Int. J. Pharm. Investigation, 2022; 12(2) : 216-222

Original Article

Pharmacological Screening of Gentisic acid for Antidepressant Activity in Unstressed and Stressed Mice

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ABSTRACT

Background: Gentisic acid has been reported to show neuroprotective effect. But its effect on depression is not available in the literature. So we evaluated the effect of this compound on depressive behavior in unstressed (normal) mice and stressed mice. **Materials and Methods:** Swiss albino male mice were given unpredictable mild stressors for twenty-one consecutive days to produce depressive behavior. Gentisic acid (25, 50, 100 mg/kg) and fluoxetine (20 mg/kg) were administered orally for twenty-one consecutive days. Depressive behavior was detected by tail suspension test and sucrose preference test. After behavioral testing, biochemical estimations were performed in plasma and brain. Histopathological studies on brain were also performed. **Results:** Immobility time of mice in tail suspension test was remarkably decreased by gentisic acid (50 and 100 mg/kg). Gentisic acid also restored decreased sucrose preference in mice subjected to stress paradigm. It remarkably lowered concentration of plasma nitrite, brain monoamine oxidase- A, malondialdehyde, TNF-α; and increased concentration of catalase and GSH in normal mice and also stressed mice. It also remarkably lowered plasma corticosterone concentration in stressed mice. Histopathological studies indicated protection by gentisic acid against hyperchromatic nuclei in brain. **Conclusion:** Gentisic acid produced remarkable antidepressant effect in normal mice and also stressed mice. The possible mechanisms for the observed antidepressant effect of gentisic acid might be through inhibition of brain MAO-A, amelioration of neuroinflammation and oxidative stress; and protection against hyperchromatic nuclei in brain. Moreover, antidepressant effect of gentisic acid in stressed mice might be through lowering of plasma corticosterone concentration.

Keywords: Depression, MAO-A, Neuroinflammation, Sucrose preference test, Tail suspension test.

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DOI: 10.5530/ijpi.2022.2.38

INTRODUCTION

Symptoms of sad mood, hopelessness, loss of interest and pleasure, insomnia, feeling of worthlessness and suicidal thoughts indicate depression.[1] It affects more than 280 million people worldwide.[2] Depression may be due to deficiency of monoamine neurotransmitters, dysregulation of HPA (hypothalamic-pituitary-adrenal axis), neuroinflammation and oxidative stress.[3-6] Continued exposure to stress increases oxidative stress and produced neuroinflammation in brain of humans; which may lead to depression.[7] Chronic unpredictable mild stress (CUMS) is commonly employed model to induce depression in laboratory animals.[8] CUMS leads to anhedonia, increased anxiety-like behavior, and hyperactivity of HPA axis in rodents.[9]

The conventional antidepressants presently used to treat depression in allopathic system of medicine have many side effects like body weight gain, sexual dysfunction, sedation, fatigue, etc.[10] Some plants like *Hypericum perforatum*, *Melissa officinalis*; bioactive compounds such as crocin, and glycyrrhizic acid, etc. have been reported to alleviate depression in clinical trials.[11-14] Thus, there is strong need for discovering plant-based antidepressants having equal effectiveness to Allopathic medications with no or less side effects.[15]

Gentisic acid, also known as 2,5-dihydroxybenzoic acid, is found in citrus fruits (*Citrus species*), *Gentiana species*, *Vitis vinifera*, red sandal wood (*Pterocarpus santalinus*), *Hibiscus rosa-sinensis*, *Olea europaea*, *Sesamum indicum*, Madagascar rosy periwinkle, avocados, kiwi and apples.[16-17] Gentisic acid has anti-Alzheimer, anti-Parkinsonian, antioxidant, anti-inflammatory and cardioprotective properties.[18-22] Since gentisic acid possesses antioxidant and neuroprotective activities, so the current research was intended to study effect of gentisic acid on depressive behavior in normal mice and also stressed mice.

