Dual antidepressant and hepatoprotective effect of *Itrifal* in experimental models of acute depression

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Abstract

Background: Various natural treatments have been introduced into psychiatric practice due to improved compliance and fewer negative effects. *Itrifal*, an important dosage form of Unani medicine, was introduced some 1000 years ago for the treatment of mainly brain disorders. The Unani system of medicine, which is also referred to as Greco-Arab medicine, has been practiced mainly by following the fundamentals of the Hippocratic doctrine of humoral theory since ancient times. In this study, we investigated *Itrifal's* hepatoprotective and antidepressant effects against fluoxetine in an acute physically produced depression utilizing an animal model of depression (forced swim test) in rats.
Aim: The aim of this research was to conduct an experimental study on the Antidepressant and hepatoprotective effect of Itrifal in experimental models of acute depression.
Methods: In this study, all animals were trained for the experiment and then rats were divided into four different groups (6 rats each) and treated via oral route as follows; Group I: normal control (Saline group). Group II: disease control (forced swimming). Group III: fluoxetine (10 mg/kg) and Groups IV: *Itrifal* (10 mg/kg). Swimming induced depression-like behaviours. *Itrifal* (10 mg/kg, *p.o.*) and fluoxetine (10 mg/kg) were given orally. The behavioral effects, such as the open field test (OFT) and forced swimming test (FST), as well as physiological measures such as neurotransmitter (Serotonin, Norepinephrine and Dopamine) levels, were evaluated. A liver histopathology investigation was performed.
Results: *Itrifal*, has been extensively studied and shown potential as an effective dual therapeutic agent for treating both depression and liver disease. Data were presented as mean

± SEM (n=6). ###p<0.001 indicates a significant difference between the disease control group and the normal control group. ***p<0.001 and ***p<0.001 indicate significant differences in the all treatement groups caompared to the disease control group.

Conclusion: The results demonstrated that *Itrifal* (10 mg/kg, *p.o.*) has potential hepatoprotective and antidepressant-like effects when compared to fluoxetine based on behavioural and biochemical parameters. This suggests that it might be useful for the future prospect.

Keywords: *Itrifal*, Depression, Fluoxetine, Serotonin, Dopamine and Swimming

1. Introduction

Depression and liver disease are major causes of disability, morbidity and mortality worldwide, and their prevalence is increasing. Depression is more common in people with liver illness than in the general population, and it is similar to the higher rates seen in other medical comorbidities and chronic inflammatory disorders. Notably, a concomitant diagnosis of depression has a negative impact on the outcomes of liver illness [1]. Depression is more common in individuals with cirrhosis than in the general population, and it has a negative impact on clinical outcomes.18 To enhance quality of life and outcomes in this vast patient population, it is critical to screen for depression in patients with cirrhosis and understand the underlying cause of its increasing incidence [2]. Depression is a frequent and debilitating mental condition that affects around 2.5% of the general population. It has negative social implications, such as diminished employment and psychosocial impairment. According to World Health Organization studies, it is expected to become the second major cause of disability by 2020 [3]. Despite a continuous growth in the number of antidepressants over the years, the disorder's prevalence has remained stable, possibly due to uncertain pathophysiology or the variable efficacy of currently available antidepressants, which have negative side effects. However, there is a direct relationship between the catecholaminergic neural circuits and depression [4].

Hepar is the Greek word for liver, so pharmaceutical phrases connected to the liver frequently begin with hepato or hepatic. The liver is known as the "great chemical factory" of the body because it regulates, synthesizes, stores, and secretes many important proteins, nutrients, and chemicals, as well as purifies and eliminates toxins or unnecessary substances from the body. The liver secretes bile, which plays a crucial role in digestion. The danger of liver poisoning has lately grown due to higher exposure to environmental pollutants, pesticides, medications, and frequent use of chemotherapeutics [5]. Liver injury is invariably linked with cellular necrosis, increased tissue lipid peroxidation, and a reduction in tissue glutathione (GSH) levels [6]. Furthermore, serum levels of various biochemical indicators, such as serum glutamate oxaloacetate transaminase (SGOT/AST) and serum glutamate pyruvate transaminase (SGPT/ALT), triglycerides, cholesterol, bilirubin, and alkaline phosphatase, are raised [7].

