Neurochemical and Behavioural Effects of Indole Substituted Piperidine Derivatives

Asghari Ghous[1,*] and Zafer Saeed Saify[2]

[1]*Department of Biochemistry Federal Urdu University, Karachi and* [2]*HEJ Research Institute of Chemical and Biological Sciences, Karachi, Pakistan*

Abstract: The present study concerns the neurochemical and behavioural effects of indole substituted piperidine compounds. Behavioral results show that intraperitonial injection at a dose of 30mg/kg of body wt. in rats significantly decreases locomotor activity in open field apparatus. Anxiogenic effects of the compounds monitored in the light and dark activity box were significantly decreased, suggesting the anxiogenic effect of indole substituted piperidine derivatives. Moreover, stimulant activity of compounds monitored in home cage activity box was not found to be significant. The neurochemical effects were monitored by HPLC-EC, which showed that the administration of compounds PMEI and PEEI increased the level of Serotonin (5Hydroxy triptamine-5HT) in rat brain. It is suggested that these indole substituted piperidine deriatives may be useful as antidepressant drugs. Further studies using animal models of depression are required to establish the antidepressant profile of these drugs.

Key Words: Serotonin, anxiety, locomotor activity, depression, indole substituted piperidine compounds.

INTRODUCTION

Serotonin, an indole amine neurotransmitter, is involved in the regulation of several brain functions such as sleep, appetite, mood variations, body temperature, locomotor activity and memory function [4, 8].

Increased serotonergic effect produces an anxiogenic effect while reduction of serotonergic neurotransmission results in anxiolytic effect [15].

Evidence shows that the anxiogenic like effects of 5HT are due to the stimulation of post synaptic $5HT_{2C}$ receptors. M-CPP increases 5HT release [2, 7] because it stimulates post synaptic $5HT_{2C}$ receptors [6]. It also decreases the locomotor activity because of the stimulation of $5HT_{2C}$ receptor.

Buspirone is the $5HT_{1a}$ agonist used for the cure of depression. Reduction in $5HT_{1a}$ function is associated with depression [3].

Specific Serotonin reuptake inhibitors have been used for the treatment of depression and anxiety disorder. SSRIs increase extracellular level of serotonin because they inhibit the reuptake of serotonin and the level of serotonin is increased in the synaptic cleft [9]. Fluoxetine (SSRI) treated animals display enhanced anxiety and decreased locomotor activity [12].

In the present study, indole substituted piperidine derivative PMEI or PEEI was injected to test the behavioral and neurochemical activities.

*Address correspondence to this author at the Department of Biochemistry Federal Urdu University, Karachi, E-mail: asghari_ghous@yahoo.com

The naturally occurring compounds, mainly alkaloids are the main sources of piperidine derivatives. The molecular formula of piperidine is $C_5H_{11}N$ with a molecular weight 81.15. A lot of research has been performed on piperidine alkaloids and their related compounds [5, 17, 13, 14, 11].

The 3-[(2-ethoxyphenoxy) methyl] piperidine derivatives [3-5] have been synthesized & screened as potential antidepressant agents [1].

Several indole derivatives of piperidine specifically 5-(3 hydroxy butyl)-3-[(R)-(1-methyl-2 pyrrolopyridinyl) methyl]-1-H-indole (Fig. **1**) are selective $5HT_1$ like agonists useful in the treatment of migraine, cluster headache and headache associated with vascular disorder [16]. Piperidinyl ethyl amide derivatives were observed having $5HT_{1A}$ antagonists & are useful anxiolytic agent [10, 16].