MATERIALS AND METHODS

Animals

Swiss albino male mice (28-32 g, 2–3 months old) were used in the current research study. Since estrogens possesses antidepressant activity, that is why female mice were excluded.[23] The animals were kept in cages (n = 8 each) in an air-conditioned room (temperature 24-25°C) with twelve hours light and twelve hours dark cycle. The number of mice to be used for research study were approved by Institutional Animals Ethics Committee in the meeting held on March 4, 2020 (IAEC Minutes letter No. IAEC/2020/10-18, dated 04th March, 2020).

Drugs and chemicals

Gentisic acid was procured from Hi-media Laboratories Pvt. Ltd. (Mumbai). It was suspended in 1% w/v carboxy methyl cellulose (CMC). Fluoxetine was purchased from Sigma-Aldrich, St Louis, USA. Fluoxetine was dissolved in normal saline. Other chemicals employed were of analytical grade.

CUMS procedure

According to Willner *et al.* (1992) and as followed earlier in our laboratory, "Mice were given variable sequence of following mild unpredictable stressors once a day between 10:30 and 16:00 hr for 21 days:

Weeks	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7
Week-1	I	E	F	O	T2	X	T1
Week-2	I	O	X	T2	E	T1	X
Week-3	O	F	T1	X	T2	I	E

I—Immobilization for 2 hr, E—Exposed to empty water bottles for 1 hr, F—Exposed to foreign objects for 24 hr (e.g., piece of plastic), O—Food deprivation for 24 hr, T2—Pinched the tail for 60 sec, X—Tilted the cage at 45° for 7 hr, T1—Pinched the tail for 30 sec".[24-25]

Tail suspension test (TST)

This test was performed as reported by Steru *et al.* (1985) and as followed earlier.[25-26]

Sucrose preference test

As reported by Willner *et al.* (1987), "Sucrose preference test is used to assess anhedonia, which is a major symptom of depression. In training period, water and food were not given to mice for 48 hr, but were given only 1% w/v solution of sucrose. After three days, food and water were not provided to mice for 23 hr. Then, 1 hr of sucrose preference baseline test was carried out. In the baseline test, each mouse was provided two previously weighed bottles (one having 1% w/v sucrose solution and other containing normal water).

Sucrose preference was measured as mentioned below:

$$Sucrose\ preference\ (\%) = A/A+B \times 100$$

Where A is sucrose solution consumed (grams) and B is amount of water intake (grams).

Then on 21st day, this test was again carried out to find out the effects of CUMS and drug treatment".[27]

Locomotor activity measurement

Locomotor activity of mice were observed for five minutes using photoactometer (INCO, Ambala, Haryana, India).[28]

Treatment groups

Following 20 groups (n= 8 each) of mice were constituted:

For TST and locomotor activity

Groups 1 to 5: Vehicle (1%, w/v, CMC), gentisic acid (25, 50 and 100 mg/kg) and fluoxetine (20 mg/kg) respectively were given orally to mice for twenty-one days in succession. On 21st day, one hour after administration of vehicle / drugs, locomotor activity of mice were observed. TST was conducted on day 22.

Groups 6 to 10: Vehicle (1% w/v CMC), gentisic acid (25, 50 and 100 mg/kg) and fluoxetine (20 mg/kg) respectively were given orally thirty minutes before giving various stressors for twenty-one days in succession. On 21st day, 01 hr after giving stress to mice, their locomotor activity scores were observed. TST was conducted on 22nd day.

For sucrose preference test

Groups 11 to 20: Separate groups of mice were treated in similar way as groups 1 to 10. This test was performed on day 21.

Biochemical estimation in groups 1 to 10

After performing behavioral tests on 21st day and 22nd day in groups 1-10, mice were killed by cervical dislocation on 23rd day and blood samples were withdrawn from heart. To separate plasma, centrifugation of blood samples was carried out at 2500 rpm for 10 min using a cooling centrifuge (Remi, Mumbai). Corticosterone levels were measured in plasma.

