Behavioral and Biochemical Studies of Piperidine Related Adrenolytic Compound and It’s Effects on Animals

Dr. Rana Kausar¹, Nazish Waris¹, Asfia Raza², Kanza Siddiqui³

Department of Biochemistry Federal Urdu University, Karachi-Pakistan.

ABSTRACT: The present study concerns behavioral and biochemical effects of 4 amino methyl piperidine substituted 2-bromo ethyl benzene derivative compounds. With intraperitoneal injection in rats, locomotor activity was significantly decreased in open field experiment, anxiolytic effect observed in light and dark apparatus significantly increased while stimulatory activity of compound monitor in home cage apparatus significantly decreased. Moreover, blood glucose and cholesterol level also examined. Present synthetic compound decreases blood glucose level and also decreases the total serum cholesterol, suggested that these 4 amino methyl piperidine substituted 2-bromo ethyl benzene compounds may be effective as a drug for treatment of anxiety, enhancement of locomotion. It is also beneficial for cardiac and diabetic patient.

Keywords: piperidine, adrenolytic compound, locomotor activity, stimulatory activity, glucose level, cholesterol level.

I. INTRODUCTION

Piperidine is an organic compound, found in many foods like vegetables, milk, dry coffee, boiled beef, pulverized white pepper and also black pepper(1-3). In mammal’s body, piperidine synthesizes from amino acid lysine. In mammals including human, piperidine excrete in urine in several mg/L under physiological conditions.(4) Piperidine used in the manufacturing of local anesthetics, analgesics and other pharmaceutical products.(5) Due to conformational flexibility piperidine analogue possess antidiabetic, analgesic, anticancer and hypotensive activity.(6,7) Piperidine considered as a leading nucleus which have important pharmacological activities due to best receptor binding and structure activity relationships.(8) Adrenolytic compound also called adrenergic blockers or adrenergic antagonist, a type of sympatholytic agent. It is a pharmaceutical substance which inhibits the action of adrenergic nerves as epinephrine (EP) and norepinephrine (NE) (9,10). Adrenolytic drugs bind with adrenergic receptor and decreases the level of adrenergic neurotransmitters (NM), EP, NE and dopamine (DA) are collectively called catecholamine(11). EP and NE are neurotransmitters as well as hormones(12). These are produced in the neuronal cells and chromaffin cells, play an important role in fight and flight responses (13). NEP is a precursor of EP, control responding actions(14). They increase the contraction of heart (15). Adrenergic receptors are sub-divided into two types as alpha and beta depending on the basis of their responses to sympathomimetic or sympatholytic drug. Alpha receptor is classified into two types alpha 1 and alpha 2 and beta receptor are classified into three sub-types beta 1, beta 2 and beta 3(16). Adrenolytic drugs block the actions of these adrenergic receptor used in management of hypertension, vasospasm, peripheral vascular disease, shock and pheochromocytoma, heart disease, arrhythmias, migraine, glaucoma and anxiety.(17) In the present study, the newly synthesized compound is antagonist to EP and NE structurally, but the functions are different. The advantage of this compound is that it decreases the blood glucose level and cholesterol level as compare to normal mechanism.

II. Materials And Methods

Synthetic compound

The amount of 2 bromo ethyl benzene with molecular weight 185.07 solid in nature, required in 3.7 gm and 4 amino methyl piperidinewith molecular weight of 114.19, liquid in nature, required in 1.14 gm. These two reactants were dissolved in acetone separately, then mixed, reaction mixture was stirred for 84 hours at low temperature 50 to 53 °C. The process of reaction was observed through thin layer chromatography. The crude solid product was filtered and washed with acetone; the product thus obtained was purified through recrystallization by using methanol and ethyl acetate. The pure compound was dried in desiccator over anhydrous calcium sulfate.
Characterization of compounds:

**Physical state** brown color
**Melting point** 180 °C
**Yield** 71%
**UV λ (MeOH) nm** 261, 217, 193, 181.
**IR ν max (KBr) cm⁻¹** 4331, 2918, 1710, 1571, 1490, 830, 520.
**EIMSm (Z):** 437, 213, 219, 198, 183, 158, 149, 130, 78
**H-NMR (DMSO 300MHZ) r**
- 1.72-1.7 (m, 2H, H-8) 2.07 – 2.18, (m, 2H, H-9) 2.47-2.52m, 4H, H3a, H3b, H5a)

**Preparation Of Injection Of Synthetic Compound To The Rats**

Synthetic compound was dissolved in 0.9% saline. But, in saline it was partially soluble. So, by adding two drops of Dimethyl sulfoxide(DMSO) compound was completely soluble. Rats were injected intraperitoneally (i.p) with 98% compound. For the preparation of saline 4.5gm Nacl was dissolved in 500ml water and then, it was freezed to get chilled saline.

**Experimental Protocol**

Locally bred male albino Wister rats being about 180 to 210 gram on arrival purchased from animal house of research institute of Aga khan university, Karachi, Pakistan were used throughout the experiment. The study was approved by Ethical committee of Federal Urdu University of Arts Science and Technology.
The rats were placed individually in specially designed cage with saw dust cover floor in a quiet room with three axes, two cubes of standard food and water at least 4 days before starting the experiment. So, the rats could adopt themselves to new environment. The room temperature was maintained between 24°C to 25°C. Rats were categorized into 3 groups, control group (CG), test group (TG) and parent group (PG), in each group six rats were included. After 4 days, rats were injected intraperitoneally as synthetic compound was injected to test group of rats, 30mg/kg body weight, control group (CG) was injected with saline and parent group (PG) was injected with 2-bromo ethyl benzene.

