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EVALUATION OF THE AMELIORATIVE EFFECT OF *ALPINIA OFFICINARUM* METHANOL EXTRACT IN AN EXPERIMENTAL MODEL OF DEPRESSION

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ABSTRACT: The active ingredient flavonoids especially Flavones, *e.g.*, Quercetin was identified from the methanol extract of *Alpinia officinarum*. This study evaluates the antidepressant activity of *Alpinia officinarum* (L.) wild Hance (Zingiberaceae) in Swiss Albino mice models. The antidepressant activity was evaluated by using different animal models *viz*. tail suspension test, despair swim test, potentiation of norepinephrine toxicity test, 5-hydroxytryptophan potentiation in Swiss Albino mice. The methanol extract of *Alpinia officinarum* significantly increases Na^+, K^+-ATPase level in the brain in the tail suspension test model. An antidepressant effect in the despair swim test was confirmed by the increase in monoamines (NE, DA, 5HT) and γ-aminobutyric acid levels in the brain. *Alpinia officinarum* methanol extract also contributes to the normal regulation of the HPA axis by significant ($P<0.001$) decreased in plasma corticosterone level. *Alpinia officinarum* (400 mg/kg) showed antidepressant potential by increasing the number of head twitches induced by 5 HTP. Antidepressant effect of an extract of *Alpinia officinarum* at a dose of 400 mg/kg could be *via* modulation of brain monoamines and normalizing hypothalamic pituitary adrenal axis.

INTRODUCTION: Man's existence on this earth is made possible by the vital role played by the animal and plant kingdoms. Plants are a rich source of medicine. Potent therapeutic agents developed from various medicinal plants. Due to easy availability and cheaper cost, traditionally it was used as a source of medicines. Consequently, there is a need to develop new herbal medicines from plants with the lowest dose, minimum side effects, fast action and shorter duration of action.

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According to the medicinal point of view, the most important genera is *Alpinia* (Zingiberaceae) which contains many species such as *Alpinia galanga*, *Alpinia officinarum*, (Zingiberaceae) *Alpinia purpurata*, *Alpinia calcarata*, *etc*. Of these *Alpinia officinarum* Hance (A.O.), species were selected for the present study [1]. Plants containing bioactive substances have increasingly become the object of research studies, particularly those plants with therapeutic value.

Many species of the genus *Alpinia* provide a variety of medicinal properties, such as *Alpinia zerumbet* (Pers.) Burtt et Smith and *A. purpurata* (Vieill) K. Schum, which have a significant presence in Brazil. These species have been commercialized in the food and cosmetic industries.

However, their greatest importance arises from the medicinal properties of their essential oils containing flavonoids, terpenoids, and kavalactones which have been used in folk medicine to treat, for example, arterial hypertension and inflammatory processes. Also, such species are also used in multidisciplinary studies, including Phyto-chemistry, ethnobotany, and biology, indicating the key pharmacological role of this genus in everyday life.

Therefore, this work presents an *Alpinia* and its significance in therapeutic applications. Currently, plants with bioactive compounds are the main objective of the research. In everyday life, they play a key pharmacological role. In several parts of Asia, it is part of the human diet. According to Carlini (1972), *A. zerumbet* is used by agricultural workers in the Ribeirão Preto (SP, Brazil) area to treat rheumatism and heart disease [2].

In the northeast and southwest regions of Brazil, the tea made from its leaves is frequently used as an antihypertensive and diuretic medication [3]. An extensive literature survey found that the *Alpinia* genus possesses several different pharmacological activities, but antidepressant activity is not yet reported. Quercetin is the active constituent of th*e Alpinia officinarum*, and many researchers reported antidepressant activity of quercetin. In India extract is used in perfumes.