Assessment of plasma corticosterone

Corticosterone level was estimated as per the procedure discovered by Bartos *et al.* (1979) using Ultra violet –visible spectrophotometer (Thermo Scientific Genesys™ 180, USA).[29]

Biochemical assessments in brain

As followed earlier in our laboratory, "After collecting blood samples on 23rd day from the mice of groups 1-10, the brain of these mice were separated. The brain samples were washed in cold 0.25 M sucrose – 0.1 M Tris-0.02 M EDTA buffer having pH 7.4. Then, these brain samples were weighed, homogenized in nine volumes of above-mentioned buffer. The brain samples were then centrifuged two times at 2500 rpm for 10 min at 4°C in a cooling centrifuge (Remi Instruments, Mumbai). The supernatant was again centrifuged at 12000 rpm for 20 min at 4°C using a cooling centrifuge. The resultant supernatant was divided into two parts-one part containing precipitates (mitochondrial fraction) was used for determination of MAO-A activity. In the remaining supernatant, the concentrations of malondialdehyde (MDA), reduced glutathione (GSH) and catalase were estimated".[25]

Assessment of brain MAO-A, MDA, GSH and Catalase

Brain MAO-A, MDA, GSH and catalase were assessed spectrophotometrically as per reported methods using UV–visible spectrophotometer (Thermo Scientific Genesys™ 180, USA).[30-34]

Estimation of protein concentration

Protein estimation was done by Biuret method.[35]

Biochemical estimation in group 11-20

After doing behavioral tests on 21st day in mice of groups 11-20 (n= 7 each), they were killed by cervical dislocation on 23rd day. Blood samples were withdrawn from heart of sacrificed mice. Centrifugation of blood samples was carried out at 2500 rpm for 10 min to separate plasma. The plasma was used for nitrite estimation. The brain was isolated from mice after collecting blood samples.

Assessment of plasma nitrite levels

It was estimated by Green *et al.* (1982) method using UV–visible spectrophotometer (Thermo Scientific Genesys™ 180, USA).[36]

Estimation of Tumor necrosis factor –alpha (TNF-α) level

The isolated brain was washed in phosphate buffer solution having pH 7.4, so as to remove extra blood. Then, the brain sample was weighed and homogenized in phosphate buffer solution with a glass homogenizer on ice. The samples were then stored in a deep freezer at -20°C for 15 days and later centrifuged at 2500 rpm for 20 min. TNF- α level was assayed by sandwich enzyme immunoassay technique (Bioassay Technology Laboratory, Shanghai Korain Biotech Co. Ltd.). Intensity of the color produced was noted by ELISA reader (Biotek Synergy-2 Multi Detection Micro Plate Reader). The values of TNF- α were read from the standard curve.[37]

Histopathological Studies

One mouse from each group was used for histopathological studies. The separated brains were preserved in 10% v/v formalin. The brain was fixed in paraffin wax and cut into sections. These were then stained with hematoxylin and eosin dyes. The stained sections of brain were observed under bright field illumination by employing AHBT-51 microscope (Olympus Vanox Research Microscope, Japan).

Statistical Evaluation

The data were mentioned as mean ± standard error of mean (SEM). The data analysis was done by two-way ANOVA followed by Bonferroni test

using Graphpad prism software 5. Statistical significance was considered at $p < 0.05$.

RESULTS

Effect of gentisic acid on immobility time of mice in tail suspension test

In TST, CUMS remarkably increased immobility time of mice ($p<0.001$) when compared to unstressed (normal) control mice. Administration of gentisic acid (50 and 100 mg/kg) and fluoxetine remarkably reduced immobility time of unstressed mice ($p<0.01$, $p<0.001$, $p<0.001$ respectively) and stressed mice ($p<0.001$) in comparison to respective unstressed and stressed control mice (Table 1).

Effect of gentisic acid on locomotor activity

No remarkable effect on locomotor activities of mice was produced by gentisic acid and fluoxetine in unstressed (normal) mice and stressed mice when compared to respective control mice (Table 1).

