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Evaluation of Hesperidin-Zinc Complex as a Novel Therapeutic Agent for Depression Treatment in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The complex contains hesperidin and zinc. The zinc is essential micronutrient that is important for human metabolism and catalyzes over 100 enzymes. It can be used to treat Wilson disease, decreased immunity, acute and chronic diarrhea. The hesperidin is a bioflavonoid found in many citrus plants. It has numerous activities like antioxidant, antibacterial, antimicrobial, anti-inflammatory, used to treat inflammation, Hypotension, varicose veins, venous ulcer, oxidative stress. There is no scientific evidence to support the claim that the complex of hesperidin and zinc have antidepressant properties even though zinc and hesperidin both separately have been linked to antidepressant activity in the literature.
Objectives: The aim of this research is to find out whether complex of hesperidin and zinc can prevent reserpine induced depression.

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Methods: Wistar rats were used in this study after acclimatization. The induction group will be induced with reserpine to produce the depression in rats. The experiment included various treatment group of high and low dose of the test drug. Rats were used for the assessments, based on behavioral parameter like tail suspension test (TST), forced swimming test (FST), and elevated plus maze test then histopathology of hippocampus, cortical and substantia nigra region has been analysed, and along with measuring the BDNF levels.
Results: On reserpine induced depression, complex of Hesperidin and zinc at low dose (100 mg/kg) shows negligible improvement in depressed behavior, when compare with standard fluoxetine (10 mg/kg). On the other hand, high dose (200 mg/kg) of the complex shows greater improvement also showed normal morphology in all 3 regions of the brain and showed increased level of BDNF in brain compared to low dose (100 mg/kg).
Conclusions: Hesperidin-zinc complex was discovered to be a successful antidepressant drug, according to the study's findings. According to the findings, however a high dose of complex is more effective at treating reserpine induced depression than a low dose of complex.

Keywords: Wilson disease; TST; FST; Histopathology; reserpine; antidepressants.

1. INTRODUCTION

Depression can be preventive and life-threatening at same time and it's a form of mental illness that can be severe and can cause some kind of physical harm and in worst case is death. Depression has affected 21% of world population (Chopra, Kumar, & Kuhad, 2011). When viewed in this light, depression becomes clear as a serious issue: it is a prevalent mental health condition that has far-reaching consequences, including but not limited to: high economic costs, disability, and even death by suicide and other causes (Cademartori, Gastal, Nascimento, Demarco, & Corrêa, 2018). There are so many marketed drugs available An older generation of agents includes the cyclic antidepressants (CA) and irreversible monoamine oxidase inhibitors (MAOI), the initial groups of medications created for the treatment of depression. A potential lower risk of toxicity in overdose compared to the cyclic antidepressants was a driving factor in the development and marketing of the newer antidepressants (Sarko, 2000).

According to research, situations like stressful life events, genetic possibilities, personality, biological, cognitive, and interpersonal vulnerabilities, might increase a person's risk of developing depression (Hankin, 2006). At molecular level, the cellular deficiencies like decreased in level of brain derived neurotrophic factor (BDNF) in hippocampus resulted in causing depression. So, it is believed that different forms of antidepressants work by increasing BDNF expression, which helps neurons survival and neurogenesis (Garcia, 2002). Repeated administration of reserpine in an animal model can exhibit progressive development of the symptoms of depression (Ikram & Haleem, 2017).

Inadequate levels of the neurotransmitters like norepinephrine, serotonin, gamma amino butyric acid, and glutamate are known to be a major contributing factor to depression, at present inhibiting reuptake, blocking receptors, and inhibiting enzymes that breakdown monoamines, especially monoamine oxidase, are the three primary methods that modern antidepressant medicines now used to restore monoamine balance (Artigas, Nutt, & Shelton, 2002). Depressive symptoms are exhibited by low levels of 5HT (serotonin), NA (noradrenaline), and DA (dopamine). Research indicates that raising these levels has antidepressant effects. Thus TCA (tricyclic anti-depressant), SSRI (selective serotonin reuptake inhibitors), and NRI (selective noradrenaline reuptake inhibitors) medication classes were employed to regulate the monoamines. These medications work on dopaminergic receptors for dopamine, adrenergic receptors for NA, and serotonergic receptors for 5HT (Elhwuegi, 2004). Fluoxetine, better known by its brand name Prozac, is a SSRIs antidepressant whose antidepressant action is due to its capacity to inhibit the prejunctional absorption of norepinephrine and serotonin (Gram, 1994).

The major disadvantages in these medications are that they don't provide any other therapeutic advantage, whereas this complex can protect the patients from zinc deficiency diseases and many more. Because most proteins include zinc ions in their reaction centers, zinc has biological relevance in protein activity. Zinc plays an

essential role in many other bodily processes, including sensory input, cognition, enzyme activity, and protein transcription factors (Parveen, Ansari, Ahmad, Jameel, & Shadab, 2017). Nutritional deficiencies, gastrointestinal disorders, liver disease, renal disease, stunted growth, acute diarrhoea in infants and children, Wilson's disease, preventing age-related macular degeneration, sickle cell disease, and other conditions can all benefit from zinc supplementation (Prasad, 2004).

Hesperidin is a type of bioflavonoid found in citrus fruit in high concentration. Antioxidant, antimicrobial, anti-inflammatory, and anticarcinogenic effects are only a few of the many health advantages linked to its usage (Pyrzynska, 2022). It also shows analgesic and antipyretic activity, antiallergic effects, antioxidant effect, activity on haemorrhoids and IUCD-induced bleeding, immuno-modulatory activity. It also shows effect on wound healing, on haemorrhoids and IUCD-induced bleeding (Garg, Garg, Zaneveld, & Singla, 2001). Hesperidin is generally safe for both topical and systemic administrations (Man, Yang, & Elias, 2019).

