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Clenbuterol causes changes in behavior- A new model to induce anxiety in mice

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ABSTRACT

The present study aimed to study the potential of clenbuterol to induce anxiety and behavioral modification in mice. A pilot study was conducted to select the dose and duration of clenbuterol; in which clenbuterol was administered in 3 doses (0.5, 1, 1.5 mg/kg; *i.p.*) for 1, 7, 15 days. As per pilot study results, single dose of clenbuterol (1 mg/kg; *i.p.*) was selected for further evaluation. The behavioral assessment was done to quantify the level of anxiety in animals using elevated zero maze, mirrored chamber, rota rod apparatus and open field test. After behavioral evaluation, the animals were sacrificed and their brains were isolated, homogenized and centrifuged for estimation of TBARS, GSH, nitrite and catalase levels in the brain. Administration of Clenbuterol (1 mg/kg; *i.p.*) to mice caused the decrease in the time spent and number of entries in open arm but increase in latency time in elevated zero maze, increase in ambulation and grooming in open field test, decrease in number of entries & time spent in mirror chamber test and increase in the fall of time in rotarod test. Furthermore, induction of anxiety to mice caused the increase ($p<0.05$) in brain TBARS and nitrite levels. Thus, clenbuterol may prove to be a useful agent for inducing anxiety and behavioral alterations in mice.

Keywords: Clenbuterol, Anxiety, Animal model, Behavior.

1. INTRODUCTION

Anxiety is defined as, "an aversive emotional and motivational state occurring in threatening situations". The term can be used to describe an unpleasant state with an anticipation of imminent danger or fear about some defined or undefined threats. Sensations of anxiety are a part of human experience, but excessive or inappropriate anxiety can become an illness. It is an emotion that allows an individual to prepare for or respond to changes in the environment [1]. Anxiety is classified in eight different groups as (i) Generalized anxiety disorder (GAD): It is a common chronic disorder characterized by long-lasting anxiety that is not focused on any one object or situation So, person persistent fear and worry and become overly concerned with everyday matters [2]. (ii) Panic disorder: It is condition in which a person suffers from brief attacks of intense terror and apprehension, often marked by trembling, shaking, confusion, dizziness, nausea, difficulty breathing [3]. (iii) Phobias: It is single largest category of anxiety disorders which includes all cases in which fear and anxiety is triggered by a specific stimulus or situation. (iv) Agoraphobia: It is specific anxiety about being in a place or situation where escape is difficult or embarrassing or where help may be unavailable. (v) Social anxiety disorder (SAD): It is described as an intense fear of negative public scrutiny or of public embarrassment or humiliation. (vi) Obsessive-compulsive disorder (OCD): It is a type of anxiety disorder primarily characterized by repetitive obsessions (distressing, persistent, and intrusive thoughts or images) and compulsions urges to perform specific acts or rituals [4]. (vii) Post-traumatic stress disorder (PTSD): It is results from an extreme situation, such as combat, natural disaster, rape, hostage situations, more serious kinds of child abuse, or even a serious accident. (viii) Separation anxiety disorder: It is the feeling of excessive and inappropriate levels of anxiety over being separated from a person or place. Anxiety is extremely common, dramatic and debilitating disorders and it is now becoming clear that without knowledge of both clinical and biological aspects of anxiety, it is impossible to offer effective treatment strategies for the patients [5].

We use animal models as experimental preparations developed in one species for the purposes of studying phenomenon occurring in another species [6]. Mice and humans share more than 90% of their genes, and animal models seem to be a useful tool in biomedical sciences, as evidenced by a notable increase in the number of active laboratories working in the field [7]. Furthermore, animal models are particularly of help in situations when the impact of stress cannot be studied in humans because of ethical and other like reasons.

However, the choice of which biological factors correlates to study is not easy, since problems with animal models of human psychic disorders include: The difference between human and non human nervous systems, the difficulty in determining analogous behaviors among species, The need in extrapolation of results from animals to humans. Such problems most likely reflect a significant difference in etiology and complexity of anxious behaviors. In literature, the term "animal model of anxiety" is used for change in anxiety-related behavior of animals and test assays conceptualized to assess anxiety-related behavior in animals [8].

