Research Article

Antidepressant and Anti-Anxiety Effect of Ellagic Acid from *Punica granatum* L. Rind in Olfactory Bulbectomy Model in Rats.

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Accepted on: 21-07-2015; **Finalized on:** 31-08-2015.

ABSTRACT

The present study evaluated the anti-depressant and antianxiety effect of standardized extract of *Punica granatum* (HEPG) in olfactory bulbectomy (OBX) induced depression and anxiety in rats. The surgery was performed in 30 male rats and was recovered by keeping in social- isolation condition for the period of 14 days followed by the treatment for next 14 days. Fluoxetine (10mg/kg, p.o), HEPG (10, 30 and 100mg/kg, p.o) were administered 1 hr prior to study. Parameters as open field test, force swim test, novelty suppressed feeding, sucrose intake test were studied. At the end of study brain monoamine estimation was carried out. Extract in all three doses significantly reversed hyperactivity in open field as well as all the parameters of anxiety along with normalization of brain monoamines.

Keywords: Anti-depressant effect, Antianxiety effect, *Punica granatum.*

INTRODUCTION

The monoamine biochemical hypothesis of depression, proposed by Schildkraut in 1965, states that depression caused by a functional deficit of monoaminergic neurotransmitters at certain areas of brain, whereas mania results from its functional excess[1-3]. Mood fluctuation is the most prevalent symptom of mental illness. Severe forms of depression affects approximately 2%–5% of the U.S. population, and up to 20% of the population suffers from the milder forms of the mental illness. Another roughly 1%–2% population is afflicted by bipolar disorder known as manic depressive illness, which affects both females and males equally. Mood disorder is recurrent, life threatening (due to the high risk for suicide), and a major cause of morbidity worldwide. There are some clinical reports concerning oxidative disturbances in patients with major depressive disorder, including oxidative damage in erythrocyte membranes as indicated by the depletion of omega-3 fatty acids[4]; elevated level of lipid peroxidation biproducts; oxidative damage of genetic material; elavated concentrations of the endogenous inhibitor of endothelial Nitric oxide (NO) synthase, an asymmetric dimethyl arginine and decreased NO. Both clinical and pre-clinical studies suggested that major depression produced by CMS is mainly associated with excessive lipid peroxidation byproducts[5]. On the other hand, it has been reported that increased reactive oxygen species (ROS) production may cause the disruption of membrane phospholipids and ultimately affecting neuronal membranes, and consequently changes in the membrane viscosity may affect serotonergic and catecholaminergic receptor functions.

Punica granatum fruit products have been frequently used in India for centuries since ancient civilizations for medicinal purposes. Stomachic, fever, inflammation, diarrhea, bronchitis, vaginitis, dysentery, urinary tract infection, and, among others, malaria have been treated effectively using various parts of pomegranate including fruit rinds[20,22,23]. Moreover, numerous pomegranate fruit products and supplements (functional foods, therapeutic formulae and cosmetics) are also available in markets[21,22,24] The phenolic constituents, ellagi tannins and ellagic acid, are among the potent antioxidants present in peels.[20-24]

Ellagic acid is a naturally occurring polyphenolic compound which has been reported to possess a wide spectrum of pharmacological activities such as antioxidant[6], anticancer[7], antiallergic[8], antimalarial[9], antiwrinkle[10], antiglycative and anti-inflammatory[11]. Further, ellagic acid showed neuroprotective activity against oxidative damage[12], inhibited A*b* 42-induced neurotoxicity *in vitro*[13] and is also a *b*-secretase inhibitor[14]. Ellagic acid has also been reported to inhibit iNOS[15]. Ellagic acid was tested in acute animal of depression as force swim test (FST), tail suspension test (TST) where the involvement of monoaminergic system (serotonergic and adrenergic) was proved as a possible mechanism of action[16].