Effect of gentisic acid on sucrose preference test

In baseline test, no remarkable difference on sucrose preference among various treatment groups was observed. However, after 21 days, sucrose preference (%) decreased remarkably in control stressed mice in comparison to control unstressed mice. Higher doses of gentisic acid (50, 100 mg/kg) and fluoxetine remarkably ($p<0.001$) restored the decrease in preference for sucrose solution in CUMS mice in comparison to their control stressed mice. Fluoxetine also remarkably ($p<0.001$) increased preference for sucrose solution in unstressed (normal) mice when compared to control (Table 2).

Effect of gentisic acid on plasma nitrite

Plasma nitrite concentration was remarkably ($p<0.001$) increased in CUMS exposed mice in comparison to control unstressed mice. Levels of plasma nitrite were remarkably decreased by higher doses (50, 100 mg/kg) of gentisic acid and fluoxetine in unstressed ($p<0.001$) mice and also stressed mice ($p< 0.001$) when compared to their respective control mice. But 25 mg/kg dose of gentisic acid did not lead to any remarkable change in plasma nitrite concentration in unstressed mice and also stressed mice (Table 3).

Effect of gentisic acid on brain TNF-α level

CUMS remarkably ($p<0.001$) elevated TNF-α levels in brain tissue homogenate in comparison to control unstressed mice. Higher doses of gentisic acid (50, 100 mg/kg) and fluoxetine remarkably decreased TNF-α

Table 1: Effect of gentisic acid and fluoxetine on immobility time in TST and locomotor activity of mice.

Treatment for 21 days	Dose (kg^{-1})	Immobility time (sec)	No. of locomotor counts
Vehicle	10 ml	186.62±5.669	236.25±16.12
Vehicle + CUMS	10 ml	237.25±2.908***	291±36.99
Fluoxetine (U)	20 mg	89.75±6.709	269.5±36.998
Gentisic acid (U)	25 mg	172.37±6.053	274±18.109
Gentisic acid (U)	50 mg	159.37±2.859**	276.62±28.486
Gentisic acid (U)	100 mg	99.62±5.713***	253.25±27.171
Fluoxetine+ CUMS	20 mg	93.42±5.686###	300.28±32.556
Gentisic acid +CUMS	25 mg	222.50±7.069	272.87±27.524
Gentisic acid +CUMS	50 mg	197.12±3.912###	295.5±11.107
Gentisic acid +CUMS	100 mg	105.12±5.420###	258.87±8.264

n = 8 each group except fluoxetine stressed group which had 7 mice. Values are mentioned as mean ± SEM.

** $p<0.05$, *** $p<0.001$ versus control unstressed mice; ### $p< 0.001$ versus control stressed mice

For immobility period; Treatments [$F_{4,69}$=212.3; $p<0.0001$], stress [$F_{1,69}$=74.88; $p<0.0001$], treatment × stress interaction [$F_{4,69}$=9.370; $p<0.0001$].

For locomotor counts; Treatments [$F_{4,69}$=0.5339; p=0.7112], stress [$F_{1,69}$=1.844; p=0.1789], treatment × stress [$F_{4,69}$=0.8212; p=0.3814]. CUMS- chronic unpredictable mild stress; U –unstressed mice

Table 2: Effect of gentisic acid and fluoxetine on sucrose preference test.

Treatment for twenty-one days	Dose (per kg)	Sucrose preference (%) [Baseline test]	Sucrose preference (%) [After 21 days]
Vehicle	10 ml	63.08±0.2216	41.01±0.4951
Vehicle + CUMS	10 ml	62.50±0.3199	28.67±0.1574***
Fluoxetine (U)	20 mg	62.528±0.4365	50.42±0.6573***
Gentisic acid (U)	25 mg	64.49±1.139	41.79±0.8690
Gentisic acid (U)	50 mg	62.51±1.008	41.22±0.3684
Gentisic acid (U)	100 mg	62.42±0.6351	42.10±0.2696
Fluoxetine+ CUMS	20 mg	62.68±0.2679	45.50±0.3654###
Gentisic acid + CUMS	25 mg	58.71±3.879	29.08±0.3913
Gentisic acid + CUMS	50 mg	62.26±1.296	32.09±0.4370###
Gentisic acid + CUMS	100 mg	61.56±2.3690	35.99±0.5570###

n= 8 in every group.