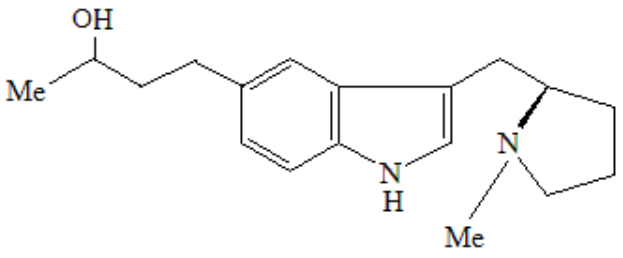

Fig. (1). Structure of 5-(3 hydroxy butyl)-3-[(R)-(1-methyl-2 pyrrolopyridinyl) methyl]-1-H-indole.

The present study concerns synthesis of indole substituted piperidine derivatives and monitoring their behavioral and neurochemical profile.

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MATERIAL AND METHODS

Compound Synthesis and Its Chemistry

1. Drugs were synthesised, each by two reactants. Reactants were dissolved in acetone (solvents) in the conical flask, placed on a hot plate (without heating) with constant stirring for two hours, after which stirring was continued with slow heating until the compound was synthsised. PMEI was synthesized after about 72 hours whereas PEBI was synthesized after 48 hours.
2. Next, the purity of compounds was checked by thin layer chromatography (TLC).
3. After elution of spots from TLC, the purity of these compounds was confirmed by spectroscopic methods such as Mass, NMR, IR & UV.

Synthesized Compounds

1. PMEI: {1-[2-(1H-indol-3-yl)ethyl]piperidin-2-yl}methanol

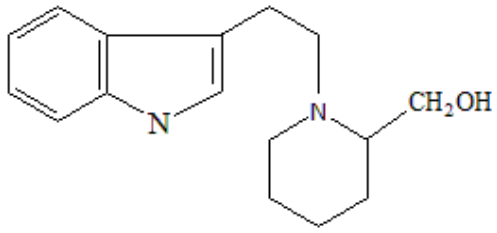


Fig. (2). Structure of PMEI.

Two basic reactants were used for the synthesis of the compound PMEI

a. Bromoethyl indole as the parent compound.

b. 2-pyridyl methanol.

1.12 gram of bromo ethyl indole was dissolved in 15-20 ml acetone whereas 0.54 gram of pyridyl methanol was dissolved in 10-15 ml of acetone. After dissolving, both reactants were mixed slowly. Reaction mixture was then stirred for 72 hours at a temperature of around 52°C-54°C. The process of reaction was checked by thin layer chromatography until a single spot was obtained. Structure was confirmed by various spectroscopic analysis.

2. PEEI: 2-{1-[2-(1H-indol-3-yl)ethyl]piperidin-2-yl}ethan-1-ol hydrate

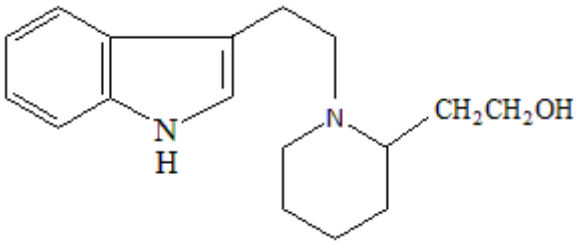


Fig. (3). Structure of PEEI.

Apart from the parent compound (bromoethyl indole), the second reactant used was 2-(pyriidyl-(2)] ethanol. Again the two reactants were dissolved in acetone (15-20ml) separately and then mixed together after dissolution. No color change was observed. A temperature of 52°C-54°C was maintained for the reaction mixture while it was continuously being stirred, this time for 48 hrs. After 48 hrs, a brown colored product was obtained. Here again, the structure was confirmed by various spectroscopic analysis.

Animals

Male Albino Wistar rats, weighing 180-200gm, were purchased from Agha Khan University Hospital. Before the experiment, the rats need to be famialiarised with the new environment for 2 to 3 days so that they start behaving normally before they are subjected to the experiment. The cages they are kept in should be quiet, along with a soft floor laid with sawdust with sufficient standard rodent diet and water. All experiments were performed according to a protocol approved by the local animal care committee.