Alpinia officinarum, also known as lesser galangal is a perennial herb with reddish-brown rhizomes, lineolate leaves, and showy white flowers in racemes. Conventionally, it has been used in Ayurveda and China and Europe since the Middle Ages [4, 5]. In China it has been used for relieving treating colds, stomach ache, invigorating the circulatory system, and reducing swelling. It has been used for their antioxidant, antidiarrheal, anti-emetic, antidiabetic, antiulcer, analgesic, anti-inflammatory, and anticoagulation effects [6, 7]. There is a major issue with the conventional synthetic drug such as low remission rate, slow action and major side effect [8]. Drugs of natural origin are considered green medicine, are always supposed to be safe. Consequently, there is a need to develop new agents with minimum side effects and fast remission from medicinal plants. Hence, this work was planned to study the antidepressant activity of methanol extract of *Alpinia officinarum* in male Swiss albino mice.

MATERIALS AND METHODS:

Experimental Animals: Male Swiss albino mice (20-25g) were housed in groups of six animals per polypropylene cages. The standard environmental conditions 25 ± 2 °C, relative humidity of 50 ± 5%, 12 h light/ dark cycle were maintained throughout the housing and during the experiments. The animals had free access to food (Amrut Laboratory Animal Feed, Sangali, Maharashtra, India) and water. Total of 136 Swiss albino mice were used, and a total number of the group was 23. All the animals were acclimatized for 10 days to the experimental laboratory conditions before the start of the experimental protocol. The research protocol (DYPIPSR/IAEC/2015-16/P-16) was approved by the Institutional Animal Ethical Committee (IAEC), constituted under the "Committee for the Purpose of Control and Supervision of Experiment on Animals". All experiments were conducted between 12:00-16:00 h.

Drugs and Chemicals: Fluoxetine hydrochloride (fluoxetine) and desipramine hydrochloride were obtained as gift samples from Alembic Pharmaceuticals Ltd., and Cadila Pharmaceuticals, Gujarat, India, respectively. Serotonin, dopamine hydrochloride, norepinephrine hydrochloride, and N- N –dimethyl l-4nitrosoaniline were purchased from Sigma-Aldrich-(St. Louis, MO, USA), 5-hydroxyl- L-tryptophan (SRL Research Laboratory Pvt. Ltd., Mumbai-India), noradrenaline bitartrate (ADRENORTM) Samarth Pharma Pvt. Ltd., Mumbai, India. Moclobemide® (Trima 150) Intas Pharmaceuticals Ltd., Mumbai, India. Trichloroacetic acid, adenosine-5-triphosphoric acid (ATP) from Loba Chemie Pvt. Ltd., Mumbai.Tris, EDTA, thiobarbituric acid (Hi-Media Laboratories, Mumbai, India). The other chemicals were purchased from local vendors.

Preparation of Drug Solution: The rhizome of *Alpinia officinarum* was obtained from Tirupati, Andhra Pradesh. The rhizome of the plant was authenticated from, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. Successive solvent Extract (Methanol) and moclobemide were suspended in 1% sodium carboxymethylcellulose (CMC)

solution fluoxetine and desipramine dissolve in normal saline. The selection of doses of *Alpinia officinarum* methanol extract of 100, 200 and 400 mg/kg was based on the acute toxicity study.

Acute Toxicity Study: An acute toxicity study was carried out as per OECD guideline- 425. The methanol extract of *Alpinia officinarum* at 100, 200 and 400 mg/kg, were administered in healthy female Wistar rats, orally at one dose level of 2000 mg/kg body weight. The animals were continuously observed for 30 min, intermittently for the 4 h for any toxicity signs, behavioral changes and then periodically during 24 h and daily for total 14 days for the sign of toxicity and mortality. The LD_{50} was calculated by using AOT-425 statpgm (Version: 1.0), Acute Oral Toxicity (OECD Test Guideline (425) Statistical Program (AOT 425 Report).