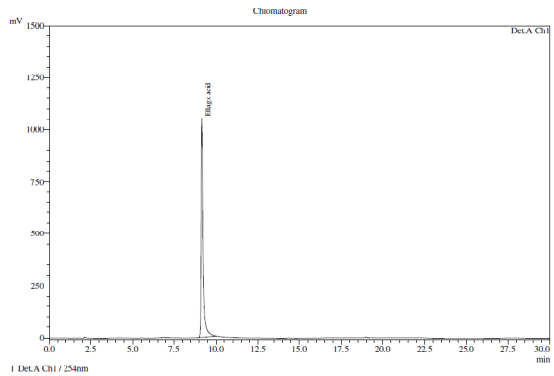
Olfactory bulbectomy is a well validated model to study the symptoms resembling clinical depression. The symptoms simulating with clinical depression are produced by the surgery[17]. There are reports concerning the depletion of neurotransmitters at synapse[17]. The effect of acute oral administration of ellagic acid was studied, where the involvement of monoaminergic system was proven to be the mechanism of antidepressant action of ellagic acid[16]. So, we found it

interesting to study the effect of sub acute administration of ellagic acid in olfactory bulbectomy induced behavioral depression in rats.

MATERIALS AND METHODS

HPLC chromatogram of HEPG

Chromatogram of standard Ellagic acid

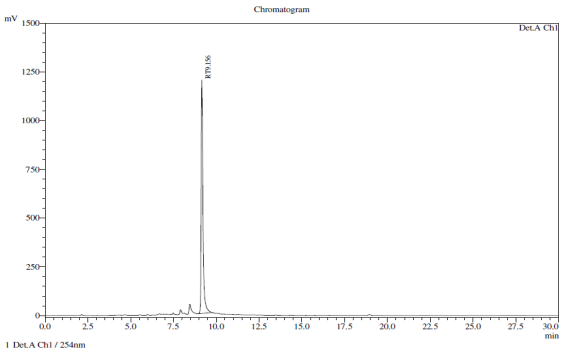


PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Name	Area	Area %	Tailing Factor
1	9.157	Ellagic acid	8254897	100.000	1.882

Chromatogram of extract



PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Name	Area	Area %	Tailing Factor
1	9.156	RT9.156	8741700	100.000	1.703
Total			8741700	100.000	

Animals and experimental groups

Experiments were conducted with 2–3-month old male Sprague Dawley rats weighing 250–270 g. All procedures were carried out with the previous approval of the Institutional Animal Ethical Committee of Maharashtra Institute of Pharmacy, Pune university (Approval number: MIP/IAEC/2013-14/M1/0012). Animals were housed in climate controlled rooms with 12 h light–12 h dark cycle. Food and water were provided ad libitum. The social isolation procedure was performed during four weeks, with recovery period of 14 days followed by 14 days of treatment.

Olfactory bulbectomy[18]

Bilateral olfactory bulbectomy (OBX) was performed on rats anesthetized with ketamine (80%; CBC, India). In brief, the head was shaven and a sharp midline sagittal incision was made with scalpel in the skin overlying the skull. Two burr holes were drilled through which both olfactory bulbs were aspired by a suction pump. Finally, the burr holes were filled with dental cement in order to avoid further bleeding. At the end of the experiment, all animals were sacrificed and the lesions were verified. Sham operation was done in the same way, but with the bulbs left intact. Animals were given diclofenac sodium (25mg/kg/day; i.p; Triveni Chemicals, Gujarat, India) immediately after surgery followed by further 3 days to avoid unnecessary suffering.

Treatment schedule in olfactory bulbectomized (OBX) rats

Group I rats were treated as sham control rats (had surgery but no OBX) and were administered with the saline. After recovery of 14 days, OBX rats were divided into following groups of six rats each and received oral drug treatments as follows. Group II was OBX- Isolated group and received distilled water. The OBX-Isolated rats in Group III were administered with fluoxetine (30 mg/kg). Group IV, V and VI OBX rats were treated with HEPG (10, 30, 100 mg/kg). All the treatments were administrated orally once daily for 14 days.

Behavioral Assessment

Each experimental group was subjected to a battery of tests in order of least to most stressful condition, and leaving the sufficient interval between them to avoid any potential order effects. Tests were performed during the light phase of cycle and the animals were transported to the experimental room before 30 minutes of start of each experiment for acclimatization purpose.