*** $p< 0.001$ versus control unstressed mice; ### $p< 0.001$ versus control stressed mice.

For baseline test, treatments [$F_{4,70}$=0.1806; p=0.9477], stress [$F_{1,70}$= 2.098; p=0.1519], treatment × stress interaction [$F_{4,70}$=1.172; p=0.3305].

After 21 days; treatments [$F_{4,70}$=239; $p<0.0001$], stress [$F_{1,70}$=843; $p<0.0001$]; treatments × stress [$F_{4,70}$=25.3; $p<0.0001$]. U – unstressed mice

Table 3: Effect of gentisic acid and fluoxetine on plasma nitrite level and brain TNF-α level.

Treatment for 21 days	Dose (kg^{-1})	Plasma nitrite level (µg/ml)	Brain TNF-α level (ng/ml)
Vehicle	10 ml	33.71±0.6809	74.57±0.8123
Vehicle + CUMS	10 ml	53.36±1.0210***	131.71±0.8081***
Fluoxetine (U)	20 mg	24.31±1.226***	55.57±0.7514***
Gentisic acid (U)	25 mg	31.42±0.3515	74±1.254
Gentisic acid (U)	50 mg	28.89±0.3346***	71.85±1.610
Gentisic acid (U)	100 mg	25.98±0.2970***	54.24±1.270***
Fluoxetine+ CUMS	20 mg	34.04±1.090###	80.42±0.5714###
Gentisic acid +CUMS	25 mg	51.91±0.4466	126±1.397
Gentisic acid +CUMS	50 mg	46.98±0.4803###	115.14±4.458###
Gentisic acid +CUMS	100 mg	41.15±0.5300###	91.57±3.108###

n = 7 in every group.

*** $p<0.001$ versus control unstressed mice; ### $p< 0.00$ versus control stressed mice

For plasma nitrite level; treatments [$F_{4,60}$=132.1; $p<0.0001$], stress [$F_{1,60}$=1321; $p<0.0001$], treatment × stress [$F_{4,60}$=18.13; $p<0.0001$]

For brain TNF-α level; treatments [$F_{4,60}$=133.8; $p<0.0001$], stress [$F_{1,60}$=1189; $p<0.0001$, treatment × stress [$F_{4,60}$=21.60; $p<0.0001$]; U- Unstressed mice

level in stressed mice (p<0.001) in comparison to their control mice. However, 100 mg/kg dose of gentisic acid and fluoxetine remarkably (p<0.001) decreased TNF-α level in unstressed mice when compared to their control (Table 3).

Effect of gentisic acid on plasma corticosterone

Plasma corticosterone concentration was remarkably decreased (p<0.001) by fluoxetine and gentisic acid (25, 50 and 100 mg/kg) in stressed mice in comparison to control stressed mice. No remarkable effect on plasma corticosterone concentration was produced in unstressed mice on administration of gentisic acid and fluoxetine when compared to their control (Table 4).

Effect of gentisic acid on brain MAO-A

Brain MAO-A activity increased remarkably (p<0.001) in control stressed mice when compared to control unstressed (normal) mice. MAO-A activity was decreased remarkably (p<0.001) by fluoxetine and higher doses (50, 100 mg/kg) of gentisic acid in stressed mice in comparison to control stressed mice. In unstressed mice, fluoxetine and 100 mg/kg dose of gentisic acid remarkably decreased (p<0.001) MAO-A activity when compared to their control (Table 4).

Effect of gentisic acid on brain malondialdehyde (MDA)

CUMS remarkably elevated (p<0.001) the concentration of MDA when compared to control unstressed mice. In stressed mice, gentisic acid (25, 50, 100 mg/kg) and fluoxetine remarkably decreased (p<0.05, p<0.001, p<0.001, p<0.001 respectively) MDA concentration in comparison to control stressed mice. Fluoxetine and gentisic acid (50 and 100 mg/kg) remarkably decreased (p<0.001, p<0.001, p<0.01 respectively) the concentration of MDA in unstressed mice when compared to their control (Table 5).