Tail Suspension Test: The Tail suspension test was conducted as described earlier by Steru *et al*., (1985) [9]. In brief, mice were divided into 6 groups of six animals each. The Group I served as vehicle control, received vehicle (1% CMC). Group II, III and IV were treated with *Alpinia officinarum* extract (100, 200 and 400 mg/kg p.o.) and Group V and VI were received standard drug moclobemide (50 mg/kg p.o.) and fluoxetine (30 mg/kg p.o.), respectively. The treatment was given once daily, continuously for 15 days. On the 15th day 60 min after the regular drug treatment, the individual mouse was suspended on the suspending rod, 58cm above a table top by adhesive tape placed approximately 2 cm from the tip of the tail. The head of a mouse was 50 cm away from the nearest object and was both acoustically and visually isolated. The duration of immobility is recorded for periods of 6 min. Mice were considered immobile when they remain passively and completely motionless for at least 1 min.

Estimation of Na^+K^+ATPase: Na^+K^+-ATPase was assayed by taking 0.2 ml of tissue homogenate and 250 µl of tris HCl buffer followed by the addition of 50 µl of 600 mM NaCl, 50 µl of 50 mM KCL, along with 50 µl of 1 mM Na EDTA, and 50 µl of 80 mM ATP. The reaction mixture was pre-incubated at 37 °C for 10 min. The addition of 10% TCA immediately arrested the reaction. The precipitate was removed by centrifugation at 8000 rpm for 05 min at 4 ºC. To 50 µl of the supernatant, 1075 µl of distilled water, 125 µl of Ammonium molybdate and 50 µl of ANSA were added and incubated for 20 minutes at 37 °C. The intensity of blue color was read at 640 nm using spectrophotometer (JASCO, Japan) against a blank that contained all the reagents minus the supernatant. The results are expressed in liberated µmoles/mg of protein [10].

Biochemical Estimations: On the 15th day, after completion of tail suspension test animals were sacrificed by cervical dislocation, the brain was removed immediately, blotted, weighed and cross-shopped in a fine slice with a surgical scalpel. The slices were suspended in chilled 0.25M sucrose solution and quickly blotted on a blotting paper. Then the slice was minced and homogenized at 3000 rpm in chilled tris buffer (10 mmol/L, pH 7.4) at a concentration of 10% (w/v). The homogenate was centrifuged at 10000 rpm at 0 ºC for 20 min the supernatant was separated and used to estimate total protein content by the method of Lowry *et al*., (1951) and Na-K-ATPase (0.2 ml) by using the method of Bonting (1965) [11].

Forced Swimming Test (FST): Mice were divided into 6 groups, six in each group. The group I served as vehicle control, received vehicle (1% CMC), Group II, III and IV were treated with methanol extract of *Alpinia officinarum* (100, 200 and 400 mg/kg p.o.) respectively, and Group V and VI were received standard drug moclobemide (50 mg/kg p.o.) and fluoxetine (30 mg/kg, p.o.) respectively. The treatment was given once daily, continuously for 15 days. On the 14th day 60 min after the regular drug treatment, the individual mouse was subjected to forced swim test as described by Porsolt *et al*., (1978, 1979) [12, 13]. A mouse was individually placed in vertical Plexiglas cylinder (38 cm × 75 cm) containing water maintained at 26 ± 1 ºC. Two swimming sessions were conducted as pre-test session (15 min habituation) and 24 h later the test session (6 min). During the pre-test session, the mouse was allowed to swim for 15 min and removed after that and dried for 15 min in a heated enclosure (32 ºC) and then returned to home cages. Water in the cylinder was changed after subjecting each animal to FST because used water has been shown to alter the behavior [14]. After 24 h of the pre-test session, *i.e.*, on the 15th-day animals were

treated with scheduled regular treatment and test session was commenced by placing the individual mouse in a cylinder with the same condition as in pre-test session. The duration of immobility was recorded during the next 4 min of the total 6 min testing period.

Biochemical Estimations: On the 15th day, after completion of the test session of forced swim test blood samples were collected by retro-orbital plexuses and centrifuged (Remi Centrifuge, Mumbai, India) at 2500 rpm for 10 min to separate plasma for measurement of corticosterone [15]. The animals were sacrificed by cervical dislocation, and immediately brain was removed for measurement of monoamines (5HT, DA, and NE) and γ amino-butyric acid (GABA).