2. OBJECTIVES

The objective of this research is to find out whether complex of hesperidin and zinc can prevent reserpine induced depression. Using various behavioural tests and histopathology of brain and measuring the BDNF levels in brain.

3. METHODS

3.1 Materials

Reserpine, Fluoxetine, $Zncl_2$, Hesperidin, methanol, NaOH, were obtained from Krupanidhi college of pharmacy, Bengaluru, India.

3.2 Preparation of Complex

The 4:1 ratio of 1.00×10^{-3} mol/L hesperidin to 1.00×10^{-3} mol/L $ZnCl_2$ was used to prepare the complex. Next, 500 mL of the blended liquid was added to a round-button flask. The mixture was refluxed and heated to 70°C for 3 hours until it was fully dissolved. The pH of the mixture was then adjusted to 10.50 by adding 0.1 mol/L of NaOH. The solution was passed through a series of washes with 100% methanol to eliminate any remaining contaminants after being concentrated to 50 mL using a rotary evaporator after 5 minutes. Finally, the complex was dried under for 12h (Chen & Zhu, 2018).

3.3 Animals

Wistar rats weighing 100 - 150 grams were taken from Krupanidhi College of Pharmacy, Bangalore, India. They were kept in animal housing with good ventilation while they acclimatized. Prior to the experiment for 10 days were spent in a controlled laboratory setting with a 50-60% with 12 hrs light and dark cycle with food and water as per the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animal (CPCSEA). The Institutional Ethical Committee approved the experiment protocol by the reference number KCP/IAEC/PCOL/124/AUG-2023.

3.4 Criteria for Dose Selection

Acute oral toxicity study has been conducted before commencement of the actual research as per the OECD guidelines 423 and the dose was fixed after that (OECD, 2002).

3.5 Experimental Procedure

40 male Wistar rats, weighing 130–150 g, were grouped into 5 groups (n=8), housed in plastic cages under natural light and dark cycles at normal room temperature with availability to feed and drink ad libitum. Group-A (Negative control) received normal diet and water. Group-B (Positive control) intraperitoneally received 0.2 mg/kg of reserpine for 14 days, while Group-C (Standard) intraperitoneally received 0.2 mg/kg of reserpine for 14 days followed by orally received 10 mg/kg of fluoxetine. Group-D (low dose) which received a low dose of hesperidin-zinc complex(100mg/kg), was given after 60 mins,0.2 mg/kg of reserpine intraperitoneally for 14 days. Group-E (high dose), on the other hand, received a high dose of hesperidin-zinc complex(200mg/kg), after 0.2 mg/kg of reserpine intraperitoneally with gap of 60 mins for 14 days, they were then evaluated for behavioral parameters utilizing techniques such as the raised plus maze test, the forced swimming test, and the tail suspension test. The conclusion of the experiment, all animals were killed by cervical dislocation. The brains were then carefully removed, examined under a microscope, and analysed for factors such as BDNF levels and histology (Olanrewaju et al., 2020).

3.6 Tail Suspension Test

This test was conducted on the 1st and 14th days following reserpine treatment, respectively. This behaviour despair model is one of the most widely used model of depression, done in animal to determine the potency of antidepressant by observing the immobile phase of animals induced by different kinds of antidepressant medications. The experiment was conducted on the 1st and 14th days following reserpine treatment, respectively. The rat was held 50 cm from the ground in this experiment using adhesive tape that was placed about 1 cm from the tail tip. To alleviate strain on the rat's tail, a square plywood platform was laid out horizontally 15-20 cm (based on its size) beneath the bench, under its front paws. The only time rats were considered immovable was when they were lying motionless and passively hanging. Over the course of five minutes, we tracked how long the tail was suspended, which rendered it immobile. Reserpine was administered via IP 60 minutes before to the test drug was given in all groups except negative control and positive control (Shinde, Yegnanarayan, Shah, Gupta, & Pophale, 2015).

3.7 Forced Swim Test

This test was created by Porsolt and colleagues to test the potency of antidepressant drugs in rats and mice. One by one the rats were placed in a vertical cylinder with 15 cm of water maintained at 25°C and allowed to swim freely within. Initial stage rats to be placed in the cylinders exhibited greater activity and mobility, they were showing actions like aggressive swimming in circular motion, trying to climb the wall or falling to the bottom. After 2-3min activity tends to subside and shows phases of immobility and floating action. When the rats remained motionless for almost 80% of the time, the immobility reaches a climax after 3 minutes. The rats were taken out of the water after 5 minutes and dried in a heated chamber (32°C) before being put back in their original cages. This test was done out at 1st and 14th day. In drug treated groups the reserpine was delivered 60 min before the test drug and test was performed in all five groups (Carr & Lucki, 2010).

3.8 Elevated Plus Maze Test

Two open arms measuring (50 × 10 cm), two enclosed arms measuring (50 × 10 × 40 cm), and a center platform measuring (10 × 10 cm) make up the equipment. A height of fifty centimetres above the floor was maintained for the equipment. The experiment began with the rat being placed on the center platform with its back to the open arm. The test lasted 5 min. Standard metrics that was calculated are: total arm entries, (in sec) spent in the open arms of the apparatus. This test was done out at 1st and 14th day. In drug treated groups the reserpine was delivered 60 min before the test drug and test was performed in all five groups (Pechlivanova, Tchekalarova, Nikolov, & Yakimova, 2010).

3.9 Statistical Analysis

All the values were presented as mean ±SD (Standard Deviation). Data was examined using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

3.10 Histopathology

Cervical dislocation method was used for euthanising the animal and brains were dissected from them and later they are used for analysis of histopathology and BDNF levels.