Drugs and Injection

Indole substituted piperidine derivatives PMEI and PEEI were injected intraperitonially to the test group of animals. Control animals were injected with saline, while the parent animals were injected with bromoethyl indole.

Experimental Protocol

A total of twenty one male Albino Wistar rats were obtained and randomly selected to be divided into three groups, containing seven rats each, namely control (vehicle administered), test (synthetic compound administered) and parent (bromoethyl indole administered).

With the completion of these tests, the animals were returned to their cages. 4 hours after the injection of the drug/vehicle, the animals were decapitated to obtain the brain for neurochemical estimation of Dopamine, 5HT and their metabolites HVA and 5HIAA respectively using HPLC-EC.

Behavioral Analysis

Open Field Activity or Novel Environment Test

The locomotor activity and exploration was determined by the open field method. Before starting the experiment, the animals were randomly selected for each drug as seven controls (C), seven test (T), seven Parent (P). The open field apparatus consisted of box, having square area 76*76cm with walls of 42cm high. Floor of the apparatus was divided by lines into 25 squares having equal size. Experiment was performed under white light. Animals were taken out one by one from their home cages and were placed in the central square of the open field apparatus. The activity was noted as

number of squares crossed with four paws was counted for 5 min.

Home Cage Activity or Skinner's Box Test

Stimulatory activity was determined by Home Cage apparatus. Home cages or skinner's boxes have been specially designed made up of Perspex (26, 26, 26cm) floor was soft due to saw dust. Inside each cage few pieces of special rat food were also placed. In the home cage the activity of rat was observed for 5 minutes. Activities were determined as the number of cage crossing. In this activity for each drug 21 rats were used, 7 each for control test and parent activity.

Light and Dark Activity

Anxiety was determined by light and dark test. The light and dark apparatus is made up of two compartments and small passage is present between two compartment due to which rat can easily move to either compartment.

Experiment was performed under normal light. The rat was placed in the dark compartment and then checked that how many times the rat enter in the light compartment and also the time spent in the light compartment within five minutes. All the control (7) test (7) and parent (7) are placed in the dark compartment one by one and checked the time and attempt in five minutes.

Neurochemical Analysis

(HPLC-EC determination of dopamine 5HT and their metabolites HVA and 5HIAA)

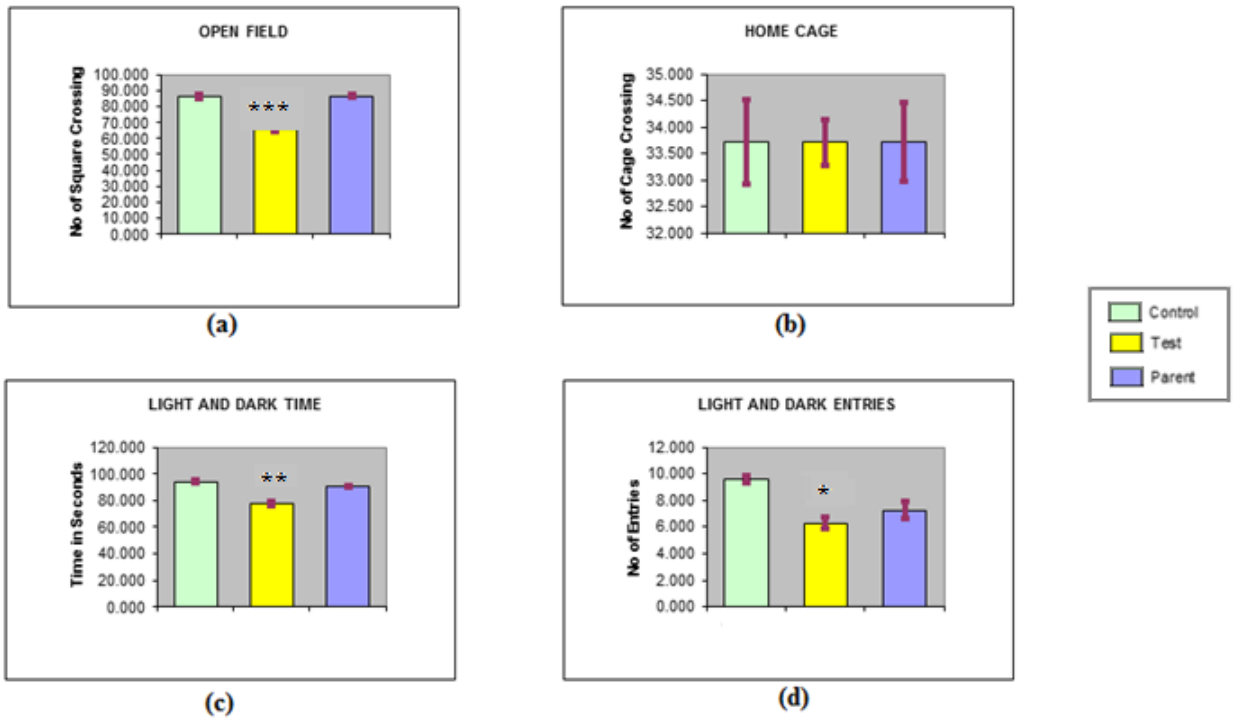
For HPLC-EC determination, a 5µm shim-pack ODS separation column of dimensions 4.5mm (internal diameter) x15cm (length) was used. Methanol (18%), octyl sodium sulphate (0.023%) and EDTA (0.05%) in 0.1M phosphate buffer was used as the solvent system. An operation potential of 0.8V (glassy carbon electrode vs. Ag/AgCl) was used for electrochemical detection.

