BEHAVIOURAL, BIOCHEMICAL AND HAEMATOLOGICAL STUDY OF PIPERIDINE RELATED ADRENERGIC COMPOUND AND IT’S EFFECTS ON RATS

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ABSTRACT

The present study concerns behavioural, biochemical and haematological effects of piperidine substituted 2 chloro 3,4, dihydroxyacetophenone derivative compounds. Interperitonial injection in rats significantly increases locomotors activity in open field experiment, stimulatory activity in home cage and anxiolytic effects of the newly synthesized compounds observed in light and dark apparatus were significantly increased. Biochemical effects of glucose and cholesterol were checked by kit (CHOD-PAP) method, which showed that after administration of compound, Concentration of glucose was significantly decreased and concentration of cholesterol was increased (but remain under normal range) as compare to control. Haematological study was also done by RBC, the value of RBC was also significantly increased, show proper function of RBC in microcirculation. Studies suggested that these piperidine substituted 2 chloro 3,4, dihydroxyacetophenone derivative compounds may be effective as a drug for treatment of hyperglycemia, depression, enhancement of locomotion and stimulation.

Key words: Glucose, Cholesterol, RBC, locomotion, stimulation and anxiety.
INTRODUCTION

Piperidine is an organic compound. Piperidine occurs naturally in various plants and roots, in tobacco leaf, (1) and is commonly detected in other natural food(2). Piperidine derivative has been found to occur in brain, skin and urine of animals and in the brain, cerebrospinal fluid, and urine of humans (3). Piperidine derivative has number of effects in human body. It is also used to synthesized adrenergic (EP and NEP) related compound.(4) Which has various pharmacological and therapeutic effects by increasing or decreasing the activity of several components of sympathetic division of the autonomic nervous system.(5) Similar effects are produced to stimulate the sympathetic nervous activity are known as sympathomimetic or adrenergic stimulant. (6) Epinephrine (EP) & Nor epinephrine (NE) are biosynthesized in certain neurons of CNS known as Adrenergic neurotransmitters .(7) It was previously studied that if EP & NE like drugs was administered in the body, it produces no effect on the CNS because it is unable to cross the blood brain barrier (BBB). (8) Where as only precursor has ability to cross the BBB. Adrenergic receptor agonists have number of role in the body, play important role for relief of nasal congestion and asthma. They also increases ionotropic activity of the heart(9). Another major role is to increase blood glucose level(10). β adrenergic receptor agonist have also various functions , majorly these types of agents effects, bronchospasm, broncheodialator, and also use for fresh mood. Anxiety and depression also depend upon the secretion of these EP and NE ,if the secretion are not proper which was observed are general in all region of the world.(11) Previously, it was reported that some drugs were synthesized to remove these problems and it was also previously reported that naturally occurring compounds are catecholamines containing catechol nucleus that have β phenyl amine, on the meta and para position of the aromatic ring , and on the amino α and β. In the present study the newly synthesized compound was very closely related to EP and NE structurally and the functions are also similar but the advantage of this compound is that it decreases the blood glucose level as compare to normal mechanism.

MATERIAL AND METHOD

SYNTHETIC PROTOCOL OF ADRENERGIC RELATED COMPOUND

4 methyl piperidine and 2 chloro 3,4 dihydroxyacetophenone, These two reactants were dissolved in acetone separately, then mixed, reaction mixture was stirred for 60 hours at low temperature 53 to 54 °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 recrystalization by using warm methanol and ethyl acetate. The pure compound was dried in desiccator over anhydrous Clciumsulfate.

REACTION
CHARACTERIZATION OF COMPOUNDS

(3,4-dihydroxyphenyl)-2-(3-methylpiperidin-1-yl)ethan-1-one (I)

Color and physical state; dark brown

Melting points: 217±2°C
Yield; 79%

MW = 235.322s

UV λmax (MEOH) 206, 234 nm.