4. RESULTS

4.1 Tail Suspension Test

The tail suspension test (TST) was carried out to determine the immobile phase time of animal when each animal was lifted by its tail. Prior to exposing the rats to depression by reserpine in the TST, there was no discernible change in the length of immobility in the rats of any of the five groups at 1st day. However, all four groups of rats excluding negative control group that received reserpine for 2 consecutive weeks without therapy saw a substantial rise in the time of immobility. However, low dose 100 mg/kg and high dose 200 mg/kg of complex along with reserpine induced in rats demonstrated a substantial drop in immobility when correlated to the positive control group after 2 weeks of therapy.

4.2 Forced Swim Test

Here duration of immobile phase was assessed by the FST. At 1st day there is no significant difference in time duration of immobility in the rats of all the 5 groups prior to subjecting the rats to reserpine induced depression. However, we observed a significant increase in duration of immobile phase in the rats of all the 4 groups

except negative control group subjected to reserpine for two consecutive weeks without treatment. After 2 weeks of treatment with complex of low dose 100mg/kg and high dose 200mg/kg along with reserpine induced rats showed significant decrease in immobility as compared to positive control group.

4.3 Elevated Plus Maze Test

The Elevated Plus Maze Test was carried out to determine the anxiety of individual animal. Each one of them were given freedom to roam freely in the apparatus for 5minutes and time spent in open arm is measured. After drug treatment the time spent in open arm has increased significantly.

4.4 BDNF(Brain-Derived-Neurotrophic-Factor) Levels

The 'neurotrophin hypothesis of depression' gives idea that brain derived neurotrophic factor (BDNF) is either decreased or increased in response to stress or antidepressant medication. Numerous studies have also documented that stress lowers the level of BDNF in the region of brain (Martinowich, Manji, & Lu, 2007).

Table 1. Effect of hesperidin-zinc complex on Tail Suspension test

Groups		Total Duration of Immobility (sec.) (8 animals) Observation time 5 mins								Mean±S.D
		NUMBER OF ANIMALS =8								
Negative control	**Day 0**	80	87	99	104	100	94	98	106	96±8.73
	Day 14	83	92	102	100	98	97	99	100	96.37±6.16
Positive control (Reserpine 0.2mg/kg)	**Day 0**	97	100	98	105	99	90	106	103	99.75±5.11
	Day 14	110	119	114	120	110	105	119	120	114.62±5.75##
Standard group (Reserpine 0.2mg/kg+Fluoxetine 10mg/kg)	**Day 0**	89	90	88	95	89	90	86	83	88.75±3.45
	Day14	55	57	52	55	56	56	58	54	55.37±1.84***
Test drug 1 (Reserpine 0.2mg/kg +Low Dose of complex 100mg/kg)	**Day 0**	87	88	90	92	89	86	90	93	89.37±2.38
	Day 14	83	81	82	83	83	82	82	82	82.25±0.70*
Test drug 2 (Reserpine 0.2mg/kg+High Dose of complex 200mg/kg)	**Day 0**	85	87	88	84	82	83	88	85	85.25±2.25
	Day 14	70	70	68	69	68	70	71	72	69.75±1.38**

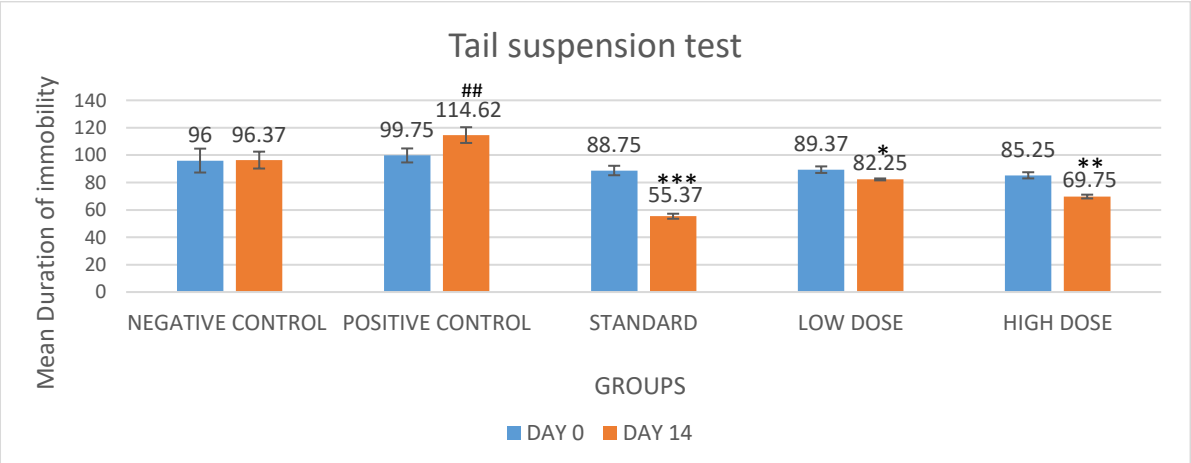

Fig. 1. This figure shows behavioral test (Tail suspension test) and evaluation of hesperidin-zinc complex effect on reserpine induced depression was recorded and graphically represented in mean ± SD (No=8)

*Statistical difference of the results was tested by comparing negative control with positive control group, standard group low dose, high dose. Here, *p < 0.05, **p < 0.01, *** p < 0.001 respectively when analysed with respect with positive control group (##)*