Biogenic Amine Estimation:

Estimation of Norepinephrine, Dopamine, and 5-hydroxytryptamine: Brain monoamine levels were estimated using the spectrofluorimetric method of Brownlee and Spriggs, (1965) and Kent and Gordon, (1971) [16]. At the end of the forced swim test, on the 15th day, 60 min after the test session of forced swim test, animals were sacrificed by cervical dislocation and brains excised immediately, blotted, weighed and cross-shopped in a fine slice with a surgical scalpel. The slices were suspended in chilled 0.25M sucrose solution and quickly blotted on a blotting paper. The tissues homogenized in ice-cold acidifying butanol using glass homogenizer.

The homogenization was performed for 1 min, and the homogenate was centrifuged at 10000 rpm at 0 ºC for 20 min the supernatant was used. From this 3 ml of the homogenate was used for estimation of GABA and the remaining homogenate was then transferred to tubes containing 1.6 ml of 0.1N hydrochloric acid and 5 ml heptane. After mixing on a vortex mixer for 30 sec, the aqueous phase/acid extract was recovered by centrifugation at 3000 rpm for 5 min. The organic supernatant phase was aspirated and discarded, including the tissue disc at the interface of the sample tubes. Aqueous phase or acid extract was used for estimation of nor-epinephrine, dopamine, and 5-HT using spectro-fluorimeter (JASCO, Japan).

Estimation of GABA: Swiss albino male mice were divided into 6 groups and treated orally for 15 days with vehicle (1% CMC), test compound treated groups received three different doses of *Alpinia officinarum* (100, 200 and 400 mg/kg p. o.) respectively, and standard drugs treated with moclobemide (50 mg/kg p.o.), fluoxetine (30 mg/kg, p.o.) respectively for fifteen days. On the 15th day after 60 min, oral administration of the drug, the brain was removed immediately, weighed and transferred to homogenization tube containing 5 mL of 0.01N hydrochloric acid and homogenized. The brain homogenate was transferred using a micropipette into Microcentrifuge tubes (Eppendorf, Tarsons Products Pvt. Ltd., Kolkata, India) containing 8 mL of absolute ice-cold alcohol and allow to stand for 1 h at 0 ºC then centrifuged for 10 min at 12 000 rpm, supernatant was separated and the precipitate was washed with 3-5 mL of 75% alcohol three times and washes were combined with the supernatant. Contents in Petri dishes were evaporated to dryness at 70-90 ºC on a water bath under a stream of air. To the residue 1 mL water, 2 mL methanol and 2 mL chloroform was added and centrifuged at 2000 rpm. Upper phase containing GABA was separated and 10 µL was spotted on Whatman paper no. 41.

The paper was mounted in the chromatographic chamber pre-saturated for 30 min with a mobile phase composed of n-butanol (50 mL) acetic acid (12 mL) and water (60 mL). The paper chromatogram was developed with ascending technique. The paper was dried in hot air and then sprayed with 0.5% ninhydrin in 95% ethanol. The paper was dried for 1 h at 90 ºC. Blue color spot developed on paper was cut and heated with 2 mL of ninhydrin solution in a water bath for 5 min. The piece of paper was removed from this solution and water (5.0 mL) was added with occasional shaking. After 1h, supernatant (2 mL) was decanted and optical density measured at 570 nm [17].

Estimation of Plasma Corticosterone: Swiss albino male mice were divided into 6 groups and treated orally with vehicle (1% CMC), test compound treated groups were received *Alpinia officinarum* (100, 200 and 400 mg/kg p.o.) respectively, and standard drugs moclobemide (50 mg/kg p.o.) and fluoxetine (30 mg/kg, p.o.) respectively for 15 consecutive days. On the 15th day, after completion of forced swim test, blood

samples were collected by retro-orbital plexus and centrifuged (Remi Centrifuge, Mumbai, India) at 2500 rpm for 10 min to separate plasma for measurement of corticosterone [15]. To 1 ml of plasma sample, 1.0 ml of ethanol, 0.5 ml of 0.1% solution of p-nitroso-N, N-dimethyl aniline in ethanol was added. The tubes were immersed in ice water for 5 min, and 0.5 ml of 0.1N sodium hydroxide was added. The tubes were plugged with cotton-wool and kept for 5 h at 0 °C protected from light add 2.0 ml of Clark and Lubs buffer for the adjustment of pH 9.8 and 5.0 ml of 0.10% solution of phenol in ethanol and 0.5 ml of a 1.0% aqueous solution of potassium ferricyanide was added. The tubes were kept in a water bath at 20 ± 2 ºC for 10 min. The change in absorbance was recorded by a double beam spectrophotometer (JASCO, Japan) at a wavelength of 650 nm against the blank [18].