Depression is significantly more common among people with liver problems than in the general population. For example, depressive disorders are at least three times more common

in patients with liver illness, with up to 17% of this population affected compared to 5% of the overall population [8]. The link between liver illness and depression is multifaceted and bidirectional. Chronic liver illnesses can cause systemic inflammation, which has been linked to the development of depression. Furthermore, the gut-liver-brain axis may play a role, because abnormalities in this system can contribute to depressive symptoms in cirrhosis patients [9].

Since ancient times, the Unani system of medicine, also known as Greco-Arab medicine, has primarily adhered to the basics of the Hippocratic philosophy of humoral theory [10]. In Unani medicine, the concept of compounding drugs was postulated by Galen (131 c.–201 AD), who is regarded as the father of polyherbo-mineral mixtures [11]. Unani classical literature describes around 90 dose forms, which can be solid, semisolid, liquid, or gaseous [12]. Certain dose forms are specially created for the prevention and treatment of diseases in distinct systems. For example, Basalıqun (fine powder) for eye diseases, Tiryaq (antidote) for poisoning, Jawarish (semisolid preparation) for gastrointestinal disorders, Khamıra (semisolid preparation) for cardiac diseases, Zaruni (semisolid preparation) for renal diseases, Lauq (linctus) for respiratory diseases, Mufarrih (semisolid preparation) for cerebral and cardiovascular disorders, YaqutI (semisolid preparation) for cardiac ailments, and Labub (semisolid preparation) for sexual dysfunction [13]. and *Itrıfal* (semisolid preparation) for the diseases of brain and stomach [14]. Itrıfal is a dosage form containing three plant drugs: Emla (Emblica officinalis Gaertn.), Halela (Terminalia chebula Retz.), and Balela (Terminalia bellirica Roxb.) [13]. Itrıfal is derived from the Sanskrit term 'triphala', which means 'three fruits'. It is produced as a confection by adding honey or sugar [14]. Unani physicians recommended that Itrifal be administered after two months of preparation for optimum therapeutic efficacy [15].

Chemicals such as hydrogen peroxide and acrylamide generate oxidative stress, which is connected with a variety of neurological diseases. Itrifal, also known as Triphala, is an antioxidant, antihistaminic, anti-inflammatory, and anticancer supplement. According to one study, Triphala has considerable neuroprotective effects against H2O2 toxicity by inhibiting cell death and increasing cellular proliferation. Triphala also reduced the activation of the mitogen-activated protein kinase signal pathway and enhanced the concentration of antioxidant enzymes, such as superoxide dismutase and catalase, against HO-treated SH-SY5Y cells [16]. Itrifal polyphenols have been reported to be beneficial in cognitive impairment and psychiatric illnesses by influencing 5-HT, brain-derived neurotrophic factor, antioxidant-related signaling pathways, and gut microbiota [17]. The hydro-alcoholic extract of E. officinalis improves cognitive functioning via raising TNF-α levels in brain tissues [18]. Similarly, a hydro-alcoholic extract derived from E. officinalis at 700 mg/kg has been shown to reduce kainic acid-induced convulsions, cognitive impairment, and excessive oxidation in rat brains. Phyllanthus fruit contains bioactive substances such as gallic acid, ellagic acid, emblicanins A & B, corilagin, furosin, and geraniin, which contribute to its pharmacological properties [19]. Ellagic acid is a polyphenolic compound found in many plants, including three myrobalan fruits, and has demonstrated notable neuroprotective effects through its free-radical scavenging property, ion chelation activity, mitigation of abnormal mitochondrial function, and improvement in the normal functions of various signaling pathways in the

nervous system [20]. E. officinalis fruit has been observed to prevent degeneration in nerve cells in fly models of Alzheimer's and Huntington's illness [21]. The ethanolic extract of *E. officinalis* improved mitochondrial membrane function, reduced apoptosis and oxidative stress, decreased VEGF, and increased PGC-1α against a human retinal pigment epithelial age-related macular degeneration transmitochondrial cybrid cell model, indicating cytoprotective potential [22]. The aqueous extract of E. officinalis has been shown to have promising antidepressant effects by inhibiting MAO-A and GABA pathways. In fluoride-intoxicated rats, the powder of E. officinalis fruit demonstrated significant memory-enhancing effects [23].

Table 1. Itrifal's preparations

Itrifal	**Ingredients**	**Dose**	**Traditional uses**	**References**
Itrifal Ustukhuddus	Terminalia chebula, Terminalia bellirica, Emblica officinalis, Rosa damscena, Lavandula stoechas, Polypodium vulgare, Cuscuta reflexa, Vitis vinifera	5–10 g	Headache, paralysis, epilepsy, chronic rhino-sinusitis	[24]
Itrifal Saghir	Terminalia chebula, Terminalia bellirica, Emblica officinalis	10–15 g	Cerebral weakness, dementia, haemorrhoids	[25]
Itrifal Kishniz	Terminalia chebula, Terminalia bellirica, Emblica officinalis, Coriandrum sativum	10–30 g	Headache, conjunctivitis, chronic rhinosinusitis, otalgia, flatulence, haemorrhoids	[26]
Itrifal Kabir	Terminalia chebula, Terminalia bellirica, Emblica officinalis, Tanacetum umbelliferum, Myristica fragrans aril, Plumbago zeylanica, Pastinaca secacul, Cheiranthus cheiri, Matthiola incana, Wrightia tinctoria, Centaurea behen, Salvia haemotodes	5–10 g	Cerebral weakness, chronic rhino-sinusitis	[27]
Itrifal Aftimun	Terminalia chebula, Terminalia bellirica, Emblica officinalis, Operculina terpethum, Cuscuta reflexa, Cassia senna, Plumbago zeylanica, Polypodium vulgare, Lavandula stoechas, Rosa damscena, Pimpinella anisum	10 g	Melancholia, insanity, psychosis	[28]

2. MATERIAL AND METHODS

2.1. Animals

The present experimental study was conducted on Wistar male rats weighing between 150 and 250 grams. The rats were obtained from SRMSCET (Pharmacy), Bareilly, Uttar Pradesh, India, at the animal housing facility. The animals were housed in a sterile polyacrylic cage and kept in air-conditioned animal housing with typical laboratory settings (ambient temperature 25 ± 3°C, relative humidity 55-60%, and a 12-hour light/dark cycle). They allowed everyone unfettered access to food and water. Every behavioral exam lasted from 9:00 to 17:00. The study followed the CPCSEA guidelines for the use and care of experimental animals, which were approved by IAEC committee no. (IAEC/SRMS/2022/II/10).

2.2. Drugs and Chemicals

Itrifal was purchased from Sigma Aldrich St. Louis, Missouri, USA. Every day before the experiment was conducted; freshly distilled water was used in this study. Every additional chemical utilized in this investigation was newly produced and of analytical grade (AR).

2.3. Experimental design

Rats were divided into four different groups (6 rats each) and treated via oral route as follows; Group I: normal control (Saline group). Group II: disease control (forced swimming). Group III: fluoxetine (10 mg/kg) and Groups IV: *Itrifal* (10 mg/kg).

2.4. Experimental procedure

Successfully depression induced by swimming in Groups II, III and IV. Group (1). Group (1) was kept in separate cages and had free access to food and water till the end of the experiment. All other groups received the corresponding drugs orally for the following 3 days. On day 2 behavioral tests, namely open field test (OFT) and forced swimming test (FST) were performed. Twenty-four hours later, the rats were sacrificed by cervical dislocation 30 min after the last drug administration. Brain and liver tissues were isolated and each brain or liver was washed with cold sterile physiological saline, blotted between two damp filter papers and stored at -80 ºC for further biochemical analysis. Parts of the liver tissues were isolated in formalin and used for the histopathology.

3. Behavioral tests

3.1. Open field test

The open field test was carried out in a square wooden arena (80 cm · 80 cm · 40 cm high) with red walls and white smooth polished floor divided by black lines into 16 equal squares.

The test was performed under white light in a quiet room. Each rat was placed at the same corner square and observed during 5 min. The floor and walls were cleaned after testing each rat. The following parameters were recorded during the 5 min observation period; latency: time taken by each animal till it starts moving in the arena, ambulation frequency: number of squares crossed by the animal, rearing frequency: number of times the animal stood stretched on its hind limbs with or without forelimb support [29-31].
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3.2. Forced swimming test

The forced swimming test was performed according to the method described by Porsolt et al. Each rat was placed for 5 min in a cylindrical water tank (70 cm high, 40 cm diameter) where, water level was about 40 cm and water temperature were maintained at 23–25 C. The total duration of immobility of each animal was recorded. The tank was emptied and washed with fresh water flush between each rat to remove any traces of urine or feces [32].

4. Biochemical analysis

Determination of brain monoamine contents Each brain tissue sample was weighed and homogenized in 75% aqueous HPLC grade methanol (10% w/v). The homogenate was spun at 4000 r.p.m. for 10 minutes, and the supernatant was separated. HPLC was used to detect brain monoamines described by Pagel et al [33].