Table 2. Effect of hesperidin-zinc complex on Forced Swim Test

Groups	Duration of immobility (sec.) (8 animals)									Mean ± S.D
	Number of animals =8									
Negative control	**Day 0**	10	9	8	8	10	7	9	6	8.37±1.40
	Day 14	9	10	8	7	9	8	9	7	8.37±1.06
Positive control (Reserpine 0.2mg/kg)	**Day 0**	16	15	16	20	17	14	18	15	16.37±1.92
	Day 14	22	23	22	24	24	23	22	22	22.75±0.88##
Standard group (Reserpine 0.2mg/kg+Fluoxetine 10mg/kg)	**Day 0**	10	9	9	10	11	9	8	9	9.37±0.91
	Day14	5	5	6	6	6	7	7	5	5.87±0.83***
Test drug 1 (Reserpine 0.2mg/kg+Low Dose of complex 100mg/kg)	**Day 0**	13	12	13	16	11	12	13	14	13±1.51
	Day 14	11	10	11	13	12	11	11	11	11.25±0.88*
Test Drug 2 (Reserpine 0.2mg/kg+High Dose of complex (200mg/kg	**Day 0**	10	9	9	9	9	10	9	8	9.12±0.64
	Day 14	9	8	8	8	8	9	8	8	8.28±0.48**

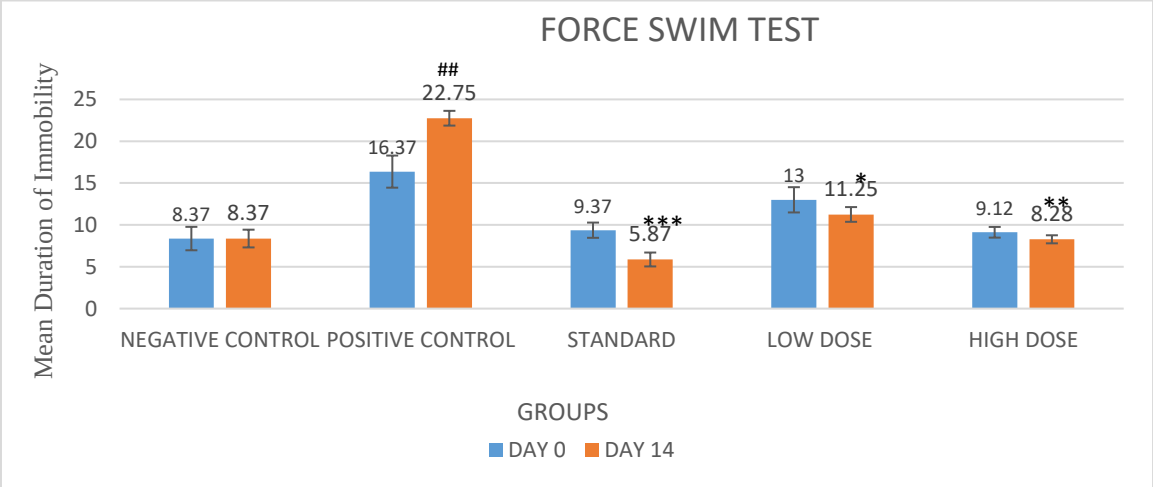

Fig. 2. This figure shows behavioral test (forced swim test) and evaluation of effect of hesperidin-zinc complex on reserpine induced depression. Immobility was recorded and graphically represented in mean ± SD (No=8)

*Statistical difference of the results was tested by comparing positive control with negative control group, standard group low dose, high dose. Here, *p < 0.05, **p < 0.01, *** p < 0.001 respectively when analysed with respect with positive control group (##)*

Table 3. Effect of hesperidin-zinc complex on Elevated Plus Maze Test

Groups	Total added Time analysed in open arm (sec.) (8 animals) Observation time 5 mins (300 sec)									Mean ± S.D
	Number of animals =8									
Negative control	**Day 0**	59	62	68	70	59	65	71	59	64.12±5.08
	Day 14	57	61	65	67	55	63	68	50	60.75±6.29
Positive control (Reserpine 0.2mg/kg)	**Day 0**	40	48	42	42	45	40	42	45	43±2.77
	Day 14	20	18	24	23	28	30	29	27	24.87±4.35##
Standard group (reserpine 0.2mg/kg + fluoxetine 10mg/kg)	**Day 0**	59	60	67	70	59	66	68	57	63.25±5.00
	Day14	80	82	85	89	87	88	83	86	85±3.11***

Groups	Total added Time analysed in open arm (sec.) (8 animals) Observation time 5 mins (300 sec) Number of animals =8									Mean ± S.D
Test drug 1 (Reserpine 0.2mg/kg+Low Dose of complex 100mg/kg)	**Day 0**	55	58	60	64	55	57	65	54	58.5±4.17
	Day 14	60	60	65	67	62	61	69	60	63±3.54*
Test drug 2 (Reserpine 0.2mg/kg+High Dose of complex 200mg/kg)	**Day 0**	60	61	68	71	59	66	69	59	64.12±4.91
	Day 14	69	70	79	79	69	70	74	75	73.12±4.25**