Today, with the growing number of medical professionals being involved in basic research, and neuroscientists involved in clinically oriented studies, an interdisciplinary view of neurobiology of anxiety and depression, linking human data to animal experimentation, is becoming extremely important.However, no sufficient studies have been carried out to explore the role of Clenbuterolin the anxiety, to best of our knowledge. Therefore, the present study was undertaken to investigate the potential of clenbuterol to induce anxiety and behavioral modification in mice.

2. MATERIALS AND METHODS

2.1. Animals

Adult Swiss mice (either sex), weighing between 20-30 g, were procured from CPCSEA registered approved breeder. The animals were kept in quarantine section till monitoring of health status of animals and subsequently transferred to the housing area. The animals were acclimatized for seven days to the housing conditions of Central Animal House Facility of ASBASJSM College of Pharmacy, BELA prior to experiments. Animals were housed in polypropylene cages with dust free rice husk as a bedding material and maintained under standard laboratory conditions with controlled temperature (23±2°C), humidity (40±10%) and natural (12 h each) light-dark cycle. The animals were fed with standard rodent pellet diet (Ashirwad Industries, Mohali) and water *ad libitum.* The experiment was carried out between 09:00 and 18:00 h. The care of laboratory animals was done following the guidelines of CPCSEA, Ministry of Forests & Environment, Government of India.

2.2. Drugs and Chemicals

Clenbuterolwas procured from the Max-tremePharma. Thiobarbituric acid, 5,5′-dithiobis (2-nitrobenzoic acid), Tris buffer, trichloroacetic acid was procured from Himedia laboratories, Mumbai. Reduced glutathione was procured from SRL. Sodium hydroxide, Sodium Dihydrogen phosphate was procured from Lobachemie.

2.3. Experimental Design

Animals were divided into 5 different groups comprised of 6 animals.A pilot study to select the dose and duration of clenbuterol was conducted using 27 mice. Clenbuterol was administered in 3 doses (0.5 mg, 1 mg, 1.5 mg/kg; *i.p.*) [9] in separate groups of animals comprising 3 mice in each group. These groups were screened for anxiogenic property of Clenbuterol on 1, 7 and 15 day. On the basis of the pilot study results, Clenbuterol (1 mg/kg) was selected for further investigations. Caffeine (25 mg/kg; *i.p.*) for 7 days served as standard anxiogenic agent. Propranolol (2 mg/kg; *i.p.*) served as standard anxiolytic agent [10]. Food intake, body weight and locomotor activity of the mice were evaluated at the start and end of experiment. The animals were evaluated for induction of behavioral alterations using Elevated zero maze, Mirrored chamber test, Rota rod test and Open field test. Afterwards, the animals were sacrificed for brain Lipid peroxidation, Catalase estimation, nitrite and reduced glutathione estimations. The results were analyzed using one way ANOVA followed by Dunnett's t-test.

Groups Employed

All the animals were divided into five groups of six animals.

Group I – (Normal Control): Normal Saline was administered to mice.

Group II – (Caffeine): 25 mg/kg;i.p. for 7 days

Group III – (Clenbuterol): 1 mg/kg;i.p. for 1 day

Group IV – (Caffeine + Propranolol): Caffeine 25 mg/kg; i.p. for 7 days + Propranolol (2 mg/kg; i.p.) 30 minutes before caffeine administration.

Group V – (Clenbuetrol + Propranolol): Clenbuterol 1 mg/kg; i.p. + Propranolol (2 mg/kg; i.p.) 30 minutes before clenbuterol administration.

2.4. Elevated Zero Maze

The elevated zero maze (EZM) is a sensitive behavioral test that reveals animal neophobia or anxiety and can be used to unveil antineophobic or anxiolytic actions of drugs. This maze is an elevated (40 cm) from ground painted black in color, annular having outer diameter of 45 cm and inner diameter of 30 cm. The runway ring where the mouse can explore is of 6 cm width, which is divided into 4 quadrants, 2 opposing "open" quadrants without walls and 2 opposing "closed" quadrants having 12 cm high walls. The open quadrants have a ridge of 2-3 mm to prevent the mouse to fall off. The walls have thickness of 0.75 cm. Animals were individually placed in closed arm facing towards the open arm and the following parameters were noted for a period of five minutes [11].