Effect of gentisic acid on brain GSH levels

CUMS remarkably (p<0.001) decreased brain GSH concentration when compared to control unstressed mice. Fluoxetine and the higher doses (50, 100 mg/kg) of gentisic acid remarkably (p<0.01, p<0.001, p<0.001 respectively) increased brain GSH concentration in CUMS mice in comparison to their control mice. But in unstressed mice, 100 mg/kg dose of gentisic acid and fluoxetine remarkably (p<0.01, p<0.001 respectively) increased GSH concentration when compared to their control mice (Table 5).

Effect of gentisic acid on brain catalase activity

CUMS remarkably (p<0.001) decreased brain catalase activity when compared to control unstressed mice. Catalase activity was significantly increased by fluoxetine and gentisic acid (50, 100 mg/kg) in both unstressed (p<0.001) and stressed mice (p<0.001) when compared to their control mice (Table 5).

Histopathological study of brain

Control unstressed mice contained very less number of hyperchromatic nuclei. Fluoxetine produced protection against hyperchromatic nuclei in unstressed mice. Gentisic acid (25, 50 mg/kg) treated unstressed mice showed less number of hyperchromatic nuclei. But 100 mg/kg dose of gentisic acid showed protection against hyperchromatic nuclei in unstressed mice. Control stressed mice contained large number of hyperchromatic nuclei. Fluoxetine produced protection against hyperchromatic nuclei in stressed mice. Gentisic acid (25 mg/kg) treated stressed mice showed decrease in number of dark stained hyperchromatic

Table 4: Effect of gentisic acid and fluoxetine on plasma corticosterone level and brain MAO-A activity.

Treatment for twenty-one days	Dose (kg^{-1})	Plasma corticosterone concentration (µg/ml)	Brain MAO-A activity (nmol/mg protein)
Vehicle	10 ml	26.66±0.5904	55.42±0.8763
Vehicle + CUMS	10 ml	36.91±0.5838**	84.95±0.5326***
Fluoxetine (U)	20 mg	23.67±0.3222	43.39±0.5221***
Gentisic acid (U)	25 mg	27.05±1.533	55.36±1.258
Gentisic acid (U)	50 mg	24.18±1.225	51.07±2.018
Gentisic acid (U)	100 mg	24.11±1.3940	44.11±1.074***
Fluoxetine+ CUMS	20 mg	24.04±1.0130$^{###}$	39.95±0.6933$^{###}$
Gentisic acid +CUMS	25 mg	28.91±2.000$^{###}$	86.15±1.501
Gentisic acid +CUMS	50 mg	26.16±1.169$^{###}$	76.39±2.702$^{###}$
Gentisic acid +CUMS	100 mg	22.82±1.6760$^{###}$	63.92±1.135$^{###}$

n = 8 each group except fluoxetine stressed group which had 7 mice.

$^{***}p$<0.001 versus control unstressed mice; $^{###}$ p< 0.001 versus control stressed mice

For plasma corticosterone levels; treatments [$F_{4,69}$=15.04; p<0.0001], stress [$F_{1,69}$=10.85; p=0.0016], treatment × stress [$F_{4,69}$=78.43; p=0.0002].

For brain MAO- activity; treatments [$F_{4,69}$=152.1; p<0.0001], stress [$F_{1,69}$=522.9; p<0.0001], treatment × stress [$F_{4,69}$=49.26; p<0.0001]; U- Unstressed mice

Table 5: Effect of gentisic acid and fluoxetine on brain MDA level, GSH level and catalase activity.