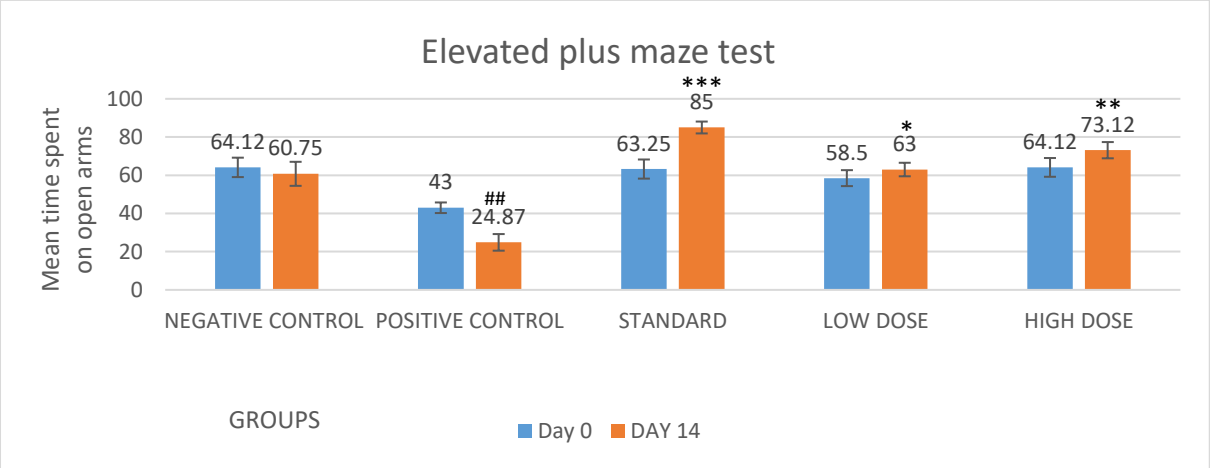

Fig. 3. This figure shows behavioral test (Elevated Plus Maze Test) and evaluation of effect of hesperidin-zinc complex on reserpine induced depression was recorded and graphically represented in mean ± SD (No=8). Statistical difference of the results was tested by comparing positive control with negative control group, standard group low dose, high dose. Here, *p < 0.05, **p < 0.01, *** p < 0.001 respectively when analysed with respect with positive control group (##)

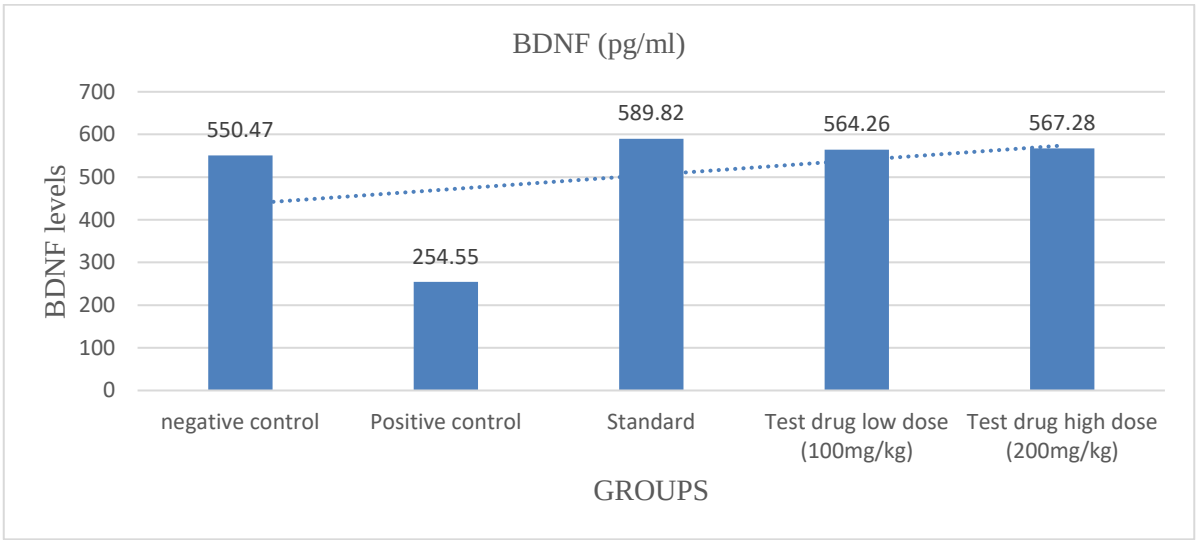

Fig. 4. The BDNF(Brain-Derived-Neurotrophic-Factor) levels in brain

4.5 Histopathology

Histopathological Report of Rat Brain (Hippocampus region)