Open field test[18]

The open field apparatus was a brightly lit (350 lx) white wooden box of dimension (50 cm × 50 cm × 30 cm) provided with white floor and luminescent walls. Rats were released in the centre of the apparatus for 5 min, and behavior was video-tracked by a video tracking system. Total distance travelled, time spent in the centre (30 cm ×30 cm), number of rearings was evaluated.

Novelty-suppressed feeding[18]

The test was performed after 24 hrs of food deprivation with water available ad libitum. The test was performed in a dimly lit (30–50 lx) open arena of dimension (50 cm × 50 cm × 30 cm) containing clean wood chip bedding and provided with a home cage food pellet (Approximately 2 g) placed in the centre of arena. Each rat was removed from its home cage, and placed in one corner of the arena. The latency to begin the feeding episode was recorded (maximum time given was 600 s) with the aid of video tracking system.

FST[18]

The rats were individually placed in the clear plastic cylinders (height 24 cm, internal diameter 12 cm) which were filled with 22 cm of fresh water at the temperature of 25–27°C. Each rat was videotaped from above for the period of 5 min and the swimming sessions were videotaped using video tracking system to determine the accumulated immobility time (floating inside the water

without struggling, and making only those movements which are necessary to keep the head above the water level). The accumulated climbing time (active vigorous movements with the forepaws inside and outside of the water, usually directed against the walls of cylinder) and swimming time (movement that is usually horizontal throughout the swimming chamber) were manually scored by an experienced observer in the blind conditions using the videotaped FST sessions.

Sucrose intake test[18]

Sucrose (Crystal Pharma, Maharashtra, India) was dissolved in drinking water to make a solution of 1% and the study rats were trained to drink the sucrose solutions during 2 days period with free access to water and sucrose solution in their home cage, and the total sucrose consumption per day was measured. Then they were deprived of any drinking solution for further 24 h and subsequently each animal was given a free access to sucrose solutions for 1 h in its home cage. The sucrose consumption (ml) for each animal was recorded and the mean sucrose intake of each experimental group was compared.

Brain monoamine estimation[19]

The whole brain was homogenized in cold acidified n-butanol solution. 2-5 ml of the supernatant solution was pipetted out into a 25 ml of glass stoppered tube and then mechanically shaken for the period of 5 minutes with n-heptane solution and HCl containing L-cysteine. Two phases were then separated followed by centrifugation and organic phase was retained for the determination of 5-HIAA.

To determine 5-HT, 0.1 ml of the aqueous phase was pipetted out into test tubes and 0.004% O-phthalaldehyde (OPT) was added in 10N HCl solution. After mixing and heating in a boiling water bath, the tubes were cooled in water and then the fluorescence was measured. Activation and fluorescent wavelengths were kept constant to 360 nm and 470 nm respectively. Blanks were prepared by reacting the OPT solution with 0.1 ml HCl and cysteine solution only.

RESULTS

Table 1: Activity in open field

Groups and treatments	Open Field-Total distance travelled (M)	Open Field-Number of rearings	Open field-Time spent in Center (sec)
Sham-Iso	05.10 ± 0.90	08.33 ± 1.30	06.83 ± 0.60
OBX-Iso	17.67 ± 1.28 $^{\#\#\#\#}$	24.67 ± 1.94$^{\#\#\#\#}$	03.16 ± 0.47^{ns1}
Fluo-Iso	12.00 ± 1.46**	16.00 ± 1.15***	05.16 ± 1.49^{ns2}
HEPG (10)-(OBX+ISO)	11.00 ± 1.82***	14.33 ± 1.28***	08.67 ± 0.49^{ns2}
HEPG (30)- (OBX+ISO)	08.83 ± 0.60***	08.00 ± 0.36***	16.33 ± 0.33***
HEPG (100)- (OBX+ISO)	09.50 ± 0.76***	06.33 ± 0.49***	15.50 ± 1.66***

The values are expressed as the mean ± SEM. The drug/vehicle treatments were administered once a day for 14 days. Figures in the bracket indicate dose in mg/kg, p.o. Open Field-Total distance travelled (M) and Open field-Time spent in Center (sec) data was analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. Open Field-Number of rearings data was analyzed by one-way ANOVA followed by Holm-Sidak's multiple comparisons test, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ as compared to sham control, $^{*}P < 0.05$, and $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to the OBX control, n = 6 per group

Table 2: Activity in persolt's apparatus, Novelty suppressed feeding and Sucrose intake test

	Novelty suppressed feeding	Sucrose intake	FST- Immobility time (sec)	FST-Swimming time (sec)	FST- Climbing Time
Sham-Iso	515.0 ± 35.19	2.28 ± 0.14	162.0 ± 2.129	125.7 ± 3.05	12.33 ± 1.66
OBX-Iso	322.2 ± 21.86$^{\#\#\#}$	0.75 ± 0.04$^{\#\#\#}$	123.5 ± 1.875$^{\#\#\#}$	164.5 ± 4.29$^{\#\#\#}$	12.00 ± 3.26^{ns}
Fluo-(OBX+ISO)	323.7 ± 48.11^{ns}	2.63 ± 0.13***	51.83 ± 3.04***	139.5 ± 3.99**	108.7 ± 5.32***
HEPG (10)-(OBX+ISO)	233.7 ± 09.80^{ns}	1.50 ± 0.22^{ns}	93.67 ± 2.459***	108.5 ± 2.99***	97.83 ± 2.49***
HEPG (30)-(OBX+ISO)	186.7 ± 07.07*	1.55 ± 0.31*	80.33 ± 4.835***	132.5 ± 6.01***	87.17 ± 4.96***
HEPG (100)-(OBX+ISO)	240.3 ± 21.36**	2.18 ± 0.14***	59.50 ± 3.106***	112.8 ± 4.88***	127.7 ± 7.36***

The values are expressed as the mean ± SEM. The drug/vehicle treatments were administered once a day for 14 days. Figures in the bracket indicate dose in mg/kg, p.o. Data was analyzed by one-way ANOVA followed by Tukey's multiple comparisons test, #P < 0.05, ##P < 0.01 and ###P < 0.001 as compared to sham control, *P < 0.05, and **P < 0.01, ***P < 0.001 when compared to the OBX control, n = 6 per group.

Table 3: Activity in elevated plus maze

	Open arm entries	Closed arm entries	Time spent in Open arm (sec)
Sham-Iso	3.167 ± 0.30	7.83 ± 0.40	86 ± 6.87
OBX-Iso	13.50 ± 0.42$^{###}$	9.33 ± 0.33^{ns}	158 ± 8.00$^{###}$
Fluo-(OBX+ISO)	6.83 ± 0.30***	7.33 ± 0.21*	107 ± 2.86***
HEPG (10)- (OBX+ISO)	12.17 ± 0.90^{ns}	9.17 ± 0.30^{ns}	147 ± 9.06ns
HEPG (30)- (OBX+ISO)	9.00 ± 0.36***	5.67 ± 0.49***	122.8 ± 3.93**
HEPG (100)- (OBX+ISO)	4.16 ± 0.47***	4.83 ± 0.47***	77.33 ± 1.99***

The values are expressed as the mean ± SEM. The drug/vehicle treatments were administered once a day for 14 days. Figures in the bracket indicate dose in mg/kg, p.o. Data was analyzed by one-way ANOVA followed by Tukey's multiple comparisons test, #P < 0.05, ##P < 0.01 and ###P < 0.001 as compared to sham control, *P < 0.05, and **P < 0.01, ***P < 0.001 when compared to the OBX control, n = 6 per group.