Potentiation of Norepinephrine Toxicity Test: The antidepressant potential based on the mechanism of action was evaluated as described by Sigg., (1959). Swiss albino male mice were divided into five groups 6 mice each. The Group I was a normal control received (1% CMC, p.o.), Group II treated with norepinephrine (4 mg/kg, i.p.) used as a norepinephrine control, Group III, IV and V received three different doses of *Alpinia officinarum* (100, 200 and 400 mg/kg p. o.) used as a drug-treated groups. Group VI received standard drug desipramine (30 mg/kg) serve as a standard group. In this model, desipramine and *Alpinia officinarum* (100, 200 and 400 mg/kg p. o.) were administered p.o. twice 24 h and 60 min before norepinephrine, injection (4 mg/kg, i.p.), some lethality's were recorded and calculated [19].

5-Hydroxytryptophan Potentiation in Mice: A modified method of Sanchez *et al*., (2007) [20] was used to investigate the possible serotonergic mechanism. The mice were divided into 6 groups of six mice each. The Group I received vehicle (1% CMC) served as a vehicle control. Group II treated with 5-HTP (75 mg/kg, p.o.) served as 5-HTP control. Group III, IV, and V received three different doses of test compound *Alpinia officinarum* (100, 200 and 400 mg/kg p.o.) Group VI treated with standard drug moclobemide (50 mg/kg p.o.). 5-HTP (75 mg/kg) was intraperitoneally administered 60 min after drug treatments. The mice were individually placed in a glass jar and 14 min later the number of head twitches was counted at 10 min interval (14, 24, 34, 44 and 54) for 2 min duration [20].

Statistical Analysis: Values were expressed as mean ± SEM and statistical analysis was carried out. Parametric data analyzed by ANOVA followed by Dunnett's test and Bonferroni post hoc test. Result considered significant at $p<0.05$.

RESULTS: The study was carried out on the methanol extract (% yield 2.7), the active ingredient flavonoids specially flavonols *e.g.* quercetin was identified from methanol extract as mentioned in published review Basri *et al*., 2017. Many researchers reported antidepressant activity of quercetin.

Acute Oral Toxicity Study: Administration of *Alpinia officinarum* was administered to five healthy female mice at the limit dose of 2000 mg/kg/p.o. Showed no behavioral abnormality during 24 h observation period and no mortality occurred during the 14 days. LD_{50} was found to be more than 2000 mg/kg body weight calculated by AOT425 statpgm (Version: 1.0), Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program (AOT 425 Report). By AOT425 report dose of *Alpinia officinarum* (100, 200 and 400 mg/kg p.o.) was selected for evaluating antidepressant activity.

TABLE 1: SUMMARY OF LONG TERM RESULT

S. no.	Dose	O	X	Total
1	175	1	0	1
2	550	3	0	3
3	2000	4	2	6
	All doses	8	2	10

O- Survival, X- Dead

Effect of *Alpinia officinarum* on Immobility Period of Mice in Tail Suspension Test for 15 Days Study: The repeated administration of *Alpinia officinarum* (100, 200and 400mg/kg p.o.) for 15 consecutive days, more significantly ($p<0.001$) decreased the duration of immobility in mice at dose 400 mg/kg and at dose 100, and 200 mg/kg less significantly decrease. The effect was dose-dependent. *Alpinia officinarum* (400 mg/kg p. o.) significantly ($p<0.001$) decreased immobility period as compared to *Alpinia officinarum* (100 and 200 mg/kg p.o.) showed less significant ($p<0.01$) decreased in immobility period. *Alpinia*

officinarum (400 mg/kg p.o.) also significantly (p<0.001) reduced immobility period as compared to the normal control group. The positive control fluoxetine (30 mg/kg) showed moderately significant [F (7, 40) = 10.04, p<0.0001] decrease while moclobemide (50 mg/kg) also significantly reduced the immobility duration (p<0.01) as compared to the normal control group **Table 1**.