IR Vmax (KBr) 2945, 2749, 1743, 1683, 1511, 1452 cm⁻¹

EIMS m/z 250{M+ -Br C14 H 21 NO2} 152, 137, 123, 112, 98, 91, 81, 70, 55.

H-NMR (300MHz, CD3OD) δ 7.52(s, 1H, H-13), 7.35-7.32(d, 1H, J=9Hz, H-17) 6.71-6.69(d, 1H, J=6Hz, H-16), 2.90-2.89(t, 2H, H-7), 2.83-2.81(t, 2H, H-8), 2.67-2.65(t, 1H, H-2), 1.79-1.77(t, 1H, H-6), 1.7-1.61(t, 2H, H-5, H-3), 1.62-1.60 (t, 2H, H-5, H-3), 0.94-0.92(d, 2H, J=6Hz, CH3).

QUALITY OF ANIMALS

Locally bred male albino Wister rats weighing about 180 to 210 gm on arrival purchased from animal house, research institute of Agha Khan University Karachi Pakistan was used throughout the experiments. The rates were housed individually in specially designed cages with saw dust cover floor in a quiet room, with free access to cubes of standard rats food and water for at least, four days before starting the experiment. So that the rates could be able to adapt them, to the new environment.

INJECTION OF SYNTHETIC COMPOUND TO THE RATS

Synthetic compound injected to test group of rats, this compound was dissolve in saline. But in saline, it was partially soluble. So, by adding two drops of DMSO (dimethyl sulfoxide) completely soluble. Rats were injected intraperitoneally (ip) with 98% compound, saline and parent compound.

EXPERIMENTAL PROTOCOL

Three groups of locally bred male albino Wister rats weighing about 180 to 210 gm on arrival purchased from animal house, research institute of Agha Khan University Karachi Pakistan. The rates were housed individually in specially designed cages with saw dust cover floor in a quiet room, with free access to cubes of standard rats food and water for at least, four days before starting the experiment. So that the rates could be able to adapt them, to the new environment. After four days, rats were injected interperitoneally 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 chloro 3,4 dihydroxyacetophenone. After fourteen hour of injection activity was monitored for ten minutes in light and dark environment. Light and dark activity is specific for anxiety the apparatus used in light and dark experiment consisted of small square area (26x26x26 cm) with an access (12x12 cm) walls of one compartment was transparent and other dark. For the next five minutes the activity was monitored in the open field, and the open field apparatus consists of a square area (76×76cm) with walls of 42cm high. The floor divided by lines into 25 equal squares.

Last ten minutes in home cage specially designed made up of Perspex (26 × 26 × 26 cm) with saw dust covered floor was used for this purpose. After monitoring these activities, the animals returned...
to their cages. Rats were decapitated after 18 hours of injection. Whole plasma was collected and stored at low temperature (-70°C) until analyzed for biochemical and hematological analysis. Concentration of glucose and cholesterol were estimated by CHOD PAP enzymatic endpoint test method. RBC was estimated by automatic hematology analyzer BC-5800, Mindray Automatic hematology analyzer.

STATISTICAL ANALYSIS

Results were represented as mean, ± SD (n= 6) significant difference by Newman-keuls test p>0.01 level from TG, CG,and PG following one-way ANOVA.

RESULTS

Fig 1(a) show synthetic compound effect on home cage of CG, TG and PG of rats. Statistical analysis by one-way ANOVA is (df2,15) (f=20.696) (p<0.01) shows that after administration of compound in TG the home cage activity significantly increased as compared to CG male rats.

Fig 1(b) treated rats, in novel environment, Statistical analysis by one-way ANOVA are (df2,15)(f=387.571) (p<0.01) 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) treated rats, Statistical analysis by one-way ANOVA (df2,15) (f=1.97) (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 rat. Fig 1(d) show synthetic compound effects on PG, TG and CG treated rats. Statistical analysis by one-way ANOVA (df2,15) (71.613) (p<0.01) show that after administration of compound in TG, the rats spend their more time in light portion as compared to CG and PG group of male rats.