Table 4: Brain monoamine estimation

	5-HT in Brain (ng/g)	5-HIAA metaboilite in Brain (ng/g)	5-HIAA to 5HT ratio	NA in Brain (ng/g)
Sham-Iso	249.44 ± 6.03	219.72 ± 4.41	0.88 ± 0.01	95.32 ± 1.52
OBX-Iso	62.92 ± 5.87$^{###}$	73.82 ± 7.33$^{###}$	1.21 ± 0.16^{ns1}	31.86 ± 2.05$^{###}$
Fluo-(OBX+ISO)	260.72 ± 2.98***	235.81 ± 5.80^{ns}	0.9 ± 0.02^{ns2}	92.48 ± 1.63***
HEPG (10)- (OBX+ISO)	42.49 ± 5.46*	81.26 ± 2.69***	2.03 ± 0.19^{ns2}	37.57 ± 3.68^{ns}
HEPG (30)- (OBX+ISO)	99.75 ± 9.32***	110.01 ± 2.57***	1.15 ± 0.11^{ns2}	76.61 ± 5.38***
HEPG (100)- (OBX+ISO)	166.06 ± 3.60***	133.26 ± 9.33***	0.80 ± 0.06^{ns2}	92.61 ± 2.04***

The values are expressed as the mean ± SEM. The drug/vehicle treatments were administered once a day for 14 days. Figures in the bracket indicate dose in mg/kg, p.o. Data was analyzed by one-way ANOVA followed by Tukey's multiple comparisons test, #P < 0.05, ##P < 0.01 and ###P < 0.001 as compared to sham control, *P < 0.05, and **P < 0.01, ***P < 0.001 when compared to the OBX control, n = 6 per group.

To determine 5-HIAA, the organic phase was pipetted out into a glass tube containing phosphate buffer (pH 7.0) and mechanically shaken for the period of 10 minutes. 0.2 ml of the aqueous phase was then pipetted into two test tubes, A and B. 1% of cysteine solution was added to test tube A and to 0.02 % sodium periodate solution was added to the test tube B. Then concentrated HCl solution was added to both A and B. After this, OPT solution and periodate solution was added to tube A. After 30 min, again the cysteine and OPT solutions were added to tube B. The tubes were then placed in a boiling water bath for the period of 10 min, cooled in water and readings were recorded at activation : 360 nm fluorescence : 470 nm.

Statistical Analysis

The data was analyzed by one way ANOVA followed by Tukey's multiple comparisons test where as the data for total distance-squares crossed in open field was analyzed by one way ANOVA followed by Holm Sidak's multiple comparisons test. Number of rearings data was analyzed by one way ANOVA followed by Dunnett's test.

Open Field Test

Total distance travelled was found to be significantly (P < 0.001; 71.14 %) increased in OBX-Isolated group as compared to Sham-Isolated group, Where as fluoxetine (32.09 %) as well as all three doses of extract (37.75 %, 50.03 %, 46.24 % respectively) significantly (P < 0.01, P < 0.001) restored the total distance travelled in open field. Rearing was also found to be significantly (P < 0.001; 66.24 %) increased in OBX-isolated group; whereas treatment with both standards as well as test drug significantly (P < 0.001; 35.15 %, 41.92 %, 67.58 %, 74.35 % respectively) normalized the rearing behavior. There was non significant reduction in time spent in center in OBX-Isolated group as compared to Sham-Isolation; whereas treatment with extract in two higher doses showed significant (P < 0.001, 416.77 %, 390.50 % respectively) increase in time spent in center and fluoxetine treatment failed to increase the same. (Table-1)

Novelty suppressed feeding

The latency to feeding in OBX-Isolated group is significantly reduced as compared to Sham-Isolated group (P < 0.001; 59.83 %). Treatment with extract at two higher doses the significantly (42.06%, 25.42 % respectively) reduced the feeding latency as compared to OBX-Isolated group. (Table-2)

Force swim test

There was significant (P < 0.001; 31.17 %) reduction of immobility time observed in OBX-Isolated group as compared to Sham-Isolated group; whereas all the

treatments significantly (P < 0.001; 58.04 %, 24.16 %, 34.96 %, 51.83 % respectively) reduced immobility duration as compared to OBX-Isolated group. Swimming time was found to be significantly (P < 0.001; 23.59%) increased in OBX-Isolated group as compared to Sham-Isolated group. Treatment with standard as well as all three doses of extracts significantly (P < 0.01, P < 0.001; 15.2%, 34.05%, 19.46%, 31.43% respectively) reduced swimming time as compared to OBX-Isolated group. Climbing time was non-significantly reduced in OBX-Isolated group; whereas treatment with standard as well as all three doses of extracts significantly (P < 0.001; 805 %, 715.25 %, 716.41 %, 964 % respectively) increased climbing time as compared to OBX-isolated group. (Table-2)