- Latency to enter the open arm (LEO):Latency is the time gap between the first entry of animal in open arm after placing it in the closed arm and signifies the behavior of animal. In the condition of anxiety the latency time increases significantly as compared to normal animals.
- Time spent in open arm (TSO): Average time spent in open arm by the animal indicates the anxiety level. Lower the anxiety level of animal, more the time animal spent in open arm.
- Total number of entries in the open arm (NEO):The frequency of entry of animal in the open arm indicates the behaviour of animal. Higher the frequency of entry in open arm lower is the level of anxiety.

2.5. Rotarod Test

The loss of muscle-grip strength is an indication of muscle relaxation. This effect can be easily studied in animals using inclined plane or rotating rods. The difference in the fall off time from rotating rod between control and treated animals is taken as an index of muscle relaxation. Mice were subjected to motor function evaluation by placing them individually on Rota rod, which was adjusted to the speed of 25 rpm. The fall-off time was recorded for each mouse and the longest period any animal was kept on the rod was 300 s [12].

2.6. Mirrored Chamber Test

The mirror chamber consisted of a wooden chamber with a mirror cube open on one side, and the mirror cube was placed into a square plexiglass box. The box (40 cm×40cm ×30.5 cm) had a white floor and opaque black walls. The mirrored cube (30 cm×30 cm ×30 cm) was made up of 5 pieces of mirrored glass with one mirrored side and an opposite side painted dark brown. In the standard configuration, the mirrored surfaces (3 side panes, a top pane and the floor pane) face the interior of the cube. Placement of the mirrored cube into the center of the container forms a 5 cm corridor completely surrounding the mirrored chamber. The animal was placed individually at the distal corner of the mirror chamber at the beginning of the test. During the 5-min test, the following parameters were evaluated [13].

- Latency to enter the mirror chamber: Latency is the time gap between the first entry of animal in mirror chamber after placing it in the corner of the mirror chamber and signifies the behaviour of animal. In the condition of anxiety the latency time increases significantly as compared to normal animals.
- Number of entries in mirror chamber: The frequency of entry of animal in the mirror chamber indicates the behaviour of animal. Higher the frequency of entry in mirror chamber lower is the level of anxiety.Total time spent in mirror chamber: Average time spent in the mirror chamber by the animal indicates the anxiety level. Lower the anxiety level of animal, more the time animal spent in mirror chamber.

2.7. Open Field Activity

Locomotor activity was studied in mice using the open-field test. Each animal was placed in the center of a square arena (60 × 60 × 35cm) with a white floor which was divided into 16 equal squares by black lines. Mice were randomly assigned to experimental groups for testing. The following parameters were noted for a period of five minutes [14].

Central area duration: The total amount of time the mouse spent in the arena centre.

Ambulation: It is the frequency with which the mice crossed one of the grid lines with all four paws.

Grooming: Duration of time the animal spent licking or scratching itself while stationary.

2.8. Brain Homogenate Preparation

All animals were sacrificed at the end of the study and brain was isolated. Tissue homogenates were prepared with 0.1 M phosphatebuffer (pH 7.4) and supernatant of homogenates was employed to estimate thiobarbituric acid reactive substances (TBARS), GSH, Catalase, nitrite level.

2.9. Measurement of Lipid Peroxidation

Brain homogenized with 0.1 M Phosphate buffer (pH 7.4) and supernatant was used for the measurement of thiobarbituric acid reactive substances (TBARS) at absorbance 532 nm by using U.V/Visible spectrophotometer (Shimadzu 1700, Singapore) [15].

2.10. Measurement of GSH

The GSH assay was performed by the method Ellman et al. Supernatant was used for the measurement of GSH at absorbance 412 nm by using U.V/Visible spectrophotometer. The concentrations were determined using a standard curve of reduced glutathione and the results were expressed as µM/ml [16].

2.11. Estimation of Nitrite Level

The nitrite levels were estimated by the acidic Griess reaction after reduction of nitrate to nitrite by vanadium trichloride according to the method described by Green et al. The concentrations were determined using a standard curve of sodium nitrate and the results were expressed as µM/mL [17].