Fig 1(e) Statistical analysis by one-way ANOVA (df2,15) (f=250.506) (p<0.01) show the synthetic compound effect on concentrations of glucose, in the whole brain of PG, TG and CG. Results indicate that after administration of compound concentration of glucose has less effect in TG as compare to PG and CG. Fig 2 (f) Statistical analysis by one-way ANOVA (df2,15)(f=73.625)(p<0.01) show that after administration of compound concentration of cholesterol has high significant effect in TG as compare to CG but remain under normal range.

Fig 1(g) treated rats, Statistical analysis by one-way ANOVA (df2,15) (F =115.2) (p<0.01) show the synthetic compound effect on concentrations of RBC, in the whole blood of PG, TG and CG. It indicate that after administration of compound concentration of RBC has good effect in TG as compare to CG.
Values are mean, ± SD (n= 6) significant difference by Newman-keuls test p> 0.01 level from TG, CG, and PG following one-way ANOVA. Fig 1. Effect of compound on (a) home cage activity, (b) Open field activity, (c) light and dark activity (entries) (d) light and dark activity (time in seconds), (e) blood glucose, (f) serum cholesterol, (g) Red blood carpuscles respectively.

**DISCUSSION**

Previously it was studied that if, parent structure for sympathomimetic drugs is substituted by β phenylamine, on the meta and para positions of the aeromatic ring, and on the amino α and β
position of the ethylamine side chain, it not only authorizes the mechanism of sympathomimetic events but also the receptor selectivity of the drug. An extensive scientist also supports modulation of glutamate and γ-aminobutyric acid (GABA) transmission by cannabinoids, enhancing proof supports an action on catecholamines, several central functions that are related compounds in his life due to pressure full work and such a person was sometimes described as getting excess energy from life. (12) which was majorly used to describe individuals who enjoyed risky behavior, for the adrenaline "junkii" (13). The major physiologic process of adrenaline release center only stresses that is physical threat, excitement, noise, bright lights, and high temperature. All of these effects are processed in the central nervous system. It was previously reported that norepinephrine caused a decrease in cell deformation at a concentration of 10^-5M, at low shear stresses. (14) It was also reported that isoprenaline, a β adrenergic agonist, improved mechanical properties at lower concentrations (10^-5M and 10^-7M) and low stresses. (14) Now present study suggested that after administration of compound stimulatory activity as shown in fig 1(a) increases, locomotor activity in fig 1(b) also increases and anxiety decreases as shown in fig 1(c) and (d), because time spend and entries in light box increases. As shown in fig 1(e) level of glucose decreases, while previous studies shows that normally, adrenergic related compound increases blood glucose level. Epinephrine is a nonselective agonist of all adrenergic receptors, including α1, α2, β1, β2, and β3 receptors. (15) Adrenaline's binding to these receptors perform various metabolic changes. When it binds to α-adrenergic receptors, inhibits insulin secretion by the pancreas, stimulates glycogenolysis in the liver and muscles. (16) It also stimulates glycolysis in muscle. (17) β-Adrenergic receptor binding initiates glucagon secretion in the pancreas, increased adrenocorticotropic hormone (ACTH) secretion by the pituitary gland (18) and increased lipolysis by adipose tissues. Fig 1(f) shows level of cholesterol significantly increases as normal process like adrenergic compound, while fig 1(g) show that level of RBC also significantly increases, indicating good effect of compound on RBC.

CONCLUSION
The present studies shown better activity in the behavioural, biochemical and haematological studies, as the behavioural studies was increased by locomotor activity stimulatory activity and decrease anxiety as compare to control. Biochemical studies was checked by glucose and cholesterol. Concentration of glucose was significantly decreased and concentration of cholesterol was increased (but remain under normal range) as compare to control. Haematological study was also done by RBC, the value of RBC was also significantly increased, show proper function of RBC in microcirculation. So, our present study conclude that this compound may be used as adrenergic drug for good performance, depression and to control blood glucose level.

REFERENCES:


