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Research Article

Anti-Depressant like Effect of Curcumin in Olfactory Bulbectomized Model of Depression in Male Wistar Albino Rats: Antidepressant Behaviour Screening Tests

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Abstract

Depression has become the most prevalent psychiatric disorder and imposes a substantial social burden. The olfactory bulbectomy (OB) in rodents results in a disruption of the limbic-hypothalamic axis with the consequence of behavioral, neurochemical, neuroendocrine and neuroimmune changes, of which many resemble changes seen in depressed patients. The main objective of the study is to investigate the antidepressant like effect of curcumin in olfactory bulbectomized model of depression of male wistar albino rats through behavior screening tests. Bilateral olfactory bulbectomy was performed with rats anesthetized under ketamine (50 mg/kg/i.p.). Twenty male albino wistar rats, weighing 150 - 220 gm were randomly allocated into four groups (n = 5) - Group A (control), Group B (Olfactory Bulbectomy; OB), Group C (Vehicle (0.9% NS, 10 ml/kg) + Olfactory Bulbectomy, OB) and Group D (Curcumin (40 mg/kg)+ Olfactory Bulbectomy; OB). Immobility Time (IT-s) in Forced Swim Test (FST), Tail Suspension Time (TST); Swimming Time (SWT-s) and Struggling Time (ST-s) in FST; Sucrose Consumption and Water Consumption and Sucrose Preference Percentage in Sucrose Preference Test (SFT) were screened. The data of the present study were expressed as mean ± S.E.M. The ANOVA test was performed for multiple comparisons and post-hoc tests to identify subsequent pair-wise differences in our study. A $p < 0.05$ was considered statistically significant. All the variables were found to have statistically significant differences ($p < 0.05$) between Group (A vs B) and Group (B vs D) but for Group (C vs D), TST (IT-s) was not significant ($P = 0.247$). Whereas, all variables were not statistically significant differences ($p > 0.05$) between Group (B vs C) and Group (A vs D) except FST (IT-s) and FST (ST-s) ($P = 0.061$). The results of the present study suggest that the curcumin has antidepressant like effect in olfactory bulbectomized-induced depression model in behavior screening tests.

Keywords: *Curcumin; Olfactory; Bulbectomized; Model*

Abbreviations

OB: Olfactory Bulbectomy; FST: Forced Swim Test; TST: Tail Suspension Test; SPT: Sucrose Preference Test; DMSO: Dimethyl Sulfoxide; I.P: Intra Peritoneal; IT: Immobility Time; SWT: Swimming Time; ST: Struggling Time

Introduction

Depression has become the most prevalent psychiatric disorder and imposes a substantial social burden. [1,2]. Curcumin, the main biologically active component of *Curcuma longa*, exhibits a wide variety of pharmacological activities including anti-inflammatory, anti-oxidant, immunomodulatory and neuroprotective activities [3-5]. Curcumin appears to exert its antidepressant action by modulating the monoaminergic system and may also inhibit glutamate release in nerve terminals from rat pre-frontal cortex, an effect, which is similar to that of the classical antidepressant fluoxetine [6,7]. The olfactory bulbectomy (OB) in rodents results in a disruption of the limbic-hypothalamic axis with the consequence of behavioral, neurochemical, neuroendocrine and neuroimmune changes, of which many resemble changes seen in depressed patients [8].

Antidepressant behaviour screening tests like the forced swim, tail suspension and sucrose preference tests are the most commonly employed behavioral paradigms of despair [9]. The forced-swimming test (FST) is based on the observation that animals develop an immobile posture in an inescapable cylinder filled with water. In this test, immobility is interpreted as a passive stress-coping strategy or depression-like behavior [10]. Tail suspension test (TST), shares a common theoretical basis and behavioral measure with the FST. In the TST, mice are suspended by their tails using adhesive tape to a horizontal bar for a certain couple of minutes, and the time of immobility is recorded [11]. In Sucrose preference Test (SPT), Rodents are born with an interest in sweet foods or solutions [12]. Reduced preference for sweet solution in sucrose preference test represents anhedonia, while this reduction can be reversed by treatment with chronic antidepressants. Although these behavioral models do not mimic the human state of major depression, they are the test models used to screen antidepressant molecules [13].

Therefore, in the present study, we utilized the olfactory bulbectomized model of depression of rat to investigate whether long-term treatment with curcumin will have anti-depressant like effect with antidepressant behaviour screening tests.The specific aims of the present study were

- To induce depression model of rat by doing bilateral olfactory bulbectomy of male wistar albino rats.
- To investigate the antidepressant like effect of curcumin in olfactory bulbectomized model of depression of male wistar albino rats.
- To perform antidepressant behaviour screening tests in these rats (forced swim test, tail suspension test and sucrose preference test).

Materials and Methods

Animals

Male Wistar rats weighing 150 - 220 gm obtained from the Animal house of BPKIHS. The present study was approved by the IRC, BPKIHS, Nepal. Regarding Animal Care, were performed in accordance with the guidelines of the 'Principles of Laboratory Animal Care' (NIH publication No. 80-23, revised 1996). Rats were housed in standard plastic cages under room temperature (22 - 24°C) with a 12-h light/dark cycle (lights on 6:00 a.m.). Unless otherwise stated standard laboratory food and water were available throughout the experiments. In this study firstly, the rats were allowed to acclimatise to the laboratory conditions for period of one week prior to the experimental procedures.

Sample size calculation done by using resource equation method

According to this method a value "E" is measured, which is nothing but the degree of freedom of analysis of variance (ANOVA). The value of E should lie between 10 and 20. If E is less than 10 then adding more animals will increase the chance of getting more significant result, but if it is more than 20 then adding more animals will not increase the chance of getting significant results. Though, this method is based on ANOVA, it is applicable to all animal experiments for quantitative characters. Any sample size, which keeps E between 10 and 20 should be considered as an adequate [14]. E can be measured by following formula:

E = Total number of animals - Total number of groups

In present study, we have to look to see the effect of curcumin and we have four groups (one group as control and three groups); with E assumed to be 16.

Then; the total number of animals or sample size (n) will be

E = n - 4

16 = n - 4

Then, n= 20;

Hence; sample size will be 5 per group.

Criteria for sample selection

Inclusion Criteria

- Healthy Male wistar albino rats
- Weight 150 - 220 gms.

Exclusion criteria

- Unhealthy male and female rats
- Weighing less than 150 gms or more than 220 gms.

Surgical procedure and experimental design

Surgery were done after one week after arrival of the animals in the laboratory for required group as mention. Bilateral olfactory bulbectomy were performed with rats anesthetized under ketamine (50 mg/kg/i.p.; ketolar®). The top the skull were shaved and swabbed with an antiseptic, after which a midline frontal incision were made in the scalp and the skin were retracted bilaterally [15]. A 5 ml syringe needle were drilled into the skull at the points 7 mm anterior to bregma and 2 mm lateral to the bregma suture, after which the olfactory bulbs were separated from the frontal cortex, removed and skin was closed with surgical sutures. Two weeks after surgery, curcumin was administered daily for 4 weeks.