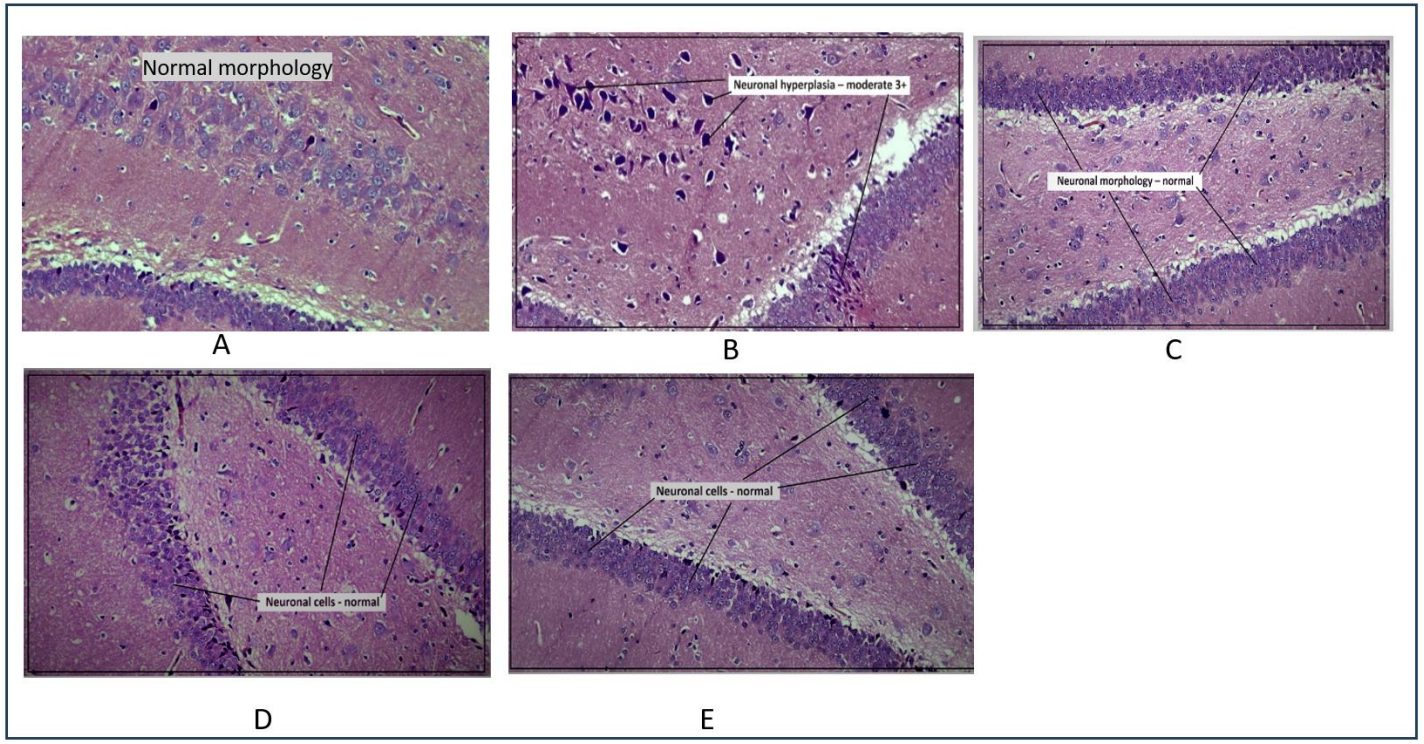

Fig. 5. Hippocampus region of all the 5 groups (negative control, positive control, low dose and high dose of hesperidin and zinc complex and standard dose i.e. fluoxetine).

- **A.** Hippocampus region of negative control group showing normal morphology.
- **B.** Hippocampus region of positive control group reserpine induced depression showing Neuronal hyperplasia = moderate 3+ (x100).
- **C.** Hippocampus region of standard group Fluoxetine (10mg/kg) showing Neuronal morphology – normal – NAD+ (X100).
- **D.** Hippocampus region of the low dose group(100mg/kg) showing neuronal-normal-NAD+(X100).
- **E.** Hippocampus region of the high dose group(200mg/kg) showing neuronal-normal-NAD+(X100).

Histopathological Report of Rat Brain (Cortical region):

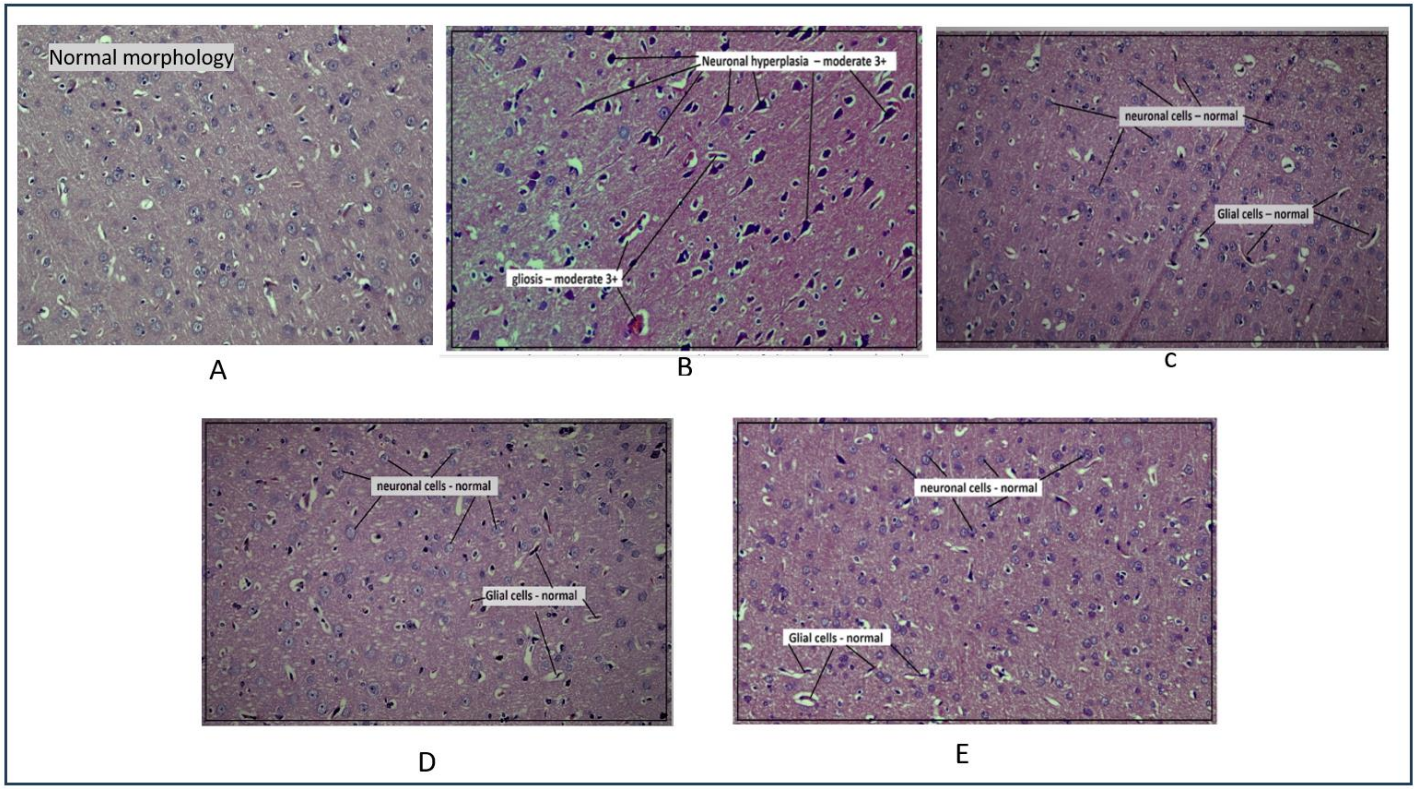

Fig. 6. Cortical region of all the 5 groups (negative control, positive control, low dose and high dose of hesperidin and zinc complex and standard dose i.e. fluoxetine)