Sucrose intake test

Sucrose intake was found to be significantly (P < 0.001, 204 %) reduced in OBX-Isolated group as compared to Sham-Isolated group. Treatment with standard (P < 0.001; 250 %) and extract in two higher doses (P < 0.5, P < 0.001; 106 %, 190.66 % respectively) significantly increased sucrose intake as compared to OBX-Isolated group.

Brain monoamine estimation

The level of serotonin (5HT), nor-adrenaline (NA), 5-hydroxy-indole-acetic acid (5HIAA) was found to be significantly (P < 0.001; 296.43%, 197.64%, 199.18% respectively) depleted in OBX-Isolated group as compared to Sham-Isolated group. Treatment with fluoxetine and all three doses of extract significantly (P < 0.01; 314.36%, 32.47%, 58.53%, 63.92% respectively) increased brain 5-HT level as compared to OBX-Isolated group. Whereas, NA level was found to be significantly (P < 0.001; 190.26%, 140.45%, 190.67% respectively) increased by fluoxetine and extract in two higher doses as compared to OBX-Isolated group. Treatment with fluoxetine as well as extract in all three doses significantly (P < 0.001; 219.42%, 10.07%, 49.02%, 80.52% respectively) increased brain 5-HIAA levels as compared to OBX-Isolated group. Whereas, serotonin turnover ratio was found to be non-significant.

DISCUSSION

Pomegranate juice contains some phytoconstituents as anthocyanins, sugar, some pharmacologically active acids such as ascorbic acid, ellagic acid, caffeic acid, gallic acid, catechin, epigallocatechin, rutin, quercetin, iron and amino acids possessing strong anti-atherosclerotic, antihypertensive, antiaging and potent antioxidant properties. Seed oil mainly contains punicic acid and sterols which possess good nephroprotective properties. The pericarp (peel, rind) contains punicalagins, flavones, flavonones, and other flavanols possessing anti-inflammatory, antifungal and antimutagenic activity. Tannins including punicafolin and punicalin and flavones glycosides like pigenin and luteolin form the major constituents of pomegranate leaves.[55] The leaves are said to have good antioxidant properties.[56] The flowers contain ursolic acid, maslinic acid and asiatic acid possessing hepatoprotective and antioxidant properties and are used as a remedy for the diabetes mellitus.[55,57] The barks and roots are known for its vermifuge and anthelmintic properties.[12] The most beneficial components of pomegranate are punicic acid, ellagitannins, anthocyanidins, flavonoids, anthocyanins and estrogenic flavones.[55]

The major polyphenolic compound, ellagic acid, present in pomegranate rind and juice is well-known for its several pharmacological activities. With reference to the proven antidepressant activity in acute animals of depression; it was tested in sub-acute model. Present study is one of the first investigations that demonstrate effects of Ellagic acid based standardized extract of *punica granatum* rind on animal model of chronic stress mediated depression.

The symptoms simulating with clinical depression were induced in rats with the help of surgical model named olfactory bulbectomy (OBX). The majority of pre-clinical antidepressant models used today have a stressful component, such as forced swim test[26], learned helplessness[27], chronic mild stress[28] restraint-induced depression[29]. However, a major disadvantage with most of these animal models (except chronic mild stress) is that antidepressant activity is detected following an acute administration, whereas in clinical depression, a syndrome, which takes several days or weeks elapse before a therapeutic effect is observed[30]. A notable exception is the OBX rat model, which detects the antidepressant activity following sub-acute and chronic, but not acute, treatment.[32-34] The model also differs from others in that, attempts have been made to correlate, the variety of physiological, neuroendocrine, neuroimmune and behavioural alterations that occur with those in clinical depression.[35,36] In the present study, anxiety-related responses of both bulbectomized and sham rats were studied in both OFT and NSF paradigms. On the one hand, the central activity was found to be reduced in bulbectomy rats which is further decreased following social isolation as reflected in the percentage of time spent in the centre. It is noteworthy that this parameter does not depend on the level of activity of each and every animal, while the central time can be affected. These findings are in very good agreement with previously reported studies demonstrating increased anxiety-like manifestations induced by olfactory bulbectomy in rodents.[37-39] In the present study, Treatment with HEPG in three different doses prompted the central activity indicating its antianxiety effects comparable to standard drug Fluoxetine.