2.12. Measurement of Catalase Activity

The assay mixture consisted of 3 ml of H_2O_2, phosphate buffer and 0.05 ml of supernatant of tissue homogenate (10%), and the change in absorbance was recorded at 240 nm. The results were expressed as micromoles of H_2O_2 decomposed per milligram of protein/min [18].

2.13. Statistical Analysis

All the results are expressed as Mean ± SEM. The data of all the groups were analyzed by oneway ANOVA followed by Tukey's test using software Graph Pad Prism 6 (Graph Pad Software Inc., USA). A value of $P<0.05$ was considered to be significant.

3. RESULTS

3.1. Effect of Clenbuterol on parameters of elevated zero maze

Administration of clenbuterol (1 mg/kg, *i.p.*) to mice showed significant ($p<0.05$) prolongation in LEO as compared to normal group which indicates successful induction of anxiety in clenbuterol group. Administration of caffeine (25 mg/kg; *i.p.*) for 7 days also showed significant ($p<0.05$) prolongation in LEO than normal group. Caffeine also showed significant ($p<0.05$) impact on LEO as compared to clenbuterol group. Co-administration of propranolol (2 mg/kg; *i.p.*) to mice before 30 minutes of clenbuterol and caffeine administration ($p<0.05$) reduced LEO as compared to respective caffeine and clenbuterol group.

The single dose of clenbuterol (1 mg/kg; *i.p.*) to mice caused a significant ($p<0.05$) decrease in NEO as compared to normal group. Administration of caffeine (25 mg/kg; *i.p.*) for 7 days showed significant ($p<0.05$) decrease in NEO than normal group. Caffeine also produce significant ($p<0.05$) impact on NEO as compared to clenbuterol group. Co-administration of propranolol (2 mg/kg; *i.p.*) to mice before 30 minutes of clenbuterol and caffeine administration significantly ($p<0.05$) antagonized the effect of clenbuterol and caffeine in EZM.

Intraperitoneal injection of Clenbuterol (1 mg/kg) showed significant ($p<0.05$) reduction in TSO as compared to normal group. Administration of caffeine (25 mg/kg; *i.p.*) for 7 days showed significant ($p<0.05$) decrease in TSO than normal group. Caffeine was able to produce significant ($p<0.05$) impact on TSO as compared to Clenbuterol group. Coadministration of propranolol (2 mg/kg; *i.p.*) to mice before 30 minutes of clenbuterol and caffeine administration significantly ($p<0.05$) increased the TSO in EZM.

3.2. Effect of Clenbuterol on fall off time in Rota-rod test

The active dose of clenbuterol (1 mg/kg; *i.p.*) to mice caused a significant ($p<0.05$) increase in FOT as compared to normal group. Administration of caffeine (25 mg/kg; *i.p.*) for 7 days showed

Table - 1: Effect of Clenbuterol on elevated Zero Maze parameters.

Groups	LEO (sec)	NEO	TSO (Sec)
Control	26.1±2.6	16±1	69.6±4.0
Caffeine	86.1±5.0[a]	4.8±1.1[a]	18±3.1[a]
Clenbuterol	52.1±4.4[a,b]	2±.1.2[a,b]	11±2.3[a,b]
Caffeine+Propranolol	68.8±4.4[b]	8.1±2.3[b]	53.3±5.6[b]
Clenbuterol+Propranolol	37.3±2.8[c,d]	5±1.7[c,d]	24.6±3.8[c,d]

Values are expressed as mean ± S.E.M. [a] denotes $p< 0.05$ compared to Control group, [b] denotes $p< 0.05$ compared to caffeine group, [c] denotes $p< 0.05$ compared to Clenbuterol, [d] denotes $p< 0.05$ compared to Caffeine + Propranolol. (One way ANOVA followed by Tukey's test).