Statistical Analysis

Neurochemical and behavioural data on the effect of piperidine derivative were analysed by one-way ANOVA. Individual differences were made by Newman Keuls test.

RESULTS

Figure (**4a**) shows the effect of PMEI on open field activity. Statistical analysis was performed on the data using one-way ANOVA (df 3,18); (F=120.20) (***p<0.01), indicating a significant effect (significant decrease). Newman-Keuls test was also performed. Individual



Effect of 30mg/kg of indole substituted piperidine derivative PMEI on (a) Open field activity, (b) Home cage activity, (c) Light and dark activity (time) & (d) Light and dark activity (entries) respectively. Values are mean±S.D. (n=7). Significant differences by Newman Keuls test: ***p<0.01 from open filed, **p<0.01 from light/dark time, *p<0.01 from light and dark entries following one-way ANOVA.

Fig. (4). Shows the behavioral effect of PMEI on rats.

difference shows that the application of PMEI resulted in a significant decrease in T as compared to P and C.

Figure (**4b**) shows the effect of PMEI on home cage activity or skinner's box activity. Statistical analysis was performed on the data using one-way ANOVA (df3,18); (F=0.0030) (p>0.05) indicating a non-significant effect. Newman-Keuls test was also performed. Individual difference shows that the application of PMEI did not result in any change in T as compared to P and C.

Figure (**4c**) shows the effect of PMEI on activity of rat on time spent in the light/dark compartment. The data on time spent in the light compartment were analyzed by one way ANOVA showed significant effect of PMEI (F=56.80) (df3,18) (**p<0.01). Analysis showed that the time spent in light compartment has decreased. Newman-Keuls test was also performed. Individual difference shows that the application of PMEI resulted in a significant decrease in T as compared to P and C.