5. Determination of brain and liver MDA and GSH levels

Each brain or liver tissue was homogenized with ice-cold saline (20% w/v) [34]. The homogenate was separated into two sections to determine the amounts of malondialdehyde (MDA) and reduced glutathione (GSH). The level of MDA was assessed using the Ruiz-Larea et al. technique. The GSH level was determined using Ellman's method modified by Bulaj et al. [35, 36].

6. Statistical analysis

All data were analyzed using GraphPad Prism (USA). The results were expressed as the mean ± standard error of the mean (SEM). Statistical significance was determined using analysis of variance (ANOVA), followed by Dunnett’s t-test for multiple comparisons.

7. Results

7.1. Effect of *Itrifal* on the open field test (No. of central square entries)

The results were represented in Figure 1. Depression-like behavior induced by swimming could be clearly demonstrated by the significant decrease in the activity of all rats compared to normal control group. *Itrifal* (10 mg/kg, *p.o.*) and Fluoxetine (10 mg/kg, *p.o.*) significantly

increased the no. of central square entries compared to the disease control group where, no. of central square entries was significantly decreased.

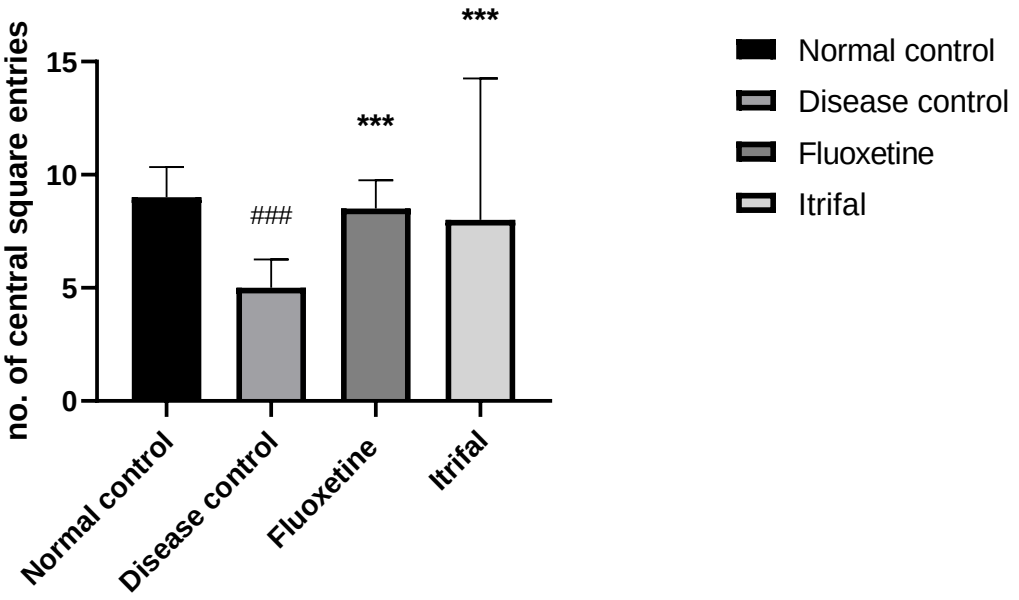

Figure 1. Effect of Itrifal on the central square entries of open field test

Data are presented as mean ± SEM (n=6). ###p<0.001 indicates a significant difference between the disease control group and the normal control group. ***p<0.001 and ***p<0.001 indicate significant differences in the all treatement groups caompared to the disease control group.

7.2. Effect of *Itrifal* on the forced swim test (Immobility)

The results were represented in Figure 2. Depression-like behavior induced by swimming could be clearly demonstrated by the significant decrease in the activity of all rats compared to normal control group. Itrifal (10 mg/kg, *p.o.*) and Fluoxetine (10 mg/kg, *p.o.*) significantly decreased the immobility compared to the disease control where, the immobility was significantly increased