- **A.** Cortical region of negative control group showing normal morphology.
- **B.** Cortical region of positive control group reserpine induced depression (0.2mg/kg) showing Neuronal hyperplasia & gliosis – moderate 3+ (X100).
- **C.** Cortical region of standard group fluoxetine (10mg/kg) showing Glial & neuronal cells **–** normal – NAD+(X100).
- **D.** Cortical region of low dose (100mg/kg) group showing Glial cells & neuronal cells normal morphology – NAD+ (X100).
- **E.** Cortical region of high dose (200mg/kg) group showing Glial cells & neuronal cells normal Morphology – NAD+ (X100).

Histopathological Report of Rat Brain (Substantia nigra (SN)region):

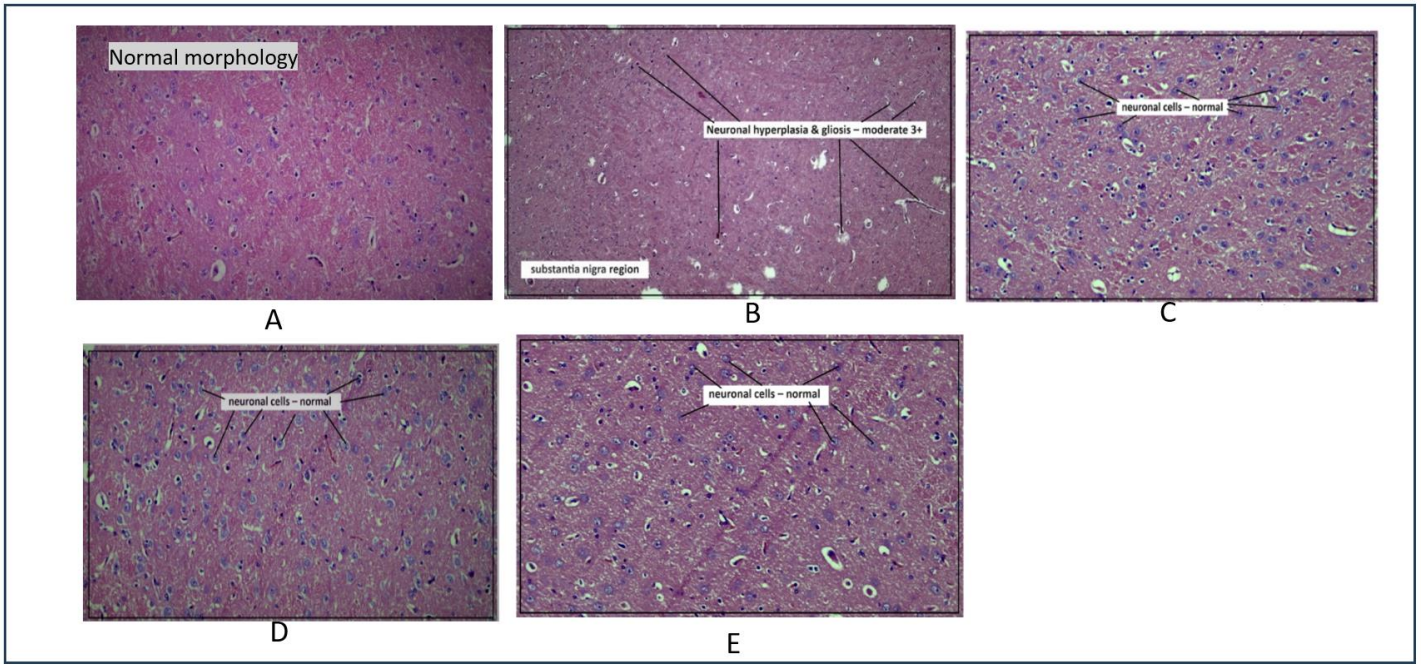

Fig. 7. Substantia nigra region of all the 5 groups (negative control, positive control, low dose and high dose of hesperidin and zinc complex and standard dose i.e. fluoxetine)

- **A.** SN region of negative control showing normal morphology.
- **B.** SN region of positive control reserpine induced depression (0.2 mg/kg) showing neuronal hyperplasia & gliosis – moderate 3+ (X100).
- **C.** SN region of standard group fluoxetine (10mg/kg) showing neuronal cells – normal – NAD+ (X100).
- **D.** SN region of low dose group (100mg/kg) showing neuronal cells – normal – NAD+ (X100).
- **E.** SN region of high dose group(200mg/kg) showing neuronal cells – normal – NAD+ (X100).

Table 4. Effect of the complex on BDNF (Brain-Derived-Neurotrophic-Factor) levels

Sl No.	Sample	BDNF (pg/ml)
2.	Negative control	550.47
1.	Positive control	254.55
2.	Standard	589.82
3.	Test drug low dose (30mg/kg)	564.26
4.	Test drug high dose (100mg/kg)	567.28

5. DISCUSSION

In this specific study, we have analyzed the effects of hesperidin-zinc complex on animals. Prior to the treatment, all animals underwent a thorough acclimatization process to ensure their baseline behavioral parameters were well-established, indicating their good health. This allowed us to compare their responses to the treatments effectively.