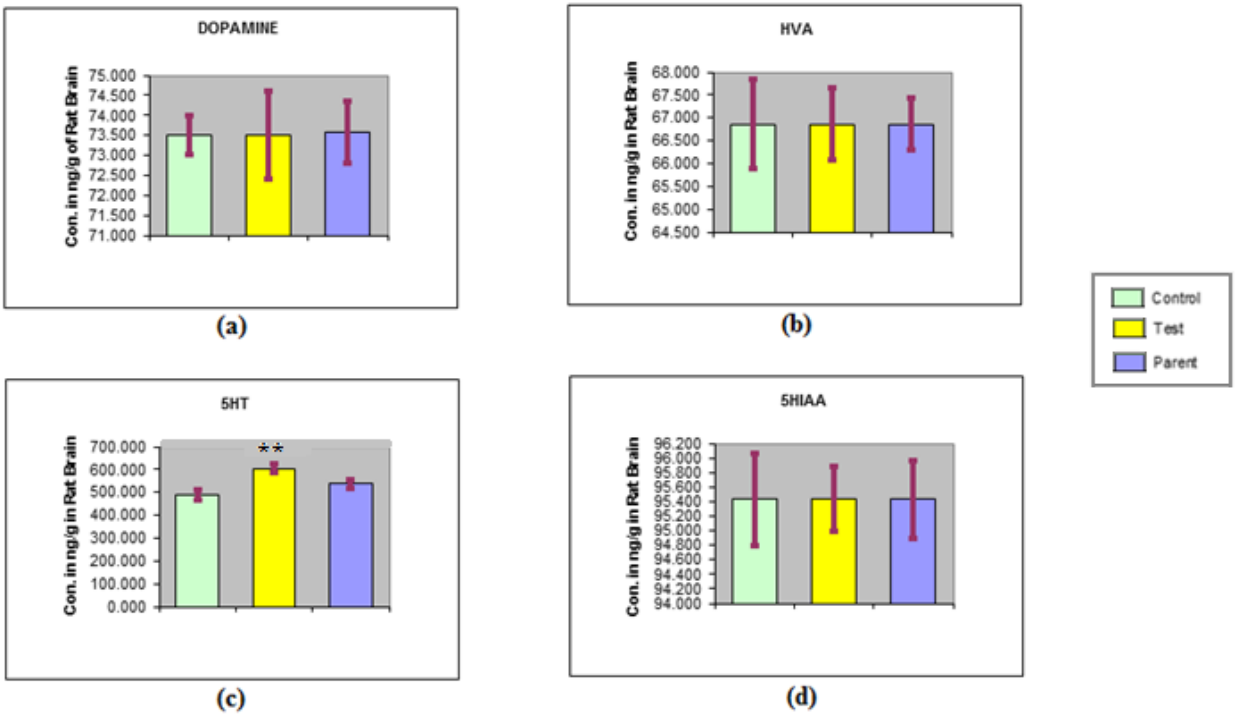
Figure (**4d**) shows the effect of PMEI on activity of rat in a light/dark box. The data on number of entries analyzed by one way ANOVA showed significant effect (F=16.70) (df3,18) (*p<0.01). Analysis showed that the entries in light/dark box are significantly decreased. Newman-Keuls test was also performed. Individual difference shows that the application of PMEI resulted in a significant decrease in T as compared to P and C.

Figure (**5a**) shows the effect of PMEI on Dopamine. The data is analyzed by one way ANOVA and showed non-significant effect; (F=0.0044) (df3,18) (p>0.05). Newman-Keuls test was also performed. Individual difference shows that the application of PMEI did not result in any change in T as compared to P and C, in the whole brain.

Figure (**5b**) shows the effect of PMEI on HVA. The data is analyzed by one way ANOVA and showed non-significant effect; (F=0.0077) (df3,18) (p>0.05). Newman Keuls test was also performed. Individual difference shows that the application of PMEI did not result in any change in T as compared to P and C, in the whole brain.