TABLE 2: EFFECT OF *ALPINIA OFFICINARUM* ON IMMOBILITY PERIOD OF MICE IN TAIL SUSPENSION TEST FOR 15 DAYS STUDY

Groups	Treatment	Oral dose(mg/kg)×14 days	Immobility period (Sec)	Percentage decrease in immobility
1	Normal control	CMC (35)	225.8 ± 6.43	
2	MEAO	100	195.63 ± 1.35*	13.36
3	MEAO	200	189.5 ± 2.64**	16.07
4	MEAO	400	142.07 ± 11.83***	37.08
5	Moclobemide	50	150.41 ± 7.71***	33.38
6	Fluoxetine	30	148.41 ± 2.21***	34.27

MEAO- Methanolic extract of *Alpinia officinarum*, CMC- Carboxy Methylcellulose, Values were expressed as mean ± SEM; n=6, the drug/vehicle treatments were administered once a day for 14 days. Data were analyzed by one way ANOVA followed by C* p<0.05, ***P*<0.01, ****P*<0.001 as compared to normal control.

Effect of *Alpinia officinarum* on Brain Na-K-ATPase Activity in Tail Suspension Test Model for 15 Days Study: Na-K-ATPase activity evaluated with measures of despair in the TST. *Alpinia officinarum* (400 mg/kg p. o.) dose showed significant (p<0.01) increase in NA-K-ATPase in the brain when compared to the normal control group. The standard treatment moclobemide (50 mg/kg) and fluoxetine (30 mg/kg) resulted in the moderately significant (p<0.001) increase in the level of NA-K-ATPase in the brain as compared to *Alpinia officinarum* (400 mg/kg p.o.) treated groups.

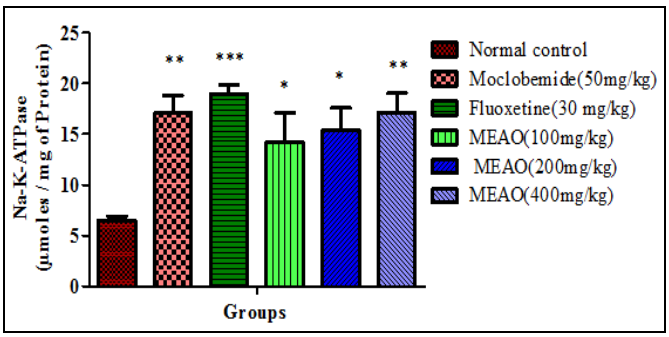

FIG. 1: EFFECT OF *ALPINIA OFFICINARUM* ON BRAIN NA^+-K^+-ATPase ACTIVITY IN TAIL SUSPENSION TEST MODEL FOR 15 DAYS STUDY. MEAO- Methanolic extract of *Alpinia officinarum*, Data was analyzed by one way ANOVA followed by Dennett's test *p<0.05 **P< 0.01, ***P<0.001 as compared to the normal control group.

Effect of *Alpinia officinarum* on Immobility Period of Mice in Forced Swimming Test Model for 15 Days Study: *Alpinia officinarum* (400 mg/kg p.o.) significantly (p<0.001) reduces the immobility period as compared to the normal control group. *Alpinia officinarum* (100 and 200 mg/kg p.o.) less significantly (p<0.01) reduced immobility period as compare to *Alpinia officinarum* (400 mg/kg). The reduction in the immobility period respectively was compared to a normal control group in mouse FST. The positive control moclobemide (50 mg/kg) and fluoxetine (30 mg/kg) showed significant (p<0.001) decrease in immobility time as compared to the normal control group.