Social isolation triggered an increase in the latency of feeding of both sham as well as bulbectomized rats in NSF while indicating a heightened anxious behavior, which is consistent to the results obtained in open field apparatus.

It must be noted that all OBX rats have shown a lower latency to feeding as compared to their sham counterparts, contrary to the behavior in depression/anxiety-like state. As the parameter was observed in a dimly lit environment, OBX rats did not display a noticeable hyperactive behavior[40]. Therefore, this reduced feeding latency might be attributed to an increased exploration or an enhanced impulsivity of this model, rather than hyper locomotion. In our studies, a decrease in the latency was observed in all OBX animals as compared with their sham counterparts. Treatment with HEPG in two higher doses further significantly reduced the latency to feeding. This discrepancy might be explained by the concurrence of the food deprivation along with the social isolation as an additional stressful environment which potentiated the aversive conditions of test, making anxiety/fear play a preferential role versus impulsivity in response of OBX-isolated rats.

Depressive-like responses in sham and OBX rats were assessed in force swim test (FST) and sucrose intake test (SIT). Outcome of sham and OBX rat was unaffected in any of the FST parameters analyzed by social isolation. As per the previous report, we confirm that isolation per se does not influence the behavior of male mice[41] and rats[42] in the FST. However, when the test was conducted in female rats, some discrepant results were obtained by some researchers[43] and during the night phase of cycle[44,45]. Interestingly, our study results demonstrated that all bulbectomized rats exhibited lower immobility time scores and higher swimming time scores than their respective sham counterparts, which is an unexpected outcome for an animal model of depression. The reduced immobility behavior has also been previously reported by some researchers[39], which is explained on the basis of the hyperactivity exhibited by animals under highly stressful situations. The same may be applied for the swimming behavior exhibited by OBX rats, though this issue has been never explored. Nevertheless, the depression-like behaviors in the FST was studied by some researchers using DDY mice[46] and rats[47] subjected to olfactory bulbectomy, suggesting that, there exists a strain and species specific differences. In our results, climbing behavior was not significantly altered in OBX-Isolated animals. Whereas, treatment with HEPG, at all three dose levels, significantly reduced immobility duration and increased swimming behavior though climbing time was unaffected by surgery. In a differentiation of the behaviors in FST, it is previously suggested that climbing involves the adrenergic neurotransmission and swimming involves the serotonergic system[48], since selective serotonin reuptake inhibitors (SSRIs) increase swimming behavior while nor adrenaline reuptake inhibitors (NRIs) increase climbing behavior[49,50]. Regional differences in brain levels of NA and 5-HT of olfactory bulbectomized animals have been reported previously.[51,52] Further, in the present study, the sucrose intake test was used as a well validated parameter to infer anhedonia in laboratory animals[53,54]; which represents a cardinal symptom of depression. Our results confirm the anhedonia-like response of bulbectomized rats as evidenced by a decrease in the consumption of sucrose solution[18], that was not due to the reduced overall fluid intake since the daily fluid consumption during the habituation period was not changed. Interestingly, extract in two higher doses significantly increased the daily sucrose consumption indicating its possible anti-anxiety effect. This possibility of the discrimination between depression and anxiety-like behaviors could be valuable while the testing of mood regulator compounds. The brain monoamines were found to be significantly normalized by all three doses of HEPG. The detailed effect of extract on individual brain parts is to be studied further in detail later.

In conclusion, HEPG exerts a good antidepressant and anti-anxiety potential in animal model of depression, the possible mechanism of antidepressant and anti anxiety effect has to be explored further.

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Source of Support: Nil, **Conflict of Interest:** None.