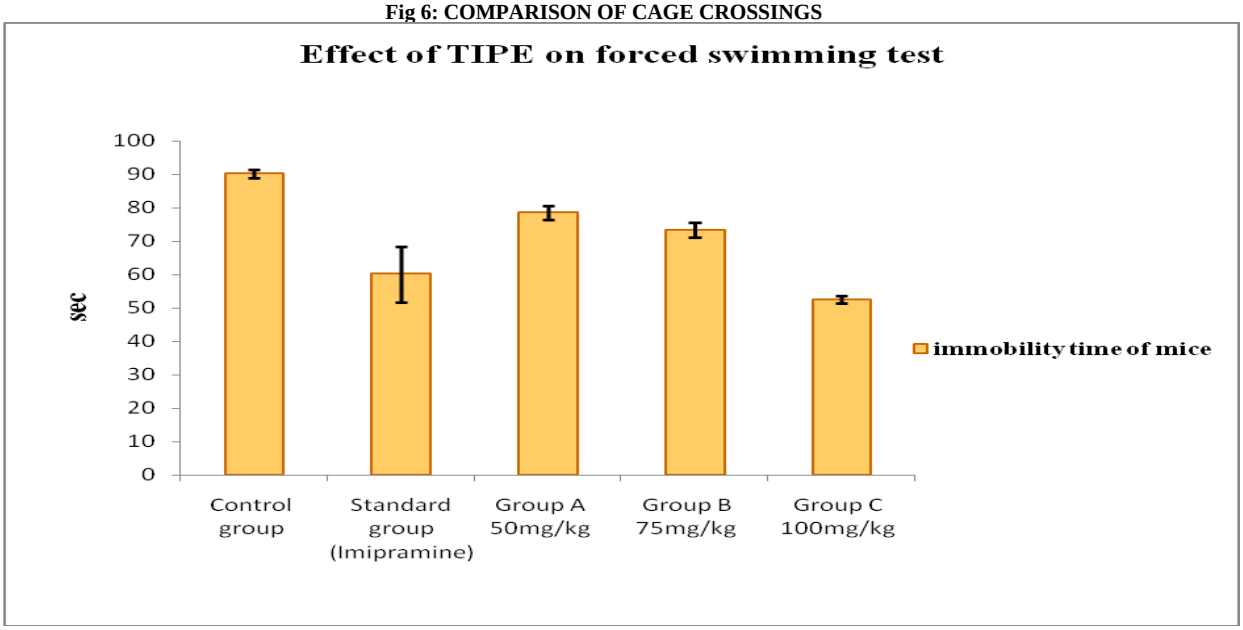

Fig 7: COMPARISON OF IMMOBILITY TIME

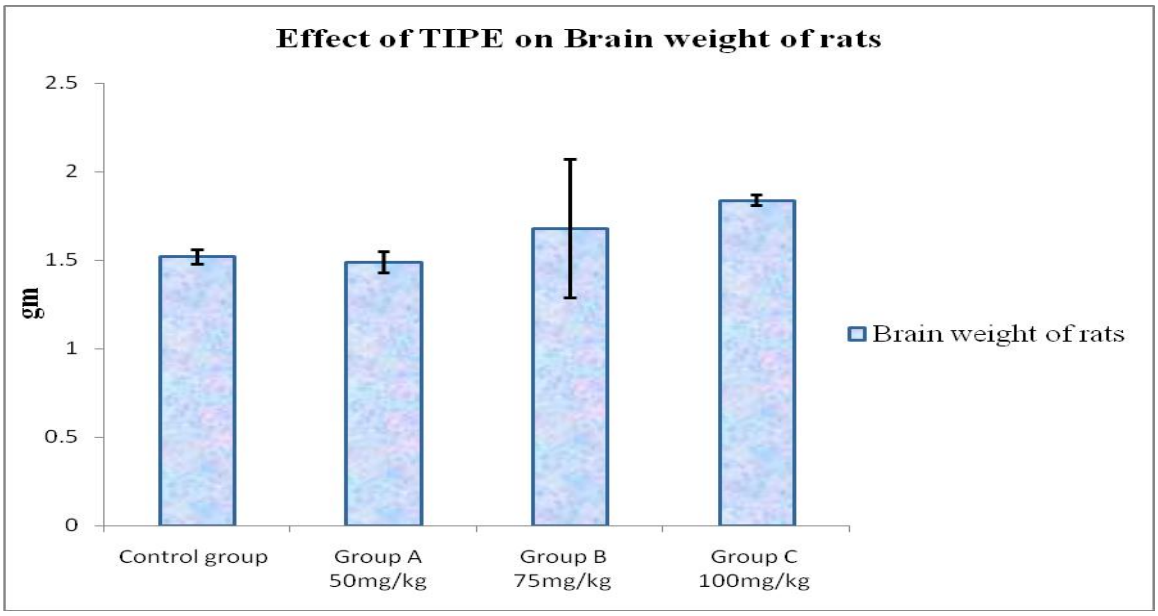

Fig 8: COMPARISON OF BRAIN WEIGHT OF RATS

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