Effect of *Alpinia officinarum* on the Estimation of Brain Monoamines (NE, DA, and 5HT) in the Brain of Mice in Forced Swimming Test for 15 Days of Study One way ANOVA showed a moderately significant (p<0.001) effect of *Alpinia officinarum* (400 mg/kg) as compared to *Alpiniaofficinarum* (100 and 200 mg/kg) to prevent the fall in the level of norepinephrine, dopamine, and serotonin respectively. *Alpinia officinarum* (100 and 200 mg/kg) showed less significant (p<0.01) effect in the prevention of fall in the level of monoamines- (norepinephrine, dopamine, and serotonin) respectively on the 15th day of study. *Alpinia officinarum* (400 mg/kg) 15 days treatment significantly (p<0.001) increase in the level of brain monoamines as compared to the normal control group in mouse FST.

Alpinia officinarum (400 mg/kg) prevent the fall in the level of norepinephrine, [F (7, 40) = 17, p<0.0001], dopamine [F (7, 40) = 5.58, P<0.0002] and serotonin [F (7, 40) = 5.4, P<0.0002] respectively. The positive control moclobemide (50 mg/kg) and fluoxetine (30 mg/kg) showed moderately significant (p<0.001) increase in the brain norepinephrine, dopamine and serotonin levels respectively as compared to the normal control group.

TABLE 3: EFFECT OF *ALPINIA OFFICINARUM* ON IMMOBILITY PERIOD OF MICE IN FORCED SWIMMING TEST FOR 15 DAYS STUDY

Groups	Treatment	Oral dose(mg/kg)×14 days	Immobility period (Sec)	The percentage decrease in immobility
1	Normal control	CMC (35)	125.63 ± 5.07	
2	MEAO	100	106 ± 5.54**	15.6
3	MEAO	200	102.55 ± 4.13***	18.37
4	MEAO	400	97.87 ± 4.47***	22.09
5	Moclobemide	50	97.93 ± 1.99***	22.04
6	Fluoxetine	30	98.71 ± 0.64***	21.42

MEAO- Methanolic extract of *Alpinia officinarum*, CMC- Carboxy Methylcellulose, Data represented as mean ± SEM; n=6 and analyzed by one way ANOVA followed by Dunnett's test, *** p<0.001, **p<0.01,*p<0.05 as compared to normal control.

TABLE 4: EFFECT OF *ALPINIA OFFICINARUM* ESTIMATION OF BRAIN MONOAMINES (NE, DA, 5HT) OF MICE IN FORCED SWIMMING TEST FOR 15 DAYS STUDY

Groups	Treatment	Oral dose (mg/kg) × 14 days	Norepinephrine (µg /g) of brain tissue)	Dopamine(µg/gm of brain tissue	Serotonin (µg/gm of brain tissue
1	Normal Control	CMC (35)	0.24 ± 0.02	0.25 ± 0.005	0.31 ± 0.01
2	MEAO	100	0.49 ± 0.01**	0.39 ± 0.04	0.51 ± 0.03**
3	MEAO	200	0.57 ± 0.03**	0.04 ± 0.02**	0.57 ± 0.02***
4	MEAO	400	0.58 ± 0.10***	0.48 ± 0.04***	0.59 ± 0.02***
5	Moclobemide	50	0.64 ± 0.03***	0.53 ± 0.06***	0.63 ± 0.00***
6	Fluoxetine	30	0.66 ± 0.01***	0.50 ± 0.01***	0.65 ± 0.02***

MEAO- Methanolic extract of *Alpinia officinarum*, CMC- Carboxy Methylcellulose, NE-Norepinephrine, DA-Dopamine, 5HT-5 hydroxytryptamine. Values were expressed as mean ± SEM; n=6, the drug/vehicle treatments were administered once a day for 14 days. Data were analyzed by one way ANOVA followed by Dunnett's test *p<0.05, **p<0.01, *** p<0.001 as compared to normal control.