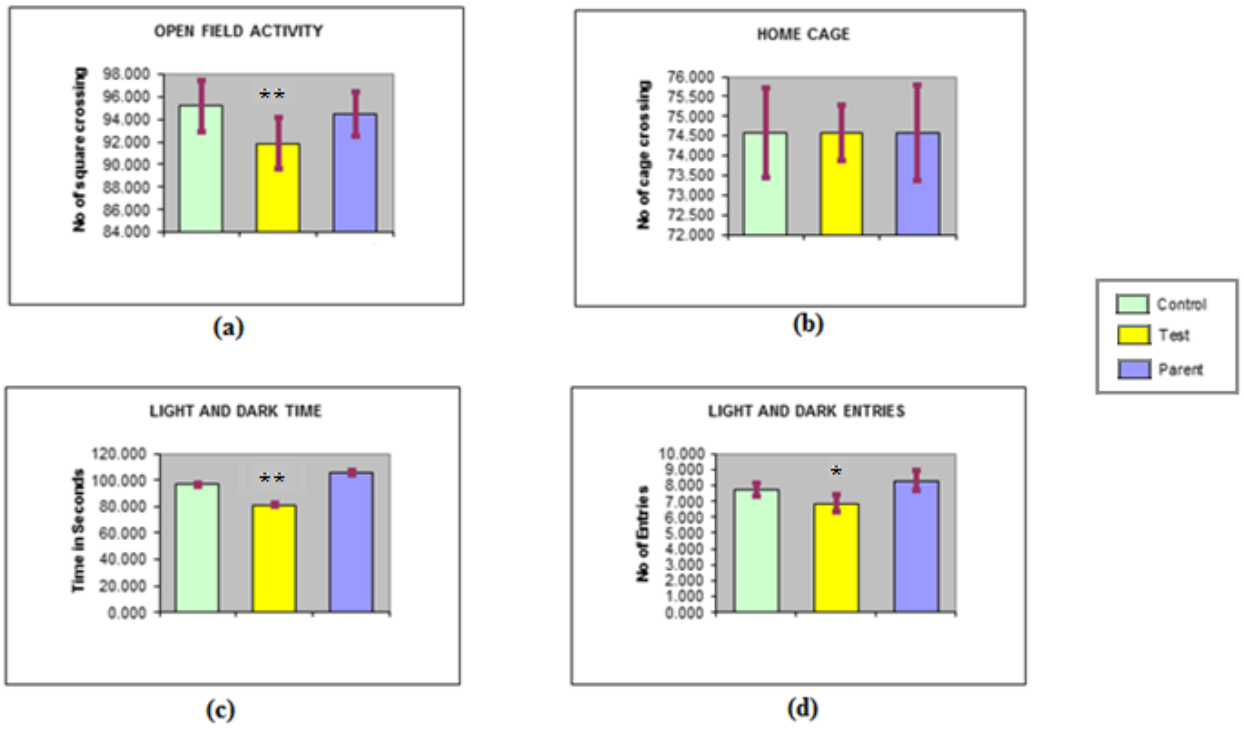
Figure (**5c**) shows the effect of PMEI on 5HT. The data is analyzed by one way ANOVA and showed a significant effect; (F=54.359) (df3,18) (**p<0.01), 5HT has significantly increased. Newman-Keuls test was also performed. Individual difference shows that the application of PMEI resulted in a significant increase in T as compared to P and C, in the whole brain.

Figure (**5d**) shows the effect of PMEI on 5HIAA. The data is analyzed by one way ANOVA and showed non-significant effect; (F=0.000508) (df3,18) (p>0.05). Newman-Keuls test was also performed. Individual difference shows that the application of PMEI did not result in any change in T as compared to P and C, in the whole brain.



Effect of 30mg/kg of indole substituted piperidine derivative PMEI on (a) Dopamine, (b) HVA, (c) 5HT & (d) 5HIAA respectively. Values are mean±S.D. (n=7). Significant differences by Newman Keuls test: **p<0.01 from 5HT following one-way ANOVA.

Fig. (5). Shows the effects of PMEI on neurochemical activity of rats.



Effect of 30mg/kg of indole substituted piperidine derivative PEEI on (a) Open field activity, (b) Home cage activity, (c) Light and dark activity (time) & (d) Light and dark activity (entries) respectively. Values are mean±S.D. (n=7). Significant differences by Newman Keuls test: **p<0.01 from open filed, **p<0.01 from light/dark time, *p<0.01 from light and dark entries following one-way ANOVA.