No animals were gone under training for behavioral parameters to avoid the adaptions of the test so that they cannot develop a certain behavioral pattern that can manipulate our results.

One of the most common mood disorders is depression. Its symptoms make it hard for a person to do things like sleep, work, study, eat, or even enjoy things that they used to enjoy. Worldwide, depressive disorder ranks as the fourth most prevalent health problem. The individual's family and financial position are affected, leading to the necessity of medical care. Epidemiological evidence suggests that major depressive disorder typically presents itself after the age of 40 (Bains & Abdijadid, 2020). Using various tests, including the tail suspension test, forced swimming test, elevated plus maze test, measuring the BDNF levels in brain and a brain histopathological analysis, this study sought to determine whether the complex of hesperidin-zinc could alleviate depression in wistar rats that had been subjected to reserpine for induction of depression.

The induction of depression in rats was achieved with the administration of reserpine. Parameters like assessment of immobility and locomotor activity was measured in all the 5 groups of study after applied 2 weeks of stress by reserpine to all the groups except negative control group and last 2 weeks' treatment along with reserpine to positive control and treatment groups for inducing depression. After treatment rats were dissected and their brain sent for histopathological study and measuring the levels of BDNF (Olanrewaju et al., 2020). Amine storage process is blocked by reserpine; this cause increased excitability of hippocampal region further it also increases blood corticoid levels. Many Previous studies have shown that the secretion of 5-hydroxytryptamine (5-HT) in the brain get decreased by reserpine (Revzin, Maickel, & Costa, 1962).

One of the most popular animal models for determining the effectiveness of antidepressant chemicals is the FST and TST, which is responsive to most of these medications after acute treatment. The hallmark behaviour indicator in FST and TST is immobility time, which reflects behavioural despondency (hopeless conduct), as seen in depressed patients. Numerous investigations have shown that rats subjected to repeated stress had longer periods of immobile phase in the FST and TST. In line with earlier findings, the current study also showed that reserpine induced depressed rats had an extended total period of immobility in both the FST and TST when analysed with to negative control rats (Bai, Li, Clay, Lindstrom, & Skolnick, 2001).

In TST and FST, at 1st day rats of all the 5 groups tried hard to escape the tail suspension and tried to swim more keep themselves floating in the water in forced swimming, and more swinging movement in tail suspension test ,which resulted in shorter durations of immobility, at 2nd week of induction of reserpine to all the groups except negative control group, rats of all 4 groups like positive control, low dose, high dose and standard dose groups shows decrease in the immobile phase time is compared to the negative control groups. This was the case because depressed people exhibit behavioural despair rather than struggle to cope with stressful circumstances in daily life. After treatment with the standard dose, high dose of complex anti-depressant effects was observed more as compared to low dose. The pharmacological action is still unknown but most of the studies shows depletion of 5-HT causing depression and while treating by fluoxetine the increase level of 5-HT was observed and further it improved the behavioral activity of the animals (O'Leary et al., 2007).

Elevated plus maze test also shows the exploratory nature of the animals, it is one of the most effective models to test behaviour of the animals. In this test, the number of explorations done by the animal in open arms were calculated, the less exploration in open arms the more animal is depressed. In this study, it shows that the except negative control group all reserpine group showed less exploration in open arms, thus they were depressed. After the treatment with standard dose and high dose of the complex it has been observed that there was significant increase in exploration by animals in open arms whereas no significant changes were seen in low dose of the complex. Studies shows that blockade of 5-HT_{2C} receptor causes depression in animals, when 5-HT_{2C} receptors gets activated by any kind of anti-depressants it starts showing the more exploratory effect in animals (Mora, Netto, & Graeff, 1997). Thus there is no conclusive evidence that our complex works on that particular sub-receptor so further studies are required.

After the 2nd week particularly 14th day of treatment, the animals were dissected, and their brain were examined. The histopathological studies confirmed that the reserpine induced animals showed abnormal morphology in the hippocampus, cortical and substantia nigra region, whereas after the treatment the studies shows the recovery of the brain tissue to normal morphology in hippocampus, cortical and substantia nigra region.

Later BDNF(Brain-Derived-Neurotrophic-Factor) levels were measured, there we can see that reserpine induced depression animals have been observed decreased levels of BDNF in brain when compared to other groups. The motive behind measuring the BDNF level was that it shows neuroplasticity and studies showed that patients suffering from depression have a lower level of BDNF (Gervasoni et al., 2005). Our results showed that decreased levels of BDNF can be observed in depression. However, there is no profound study that shows the connection between the BDNF levels and severity of depression.

Hence, this current research offers insight into the potential benefits of Hesperidin-zinc complex for the treatment of depression and related mood disorders. However, it's crucial to acknowledge the study's limitations, such as the translational gap between animal models and human depression, and the need for a more comprehensive safety assessment. This study lays the foundation for future research to explore differential effects of complex, their mechanism of action and to thoroughly examine their safety profiles and potential side effects, essential for a comprehensive understanding of their therapeutic potential in depression.

6. CONCLUSION

According to the results and the discussion that followed, it can be concluded that a proper diagnosis of depression is crucial for its successful treatment. Before contemplating any form of treatment for depression, it is crucial to identify its root cause.

Furthermore, it has been proposed that Hesperidin-zinc complex may be employed at high dose as it can maintained the normal morphology of brain and normal levels of BDNF in brain.

Although more research study is still to be done to know more about the potential side effects of the complex, its bioavailability, also how BDNF is related with depression is not yet known so there is also scope for further investigation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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