Table - 2: Effectof Clenbuterol on mirror chamber parameters

Groups	FET (sec)	No. of entries	Time spent (sec)
Control	40.8±1.4	13±3.3	66.3±4.6
Caffeine	59±4[a]	2.8±0.7[a]	22.3±3.9[a]
Clenbuterol	67±2[a,b]	1.1±0.7[a,b]	3.1±1.1[a,b]
Caffeine+Propranolol	32±1.8[b]	8.8±3[b]	33.4±6.3[b]
Clenbuterol+Propranolol	46±3.8[c,d]	6.3±1.2[c,d]	10.1±1.3[c,d]

Values are expressed as mean ± S.E.M. [a] denotes $p< 0.05$ compared to Control group, [b] denotes $p< 0.05$ compared to caffeine group, [c] denotes $p< 0.05$ compared to Clenbuterol, [d] denotes $p< 0.05$ compared to Caffeine+ Propranolol. (One way ANOVA followed by Tukey's test.

significant ($p<0.05$) increase in FOT than normal group. The result of caffeine is significantly different from clenbuterol group on FOT. Also, no difference was observed when caffeine + propranolol group was compared to clenbuterol + propranolol group.

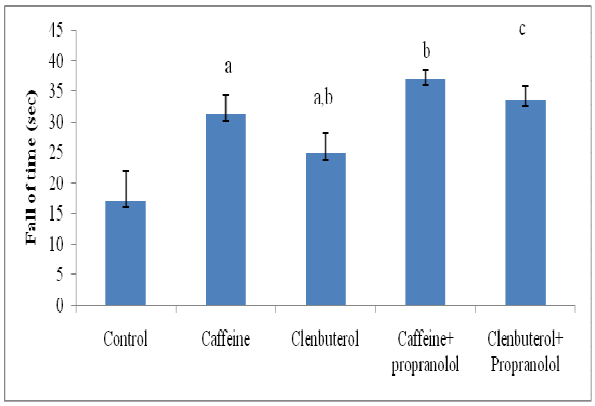

Figure - 1: Effectof Clenbuterol on fall off time in Rota-rod test [Values are expressed as mean ± S.E.M. [a] denotes $p< 0.05$ compared to Control group, [b] denotes $p< 0.05$ compared to caffeine group, [c] denotes $p< 0.05$ compared to Clenbuterol. (One way ANOVA followed by (Tukey's test)].

3.3. Effect of Clenbuterol in mirror chamber paradigm

Administration of clenbuterol (1 mg/kg, *i.p.*) to mice showed significant ($p<0.05$) prolongation in FET as compared to normal group. Administration of caffeine (25 mg/kg; *i.p.*) for 7 days showed significant ($p<0.05$) prolongation in FET than normal group. Caffeine also showed significant ($p<0.05$) impact on FET as compared to clenbuterol group. Coadministration of propranolol (2 mg/kg; *i.p.*) to mice before 30 minutes of clenbuterol and caffeine administration significantly ($p<0.05$) reduced the ability of clenbuterol and caffeine in mirror chamber.

The administration of clenbuterol (1 mg/kg; *i.p.*) to mice caused a significant ($p<0.05$) decrease in number of entries in mirror chamber as compared to normal group. Administration of caffeine (25 mg/kg; *i.p.*) for 7 days caused significant ($p<0.05$) decrease in number of entries than normal group. Coadministration of propranolol (2 mg/kg; *i.p.*) to mice before 30 minutes of clenbuterol and caffeine administration significantly ($p<0.05$) attenuated the ability of clenbuterol and caffeine in mirror chamber on number of entries.

Single injection of clenbuterol (1 mg/kg; *i.p.*) to mice showed significant ($p< 0.05$) reduction in time spent as compared to normal group. Administration of caffeine (25 mg/kg; *i.p.*) to mice for 7 days caused significant ($p<0.05$) decrease in time spent than normal group. Caffeine was able to produce significant ($p<0.05$) impact on time spent as compared to clenbuterol group. Coadministration of propranolol (2 mg/kg; *i.p.*) to mice before 30 minutes of clenbuterol and caffeine administration significantly ($p<0.05$) increased the time spent in mirror chamber.