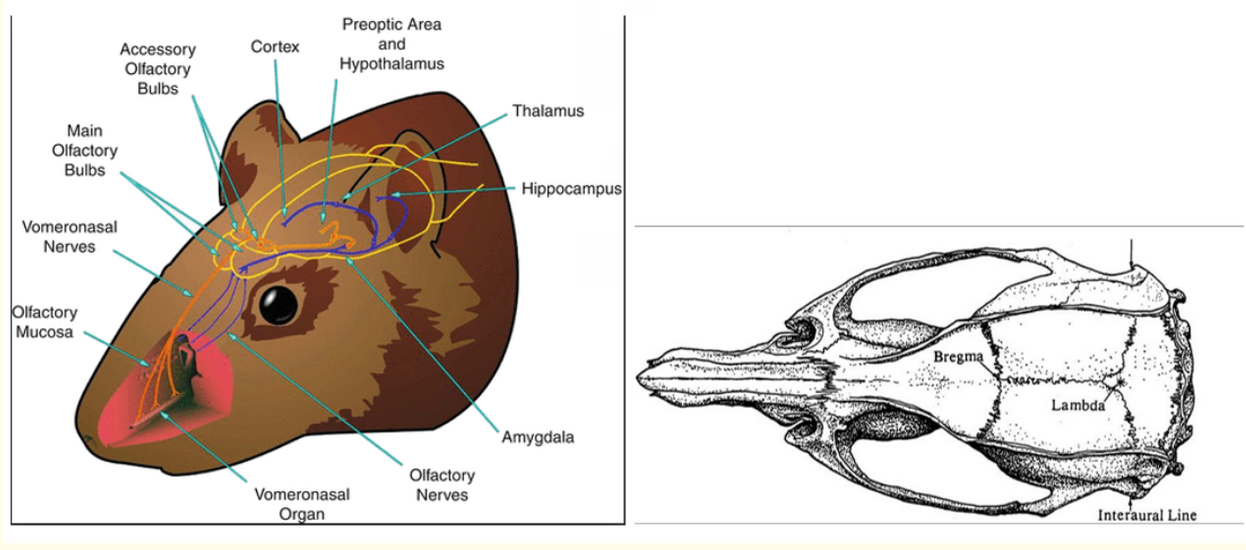

Figure 1: *Soft tissue and bony land marks for olfactory bulbectomy.*

Drugs and treatment

Curcumin powder (Sigma Aldrich, USA) was dissolved in 50% Ethanol/dmso at a concentration of 10 mg/ml. After surgery, curcumin or 0.9% Normal Saline (NS) were administered I.P. in a constant volume once a day at same time for four weeks. Animals were randomly allocated to one of the following four groups of twenty rats five in each arm:

(A) Control,

(B) Olfactory Bulbectomy (OB)

(C) Vehicle (0.9%NS, 10 ml/kg) + Olfactory Bulbectomy (OB)

(D) Curcumin (40 mg/kg)+ Olfactory Bulbectomy (OB).

Behavioural testing

Behavioural Tests were performed from the next day after six weeks of respective group treatments as mention above in sequence as follows.

Forced swim test (FST)

In brief, rats were placed individually in a glass cylinder (height: 80 cm, diameter: 30 cm) filled with 40 cm of water at 25°C for two consecutive swim sessions. In this cylinder, rats cannot touch the bottom or escape. For the first exposure, rats were placed in the water

for 10 minutes of forced swimming (training session).Twenty-four hours later, rats were placed in the cylinder again for a 5 minutes period (test session). In the test session rats were scored by an observer blind to the treatment condition of the animal for immobility time (floating with only small movements necessary to keep their head above water), swimming time (pedalling or making circular movements), and struggling time (climbing walls or diving attempts to escape). Water in the tank were changed after each session [16].

Figure 2: *Forced swimming test.*

Tail suspension test (TST)

The day after FST, we performed TST. In the TST, rats were suspended by their tails using adhesive tape to a horizontal bar for a certain couple of minutes, and the time of immobility was recorded.

Figure 3: *Tail suspension test.*

Sucrose preference test (SPT)

The sucrose preference test was performed the next day after the TST. In the first phase, rats were placed individually in the cages for an adaption period to the sucrose solution (1%, w/v) before the start of the test session. Two bottles with a 1% sucrose solution were placed in each cage for the first 24-h period, and then one of the bottles containing the 1% sucrose solution was replaced with tap water for the second 24-h period. After the adaptation period, rats were deprived of water and food for 24h. The test was performed at 9:00 a.m. with the rat being placed individually in the cages and permitted free access to two bottles, one containing 100 ml of 1% sucrose solution and one containing 100 ml of tap water. After 3h, the consumed volumes of sucrose solution and tap water were recorded. The reduced sucrose preference, which is used as an index of anhedonia, was calculated according to the following formula: sucrose consumption/[water consumption+sucrose consumption] ×100% [16].

Figure 4: *Sucrose preference test (SPT).*

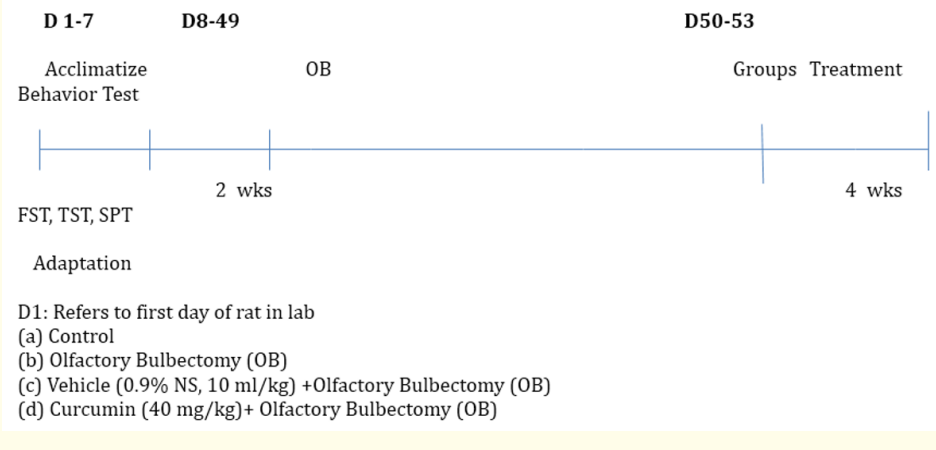

Figure 5: *Schematic figure of the treatment protocol.*

Data analysis

Data analysis along with statistical test were performed on SPSS version 11.5. All data in the present study were expressed as mean ± S.E.M generated. ANOVA test were performed for multiple comparisons and post-hoc tests to identify subsequent pair-wise differences in this study. A $p < 0.05$ was required for results to be considered statistically significant.