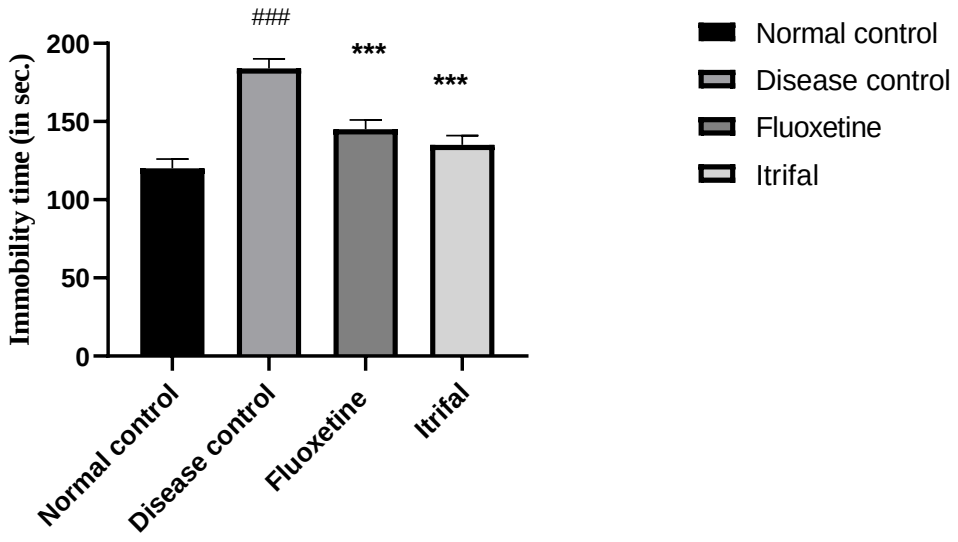

Figure 2. Effect of *Itrifal* on the immobility of forced swimming test (FST)

Data are presented as mean ± SEM (n=6). ###p<0.001 indicates a significant difference between the disease control group and the normal control group. ***p<0.001 and ***p<0.001 indicate significant differences in the all treatement groups caompared to the disease control group.

7.3. Effect of *Itrifal* on the neurotransmitter levels

The levels of monoamines detected are summarized in Table 1. Statistics revealed that both serotonin norepinephrine and dopamine levels were normalized after the administration of fluoxetine (10 mg/kg) and Itrifal (10 mg/kg) compared to the disease control group.

Table 2. Effect of *Itrifal* on the neurotransmitter levels in depressed rats

Groups	Serotonin (lg/g tissue)	Norepinephrine (lg/g tissue)	Dopamine (lg/g tissue)
Normal control	97.33 ± 4.49	95.33 ± 3.39	96.33 ± 4.18
Disease control	23.00 ± 3.74	32.00 ± 3.74	35.33 ± 4.10
Fluoxetine (10 mg/kg)	81.66 ± 3.39	82.00 ± 4.08	88.33 ± 5.79
Itrifal (10 mg/kg)	68.00 ± 1.63	72.00 ± 3.26	76.00 ± 3.26

The effect of Itrifal on neurotransmitter levels is summarized in Table 2

7.4. Histopathological examination

Results are represented in Fig. 3 Histopathological examination of the liver sections revealed that induction of depression by swimming resulted in slight hydropic degeneration of the hepatocytes. The hepatocytes showed no histological alterations at all of the fluoxetine or Itrifal treated groups.