3.4. Effect of Clenbuterolon open field activity of mice

A single injection of clenbuterol (1 mg/kg;*i.p.*) showed significant ($p< 0.05$) reduction in Central area durationas compared to normal group. Administration of caffeine (25 mg/kg; *i.p.*) for 7 days showed significant ($p<0.05$) decrease in central area durationthan normal group. Caffeine was able to produce significant ($p<0.05$) impact on Central area durationas compared to clenbuterol group. Coadministration of propranolol (2 mg/kg; *i.p.*) to mice before 30 minutes of clenbuterol and caffeine administration significantly ($p<0.05$) increasedCentral area duration, unlike those of clenbuterol and caffeine treated group.

Administration of clenbuterol (1 mg/kg, *i.p.*) to mice showed significant ($p<0.05$) increase in ambulation as compared to normal group. Administration of caffeine (25 mg/kg; *i.p.*) for 7 days showed significant ($p<0.05$) increase in ambulation than normal group. Caffeine also showed significant ($p<0.05$) effect on ambulation as compared to clenbuterol group.

Table - 3: Effectof Clenbuterol on open field parameters:

Groups	Central area duration (sec)	Ambulatory score	Grooming
Control	80.6±3.4	48.3±5	1.6±0.8
Caffeine	35.6±2.8[a]	56.3±2.7[a]	12±1[a]
Clenbuterol	28.3±5.3[a,b]	63±2.9[a,b]	7±1.6[a,b]
Caffeine+Propranolol	53±3.3[b]	67.3±2.4[b]	6.1±1.1[b]
Clenbuterol+Propranolol	45±3.4[c,d]	73±2[c,d]	2±0.6[c,d]

Values are expressed as mean ± S.E.M. [a] denotes $p< 0.05$ compared to Control group, [b] denotes $p< 0.05$ compared to caffeine group, [c] denotes $p< 0.05$ compared to Clenbuterol, [d] denotes $p< 0.05$ compared to Caffeine+ Propranolol.(One way ANOVA followed by Tukey's test).

Table - 4: Effect of Clenbuterol on brain oxidative stress parameters of mice

Groups	TBARS Conc. (µM/ml)	GSH Conc. (µM/ml)	Nitrite Conc. (µM/ml)	Catalase activity (Units/mg protein)
Control	10.19±0.6	50.9±1.9	14.6±2.0	0.85±0.03
Caffeine	36.1±2.3[a]	27.1±2.2[a]	24±2.3[a]	0.33±0.02[a]
Clenbuterol	41.7±2.6[a,b]	21.1±0.8[a,b]	28.19±1.0[a,b]	0.40±0.03[a,b]

Values are expressed as mean ± S.E.M. [a] denotes $p< 0.05$ compared to Control group, [b] denotes $p< 0.05$ compared to caffeine group.(One way ANOVA followed by Tukey's test).

Coadministration of propranolol (2 mg/kg; *i.p.*) to mice before 30 minutes of clenbuterol and caffeine administration significantly ($p<0.05$) increased ambulation in open field.

The single dose of clenbuterol (1 mg/kg; i.p.) to mice caused a significant ($p<0.05$) increase in grooming as compared to normal group. Administration of caffeine (25 mg/kg; i.p.) for 7 days showed significant ($p<0.05$) increase in grooming than normal group. Caffeine also produce a significant ($p<0.05$) effect as compared to clenbuterol group. Coadministration of propranolol (2 mg/kg; *i.p.*) to mice before 30 minutes of clenbuterol and caffeine administration significantly ($p<0.05$) attenuates the ability of clenbuterol and caffeine in open field.

3.5. Effect of Clenbuterol on brain TBARS level in mice

Administration of clenbuterol (1 mg/kg, *i.p.*) to mice showed significant ($p<0.05$) increase in higher brain TBARS levels as compared to normal group, indicating the rise in oxidative stress. Administration of caffeine (25 mg/kg; *i.p.*) for 7 days showed significant ($p<0.05$) increase in TBARS levels than normal group. Caffeine also showed significant ($p<0.05$) effect on TBARS as compared to clenbuterol group.

3.6. Effect of Clenbuterol on Brain GSH Levels in Mice

Administration of clenbuterol (1 mg/kg, *i.p.*) to mice showed significant ($p<0.05$) decrease in GSH levels as compared to normal group. Administration of caffeine (25 mg/kg; *i.p.*) for 7 days showed significant ($p<0.05$) decrease in GSH levels than normal group. Caffeine also showed significant ($p<0.05$) effect on GSH as compared to clenbuterol group.