Treatment for twenty-one days	Dose (kg^{-1})	MDA level (nmoles/mg protein)	GSH level (µmol/mg protein)	Catalase (µg/mg protein)
Vehicle	10 ml	1.001± 0.02014	0.03386± 0.00890	55.93± 0.5156
Vehicle + CUMS	10 ml	1.436± 0.08632***	0.01520± 0.000466***	31.95± 0.6257***
Fluoxetine(U)	20 mg	0.7457± 0.000946**	0.04525± 0.001036***	85.07± 0.5943***
GA (U)	25 mg	0.9755± 0.04511	0.03413± 0.000294	55.86± 0.3150
GA (U)	50 mg	0.0920± 0.03103***	0.03513± 0.000166	60± 0.8290***
GA (U)	100 mg	0.0875± 0.04224***	0.03637± 0.000117**	67.52± 0.6432***
Fluoxetine+ CUMS	20 mg	1.054± 0.015390$^{###}$	0.02640± 0.000416$^{###}$	76.68± 1.1140$^{###}$
GA +CUMS	25 mg	1.2700± 0.08069$^{#}$	0.01616± 0.000115	32.08± 0.2787
GA +CUMS	50 mg	1.1410± 0.05419$^{###}$	0.01758± 0.000212$^{##}$	37.64± 0.8183$^{###}$
GA +CUMS	100 mg	1.1210± 0.04460$^{###}$	0.01942± 0.000257$^{###}$	53.22± 0.7035$^{###}$

n = 8 each group except fluoxetine stressed group which had 7 mice.

$^{**}p$<0.01, $^{***}p$<0.001 versus control unstressed mice; $^{#}p$<0.05, $^{##}p$<0.01, $^{###}$ p< 0.001 versus control stressed mice

For MDA level; treatments [$F_{4,69}$=63.83; p<0.0001], stress [$F_{1,69}$=390.1; p<0.0001], treatment × stress [$F_{4,69}$=29.66; p<0.0001]

For GSH level; treatments [$F_{4,69}$=165.9; p<0.0001], stress [$F_{1,69}$=3193; p<0.0001], treatment × stress [$F_{4,69}$=1.215; p=0.3125]

For catalase activity; treatments [$F_{4,69}$= 1084; p<0.0001], stress [$F_{1,69}$ =1908; p<0.0001], treatment × stress [$F_{4,69}$=53.34; p<0.0001]; U- Unstressed mice

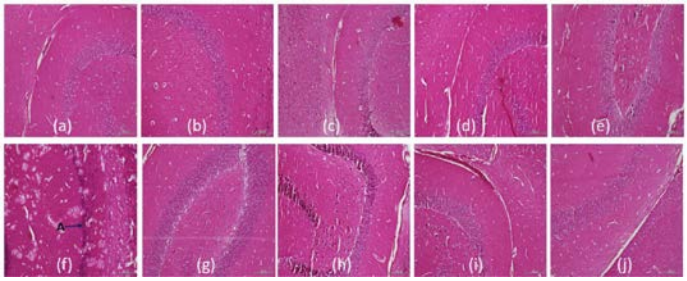

Figure 1: Effect of gentisic acid on histopathological changes in brain of unstressed and stressed mice. a-j photomicrographs (20x) of Hematoxylin and Eosin (H&E) stained brain section.
a) Control unstressed mice; (b) Fluoxetine (20 mg/kg) in unstressed mice; (c, d, e) Gentisic acid (25, 50, 100 mg/kg respectively) in unstressed mice; (f) Control stressed mice; (g) Fluoxetine (20 mg/kg) in stressed mice; (h, i, j) Gentisic acid (25, 50, 100 mg/kg respectively) in stressed mice 'A'-hyperchromatic nuclei; Calibration graph= 100µm

nuclei. But the higher doses (50, 100 mg/kg) of gentisic acid produced protection against hyperchromatic nuclei in stressed mice (Figure 1).

DISCUSSION

In the current research, gentisic acid produced remarkable antidepressant potential in unstressed mice and also in those mice subjected to CUMS. Tail suspension test is usually employed pre-clinical model to screen antidepressant action of drugs; as this test is easy to perform and it is also reliable method. In this test, immobility time of mice is evaluated which specifies helplessness behavior of mice.[38] In this study, CUMS remarkably prolonged immobility period of mice in TST, showing induction of depressive behavior in mice. Continuous administration of gentisic acid (50, 100 mg/kg) and fluoxetine for twenty-one consecutive days remarkably decreased immobility time of unstressed and CUMS mice in TST. These findings show antidepressant action of gentisic acid. Fluoxetine and gentisic acid failed to show remarkable effect on locomotor activity of unstressed mice and also CUMS mice in comparison to their respective control. Thus, there was no central nervous system stimulating effect by these drugs.