After ½ hour of injection activity was monitored for 5 minutes in home cage which was specially designed made up of Perspex (26x26x26cm), floor was soft due to saw dust. For the next 5 minutes the activity was monitored in the Open field, and the Open field apparatus consisted of box, having square area (76x76cm) with walls of 42cm high, floor of the apparatus was divided by lines into 25 squares having equal size. Last 5 minutes, in light and dark environment. The light and dark apparatus was made up of two compartments and small passage was present between two compartment due to which rats could easily move to either compartment. After monitoring these activities, the animals returned to their cages. Rats were decapitated, after 20 hours of injection. Whole plasma was collected and stored at low temperature (-70°C) until analyzed for biochemical analysis. Concentration of glucose and cholesterol were estimated by CHOD PAP enzymatic endpoint test method.

Statistical analysis:
Results were represented as mean ±SD (n=6), data was analyzed by using one-way ANOVA. Significant difference was considered by Newman-keuls test p>0.01 level from TG, CG and PG.

III. Results

Fig 1(a) shows synthetic compound effect on home cage of CG, TG, and PG, of rats. Statistical analysis by one-way ANOVA (df2,18) (f=49.65) (p<0.01). It shows that after administration of compound in TG the home cage activity significantly decreased as compared to CG and TG male rats. Fig 1(b) Statistical analysis by one-way ANOVA are (df2,18) (f=69.87) (p<0.01) respectively. It shows that after administration of compound in TG the Novel environment behavior increases as compared to PG and CG group of male rats.

Fig 1(c) Statistical analysis by one-way ANOVA (df2,18)(f=13.348) (p<0.01) show that after administration of compound in TG, the entries in light portion was increased as compared to CG and PG group of male rats. Fig 1 (d) results of Statistical analysis by one-way ANOVA are (df2,18)(f=137.91) (p<0.01) show that after administration of compound in TG, the rats spend their more time in light portion as compared to control and parent group of rats.

Fig 1(e) show the synthetic compound effect on concentrations of glucose, in the whole brain of PG, TG and CG. Statistical analysis by one-way ANOVA (df2,18) (f=26.685) (p<0.01) indicate that after administration of compound concentration of glucose has high significant effect in TG as compare to CG but remain under normal range in PG as compare to PG and CG. Fig 1 (f) show the synthetic compound effect on concentrations of cholesterol, in the whole brain of PG, TG and CG. Statistical analysis by one-way ANOVA (df2,18)(f=123.758) (p<0.01) show that after administration of compound concentration of cholesterol has less in TG as compare to PG and CG.
IV. Discussion

Previously, it was studied that adrenolytic drugs bind with adrenergic receptor but block their actions, used in management of hypertension, vasospasm, peripheral vascular disease, shock, and pheochromocytoma. (18) Beta blocker activity occurs when it contains aromatic ring and beta-ethanolamine means, these two things are necessary for the activity of beta blocker, aromatic ring may be benzo heterocyclic or heterocyclic (19). Home cage activity is used to determine the relation between activity and performance, if adrenergic system is activated and function normally the activity of rodents in home cage is normal, but if catecholamine decreases or increases then these activities are affected. Likewise, if adrenergic blocking agents as adrenolytic drugs are given to rats so, locomotor activity of rats will decrease. In the present study, results were monitored before administration of compound and after twenty hours of administration of compounds but, only the results after administering the compound were discussed. Treated group of rats show slower stimulatory activity such as (grooming, gnawing, crossing one corner to other corner, cutting their dust) but good results are produced in parent compounds treated rats as compared to control and test indicate that new synthetic compound act as antagonist. Previous study also suggest that propranolol that is beta 2 antagonist reduces exploratory activity (20). In test group, locomotor activity of rats increases as compared to control in normal day light and without any noise and stress as well as standing behavior also increases show the good response of compound. Previously, adrenolytic compound antagonize clonidine induced locomotor stimulation (21). But, this compound gives beneficial result by increasing locomotor activity of test rats. Light and dark box activity was monitored to determine the anxiogenic and anxiolytic effect. Adrenolytic compound reduced symptoms of anxiety (22). In present study, test rats spend more time in light box, their entries in light box also increased which indicates that new synthetic compound reduced anxiety in rats. Previously, Olympic marksmen used beta blocker for the enhancement of performance (23). Blood glucose level is maintained by insulin hormone, insulin secreted by beta cell of pancreatic islets. Glucagon is another hormone, which increase blood glucose level it is secreted by alpha cell of pancreatic islets, epinephrine produced same effect as glucagon which result in increased blood glucose level.
glucose level. Adrenolytic compounds block the action of epinephrine which results in decrease blood glucose level. Previous studies indicate that beta blocker inhibit insulin secretion and alpha blocker stimulate insulin secretion (24). So, it indicates beta blocker increase blood glucose level while alpha blocker decrease. Other studies also show that alpha and beta blocker reduced nicotine induced hyperglycemia (25). Other studies also show that adrenergic blocker increase plasma insulin level (26). In the present study, after administration of synthetic compound to rat's blood glucose level decreases as compared to control, so it is concluded that this compound produce hypoglycemic effect. Cholesterol level was also decreased in test group of rats. Vasodilating beta blocker reduce coronary artery diseases and more favorable effects on lipid profile such as, prazosin significantly reduced total cholesterol (27). So, the present compound is beneficial for hypertension and coronary heart diseases because it produces beneficial effect on cholesterol level.

V. CONCLUSION

It is concluded that new compound act as antagonist with good behavioral responses. As, it improves behavior, increases locomotor activity, decrease anxiety but it reduces stimulatory activity. Biochemical studies show that this compound significantly reduced blood glucose level and total cholesterol. So, it is beneficial for diabetic patient and it also reduces total cholesterol keep importance for hypertensive and cardiac patient.

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