Fig. (6). Shows the behavioral effect of PEEI on rats.

Figure (**6a**) shows the effect of PEEI on open field activity. Statistical analysis was performed on the data using one-way ANOVA (df3,18); (F=36.52) (**p<0.01), indicating a significant effect (significant decrease). Newman-Keuls test was also performed. Individual difference shows that the application of PEEI resulted in a significant decrease in T as compared to P and C.

Figure (**6b**) shows the effect of PEEI on home cage activity or skinner's box activity. Statistical analysis was performed on the data using one-way ANOVA (df3, 18); (F=0.6835) (p>0.05) indicating a non-significant effect. Newman-Keuls test was also performed. Individual difference shows that the application of PEEI did not result in any change in T as compared to P and C.

Figure (**6c**) shows the effect of PEEI on activity of rat on time spent in the light/dark compartment. The data on time spent in the light compartment were analyzed by one way ANOVA showed significant effect of PEEI (F=30.95) (df3,18) (**p<0.01). Analysis showed that the time spent in light compartment has significantly decreased. Newman-Keuls test was also performed. Individual difference shows that the application of PEEI resulted in a significant decrease in T as compared to P and C.

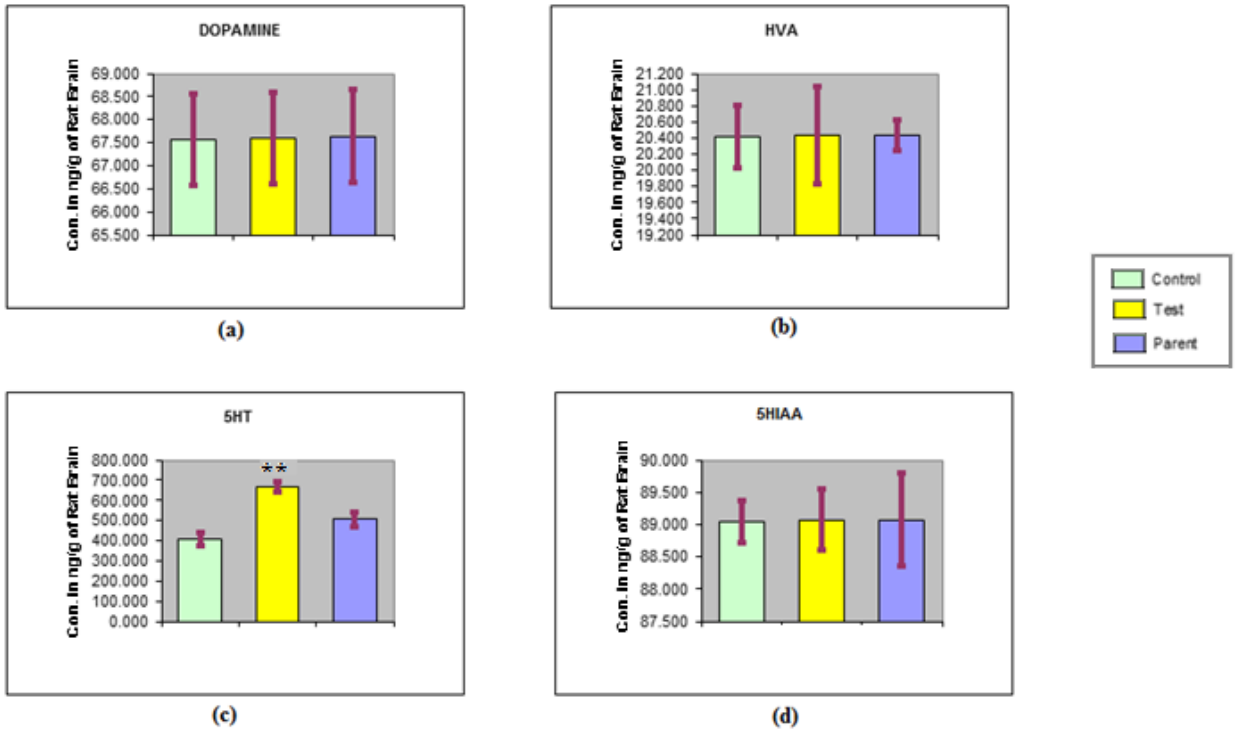
Figure (**6d**) shows the effect of PEEI on activity of rat in a light/dark box. The data on number of entries analyzed by one way ANOVA showed significant effect (F=14.382) (df3,18) (*p<0.01). Analysis showed that the entries in light/dark box are significantly decreased. Newman-Keuls test was also performed. Individual difference shows that the application of PEEI resulted in a significant decrease in T as compared to P and C.