Sucrose preference test is measure of loss of pleasure in rodent depression models. On exposure of mice to CUMS, the mice take less sucrose solution which signifies depression-like behavior.[39] In the current research, CUMS exposed mice remarkably decreased sucrose consumption in comparison to unstressed control mice. Administration of gentisic acid and fluoxetine for twenty-one continuous days remarkably restored decrease in preference for sucrose solution in CUMS mice; which also substantiated their antidepressant effects. Fluoxetine also increased sucrose preference in unstressed mice, which is also substantiated by a previous study.[40]

MAO metabolizes monoamine neurotransmitters like nor-epinephrine, dopamine and serotonin. MAO-A inhibitors are effective for treating depression.[41] In the present research work, CUMS exposed mice showed remarkable increase in brain MAO-A. It is also substantiated by a previous study.[42] Gentisic acid (100 mg/kg) and fluoxetine remarkably decreased brain MAO-A activity in stressed and unstressed mice. These results showed antidepressant activity of gentisic acid through MAO-A inhibition. MAO-A inhibition by fluoxetine is also substantiated by a previous study.[43]

In rodents and humans, stress leads to hyperactivation of HPA axis by increasing corticosterone levels, resulting in depressive disorder.[44] In the present research work, plasma corticosterone levels were remarkably elevated on exposure of mice to CUMS. It is also substantiated by observations from an earlier study.[45] Gentisic acid (25, 50, 100 mg/kg) remarkably decreased plasma corticosterone in stressed mice.

Oxidative stress results in accumulation of oxygen free radicals, leading to destruction of cellular macromolecules.[46-47] Various oxidative stress markers (catalase, malondialdehyde, GSH, plasma nitrite) are found to be altered in depressive disorders.[48] Catalase and GSH act as antioxidant defense systems against oxygen free radicals. Malondialdehyde is increased in oxidative damage.[49] In the present research work, CUMS remarkably increased plasma nitrite and brain malondialdehyde levels; and decreased brain GSH and catalase. This finding is also substantiated by a previous study.[50] Gentisic acid (100 mg/kg) significantly restored the altered levels of brain GSH, catalase, malondialdehyde; and plasma nitrite in stressed and unstressed mice. The observed antioxidant effect of gentisic acid is also supported by a previous study.[20]

Inflammatory cytokines play an important part in pathogenesis and development of depression. Stressful conditions activate the immune system that leads to increase in proinflammatory cytokines (such as TNF-α) levels.[51] In the present research work, CUMS remarkably increased TNF-α level in mice. Gentisic acid (50, 100 mg/kg) remarkably decreased TNF-α in stressed as well as unstressed mice, indicating amelioration of neuroinflammation.

In the present research work, histopathological studies indicated that stressed mice have large number of hyperchromatic nuclei in brain in comparison to control unstressed mice, which is substantiated by a previous study.[52] Hyperchromatic nuclei are darker in color when examined under microscope.[53] Gentisic acid produced protection against hyperchromatic nuclei brain of both unstressed as well stressed mice.

CONCLUSION

Gentisic acid (100 mg/kg) administered through oral route for twenty-one days in succession showed remarkable antidepressant action in unstressed (normal) mice and also stressed mice. The observed antidepressant effect of gentisic acid might be through decrease of brain MAO-A, amelioration of oxidative stress and neuroinflammation; and decrease in hyperchromatic nuclei in brain. Additionally, antidepressant effect of gentisic acid in CUMS mice might be through decrease of plasma corticosterone concentration.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Article History: Submission Date : 25-03-2022; Revised Date : 05-04-2022; Acceptance Date : 21-04-2022.
Cite this article: Bansal S, Dhingra D. Pharmacological Screening of Gentisic acid for Antidepressant Activity in Unstressed and Stressed Mice. Int. J. Pharm. Investigation. 2022;12(2):216-22.