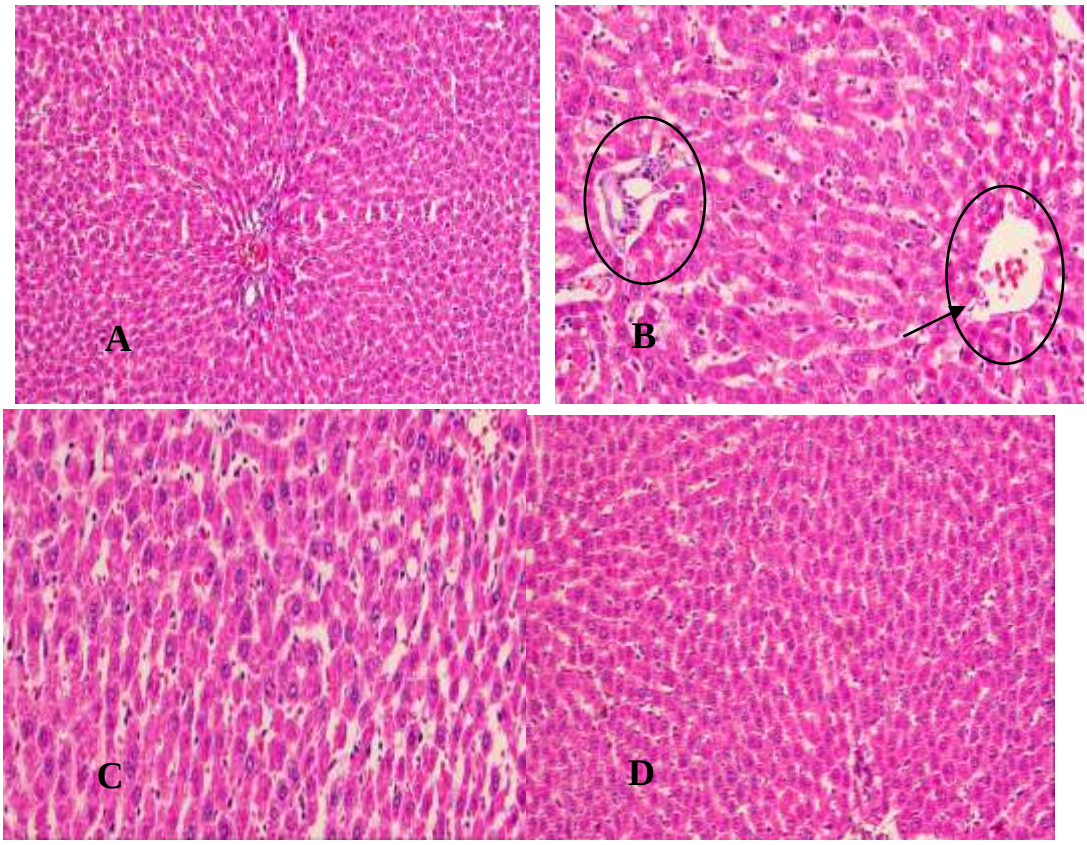

Figure 3 (A) Liver of rat from the normal control group showing no histopathological changes (H & E x 400). (B) Liver of rat from the depressed group showing slight hydropic degeneration of hepatocytes (H& E x 400). (C) Liver of rat from the Fluoxetine (10 mg/kg) group showing no histopathological changes (H & E x 400). (D) Liver of rat from the *Itrifal* (10 mg/kg) group showing no histopathological changes (H& E x 400).

8. Discussion

This study was designed to investigate the antidepressant and hepatoprotective effects of Itrifal against fluoxetine in an acute experimental model of depression in rats. Subsequent research in rats showed that physically induced behavioral depression was correlated to the depletion of brain monoamines. However, although the concentrations of serotonin (5-HT), norepinephrine (NE) and dopamine (DA) in brain remained at low levels after inducing depression. *Itrifal* (10 mg/kg, p.o.) reversed physically induced hypo-motility in the open field test and forced swimming test in a manner comparable to that of fluoxetine [37, 38]. In addition, *Itrifal* increased the level of neurotransmitters in the brain. Recent study also indicated that *Itrifal* inhibits monoamine oxidase (MAO) activity [39].

Itrifal, is recognized for its diverse pharmacological properties, including neuroprotective, vasodilatory and nootropic effects. Recent research suggests that *Itrifal* may also exert hepatoprotective and antidepressant effects, especially in models of acute stress-induced depression. *Itrifal* 's ability to scavenge free radicals and enhance antioxidant enzyme activity

can protect hepatocytes from damage caused by oxidative stress, a common characteristic in models of depression [40, 42].

Conclusion

Results revealed that acute administration of *Itrifal* (10 mg/kg) for 3 days after induction of depression by swimming, showed a hepatoprotective and an antidepressant-like effects comparable to those of fluoxetine. Further investigations are required to evaluate long term efficacy and safety of *Itrifal* as antidepressant.

List of abbreviations

5-HT = 5-Hydroxy Tryptamine
ANOVA = Analysis of Variance
CNS = Central Nervous System
CPCSEA = Committee for the Purpose of Control and Supervision of Exper- iments on Animals
DA = Dopamine
HPLC = High Performance Liquid Chromatography
MAO = Monoamine Oxidase
NE = Norepinephrine
OFT = Open Field Test
SOD = Super Oxide Dismutase
IAEC = Institutional Animal Ethics Committee
USA = United state
AR = Analytical grade
SEM = Standard error of the mean
PO = Peroral route
MDA = Malondialdehyde
GSH = Glutathione
SGOT = Serum glutamic oxaloacetic transaminase test
SGPT = Serum glutamic pyruvic transaminase
ALT = Alanine transaminase
AST = Aspartate transaminase

Ethics approval and consent toparticipate

The Institutional Animal Ethics Committee (IAEC) evaluated and approved the experimental protocol (IAEC/SRMS/2022/II/10)

Consent for publication

Not applicable.

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None.

Conflict of interest
The authors declare no conflict of interest, financial or otherwise.

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