Figure (**7a**) shows the effect of PEEI on Dopamine. The data is analyzed by one way ANOVA and showed non-significant effect; (F=0.00025) (df 3,18) (p>0.05). Newman-Keuls test was also performed. Individual difference shows that the application of PEEI did not result in any change in T as compared to P and C, in the whole brain.

Figure (**7b**) shows the effect of PEEI on HVA. The data is analysed by one-way ANOVA and showed non-significant effects; (F=0.000032) (df 3,18) (p>0.05). Newman Keul's test was also performed. Individual difference shows that the application of PEEI did not result in any change in T as compared to P and C in whole brain.

Figure (**7c**) shows the effect of PEEI on 5HT. The data is analysed by one-way ANOVA and showed a significant effect; (F=25.59) (df 3,18) (**p<0.01). Newman-Keuls test was also performed. Individual difference shows that the application of PEEI resulted in a significant increase in T as compared to P and C in whole brain.

Figure (**7b**) shows the effect of PEEI on 5HIAA. The data is analysed by one-way ANOVA and showed non-



Effect of 30mg/kg of indole substituted piperidine derivative PEEI on (a) Dopamine, (b) HVA, (c) 5HT & (d) 5HIAA respectively. Values are mean±S.D. (n=7). Significant differences by Newman Keuls test: **p<0.01 from 5HT following one-way ANOVA.

Fig. (7). Shows the effects of PEEI on neurochemical activity of rats.

significant effects; (F=0.002) (df 3,18) (p>0.05). Newman Keul's test was also performed. Individual difference shows that the application of PEEI did not result in any change in T as compared to P and C in whole brain.

DISCUSSION AND CONCLUSION

The present study shows that administration of indole substituted piperidine compounds PMEI and PEEI (30mg/kg) elicited anxiety as animals exhibited fewer entries and less time spent in the light compartment of a light/dark box. This suggests that these compounds produce anxiety. PMEI produces more anxiety than PEEI. There is no effect in the home cage activity, but the open field activity is significantly decreased by PMEI and PEEI both, showing decreased locomotor activity. The decrease in locomotor activity was more pronounced in PMEI as compared to PEEI. It has been well established that activation of serotonergic system results in anxiogenic effect and hypolocomotion.

Neurochemical studies of both compounds shows that there is no change in dopamine HVA and 5HIAA but there is increase in 5HT level following the administration of PMEI and PEEI. The increase in 5HT concentration following the administration of PMEI and PEEI suggests that the compounds may inhibit the activity of degradative enzyme MAO (monoaminoxidase) but inability of both of these compounds in increasing 5HT concentration suggests that the effect is not mediated via an inhibition of MAO-A enzyme. It is more relevant that the drug like SSRIs inhibit high affinity reuptake of serotonin to increase extracellular levels of 5HT which is now less available to MAO.

A greater central effect of PMEI than PEEI is explainable in terms of greater lipophilic nature of PMEI than PEEI. The results suggest that these compounds have potential antidepressant effects; however studies on animal models of depression are required to confirm antidepressant potential of these drugs.

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