3.7. Effect of Clenbuterol on Brain nitrite level in mice

Administration of clenbuterol (1 mg/kg, *i.p.*) to mice showed significant ($p<0.05$) increase in higher brain nitrite levels as compared to normal group. Administration of caffeine (25 mg/kg; *i.p.*) for 7 days showed significant ($p<0.05$) increase in nitrite levels than normal group. Caffeine also showed significant ($p<0.05$) effect on nitrite as compared to clenbuterol group.

3.8. Effect of Clenbuterol on brain catalase activity in mice

Administration of clenbuterol (1 mg/kg; *i.p.*) to mice showed significant ($p<0.05$) decrease in catalase levels as compared to normal group. Administration of caffeine (25 mg/kg; *i.p.*) for 7 days showed significant ($p<0.05$) decrease in catalase levels than normal group. Caffeine also showed significant ($p<0.05$) effect on GSH as compared to clenbuterol group.

4. DISCUSSION

Animal models form the backbone of preclinical research on the neurobiology of psychiatric disorders, and are employed both as screening tools in the search for novel therapeutic agents and as simulations for studies on

underlying mechanisms. More than 30 animal models of anxiety are currently in use and, while some are based on physiological (e.g., hyperthermia) or endocrine (e.g., plasma corticosterone) responses to stress, the vast majority are behavioral in nature. Behavioral models may conveniently be classified as either conditioned or unconditioned responses to stimuli which appear capable of causing anxiety in humans. The need for a new strategy in preclinical anxiety research is not only indicated by the apparent limitations of existing animal models, but also by the need for novel, safe and effective treatments for the full range of anxiety-related disorders. Nevertheless, in considering the broader question of improvements in animal modeling, it seems prudent to bear in mind that a 'balance must be struck between the proliferation of newer models and the refinement of existing ones' [19]. In the present study, we investigate the potential of Clenbuterol to induce anxiety and behavioral modifications in mice.

In the present study, the administration of clenbuterol for 1 day to mice increased muscle grip strength on rotarod apparatus as shown by increase in fall off time.Rota rod provides an excellent tool for detecting muscle grip strength of mice. It is based on the fall off time of the mice from the rotating rod which shows their muscle co-ordination [20]. In addition, clenbuterol treated mice exhibited anxiety in elevated zero maze test and mirror chamber test. Elevated zero maze is a sensitive tool to study the anxiety related behaviorswhich is based on the principle that rodents are generally unwilling to enter in the open arms of the maze, considering their aversion to open spaces [11]. Clenbuterol administration increased the latency to enter open arms, decreased the number of entries and time spent in open arm in EZM study. The same pattern of results is also seen in mirror chamber apparatus test which indicated successful induction of anxiety in clenbuterol group in mice. Open field provides a good measure of the approach response towards novelty, by assessing ambulation (when animal moves from one segment to other) also. Clenbuterol administration decreased the central square entries and increased grooming in comparison to control animals.Furthermore, the biochemical evidences of the present study suggested the elevated level of lipid peroxidation and the reduction in level of antioxidant enzymes which increases the consequences of disease progression. The increase in production of oxidative stress in anxiety condition leading to structural destruction of unsaturated fatty acids in lipid membrane resulted in the elevated levels of TBARS in clenbuterolgroup, TBARS is a quantitative measure of ROS induced lipid peroxidation [21]. In the present study, the level of naturally occurring antioxidant like GSH was significantly decreased in the clenbuterol group animals. GSH S-transferase is an enzyme that plays an important role in detoxification of electrophiles. The free electron via vicious cycles causes generation of free radical that resulted in elevation of oxidative stress. GSH is responsible for the quencher of this free electro [22]. The group treated with clenbuterol causes significant decrease in catalase activity in mice brain and increase in nitrite level.

Clenbuterol may prove to be useful model for inducing anxiety and behavioral alterations in mice by increasing oxidative stress and by interrupting with Nitrite processes. Catalase, GSH activities are significantly decreased in clenbuterol treatment.

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