Results and Discussion

Table 1 showed the mean and standard error of mean of different variables in behavior tests. There is slight decreased in immobility time in TST and FST; increase in swimming and struggling time and also increase in sucrose preference percentage between Group B and Group D showing improvement in the behavior when giving curcumin.

Groups		TST (IT-s)	FST (IT-s)	FST (SWT-s)	FST (ST-s)	SPT(%)
A	Mean	17.40	116.60	113.00	70.40	73.46
	SEM	(1.86)	(2.73)	(2.21)	(2.06)	(4.59)
B	Mean	32.00	185.00	81.80	33.20	30.50
	SEM	(2.38)	(2.12)	(2.59)	(1.65)	(4.29)
C	Mean	28.80	173.80	85.40	40.80	32.61
	SEM	(2.70)	(3.27)	(1.77)	(2.28)	(3.01)
D	Mean	21.20	139.00	104.40	56.60	60.33
	SEM	(2.63)	(2.61)	(1.63)	(2.52)	(1.06)

Table 1: *Mean and SEM of all variables of groups.*

Table 2 showed the statistically significant difference between and within groups in all variables of behavior test.

Variables		Sig.
TST (IT-s)	Between Groups	0.002
	Within Groups	
FST (IT-s)	Between Groups	< 0.001
	Within Groups	
FST (SWT-s)	Between Groups	< 0.001
	Within Groups	
FST (ST-s)	Between Groups	< 0.001
	Within Groups	
SPT (%)	Between Groups	< 0.001
	Within Groups	

Table 2 showed the statistically significant difference between and within groups in all variables of behavior test.

Table 3 showed the post-hoc test among the variables of different group.

Group	Groups	TST (IT-s)	FST (IT-s)	FST (SWT-s)	FST (ST-s)	SPT (%)
A	B	0.004	< 0.001	< 0.001	< 0.001	0.001
	C	0.025	< 0.001	< 0.001	< 0.001	0.002
	D	1.000	< 0.001	0.061	0.002	0.983
B	A	0.004	< 0.001	< 0.001	< 0.001	0.001
	C	1.000	0.061	1.000	0.144	1.000
	D	0.037	< 0.001	< 0.001	< 0.001	0.026
C	A	0.025	< 0.001	< 0.001	< 0.001	0.002
	B	1.000	0.061	1.000	0.144	1.000
	D	0.247	< 0.001	< 0.001	0.001	0.043
D	A	1.000	< 0.001	0.061	0.002	0.983
	B	0.037	< 0.001	< 0.001	< 0.001	0.026
	C	0.247	< 0.001	< 0.001	0.001	0.043

Table 3: *Post-hoc Test between the groups and within the groups of all variables.*

The study conducted in China Showed that, six weeks of CUMS, resulted in depression-like behaviour in rats, as indicated by the significant decrease in sucrose consumption and increase in immobility time in the forced swim test. The results were in agreement with our results in Sucrose Preference Test and Forced Swim Test [16].

Table 1 showed the mean and standard error of mean of different variables in behavior tests. There was slight decreased in immobility time in TST and FST; increase in swimming and struggling time and also increase in sucrose preference percentage between Group B and Group D showing improvement in the behavior when giving curcumin. Table 2 and 3 showed the statistically significant difference between and within groups in all variables of behavior test in Anova test and post hoc test.

Many ongoing research work on curcumin are justifying some interesting properties in major depression [17,18]:

- Curcumin is an inhibitor of monoamine oxidase (MAO) enzyme [19]
- Curcumin modulates the level of various neurotransmitters [19]
- Curcumin promotes hippocampal neurogenesis [19]
- Curcumin is an anti-inflammatory agent [19].

Limitations

In the present study, manual OB was done, which is blind procedure and higher investigations such as Immunofluorescence, Immunoassay and Electron microscopy study are not possible due to unavailability of necessary kits and instruments.

Conclusion

The results of the present study suggest that the curcumin has antidepressant like effect in olfactory bulbectomized-induced depression model in behavior screening tests. But further higher investigation should be done to confirm.

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- RC, BPKIHS
- RFM, BPKIHS
- Rats.

Conflict of Interest

The authors declare that there is no conflict of interest.

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