Effect of *Alpinia officinarum* on the Estimation of Brain GABA of Mice in Forced Swimming Test for 15 Days of Study: *Alpinia officinarum* (400 mg/kg p.o.) significantly increase (F7, 40 = 2.67; p<0.01) in the level of brain GABA concentration in animals as compared to the normal control group. Moclobemide (50 mg/kg, p.o.) and fluoxetine (30 mg/kg, p.o.) for 15 consecutive days induced a significant increase in brain GABA levels.

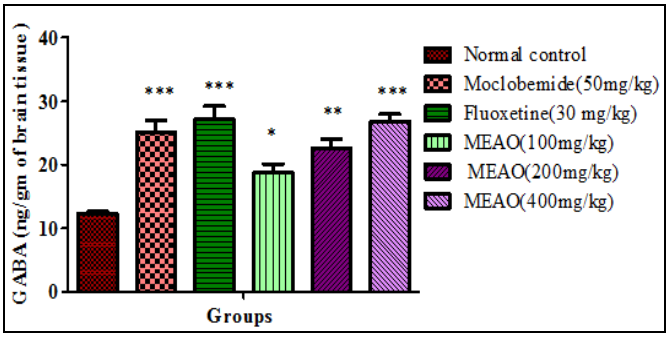

FIG. 2: EFFECT OF *ALPINIA OFFICINARUM* ON ESTIMATION OF BRAIN GABA OF MICE IN FORCED SWIMMING TESTS FOR 15 DAYS STUDY. MEAO- Methanolic extract of *Alpinia officinarum*, Data was analyzed by one way ANOVA followed by Dennett's test * p<0.05 **P< 0.01, ***P< 0.001 as compared to the normal control group.

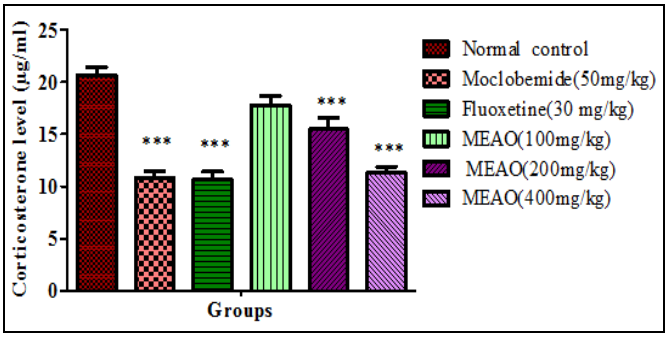

FIG. 3: EFFECT OF *ALPINIA OFFICINARUM* ON ESTIMATION OF PLASMA CORTICOSTERONE LEVEL IN MICE IN FORCED SWIMMING TEST FOR 15 DAYS STUDY. MEAO- Methanolic extract of *Alpinia officinarum*, Data was analyzed by one way ANOVA followed by Dennett's test * p<0.05 **P<0.01, ***P<0.001 as compared to the normal control

Effect of *Alpinia officinarum* on the Estimation of Plasma Corticosterone Level in Mice in Forced Swimming Test for 15 Days Study: Regarding the plasma corticosterone levels in mice, one-way ANOVA revealed a significant difference among the groups subjected to different treatments [F(7, 40) = 43, P = 0.0001]. Chronic stress produced a pronounced increase in the µg/ml of plasma corticosterone levels in mice. The administration of *Alpinia officinarum* (400 mg/kg) showed significantly (p<0001) reduce in plasma corticosterone as compared to a lower dose (100 and 200 mg/kg) showed less significant (p<0.05) reduction.

Effect of Desipramine and *Alpinia officinarum* on Potentiation of Norepinephrine Toxicity in Mice: Co-administration of norepinephrine (4.0

mg/kg, i.p.) and *A. officinarum* (400 mg/kg, p.o.) induced 33% mortality when compared with norepinephrine treated group showed 16.16% mortality. Standard drug desipramine treated animals when administered with intraperitoneal norepinephrine showed 66.66% mortality.

TABLE 5: EFFECT OF DESIPRAMINE AND *ALPINIA OFFICINARUM* ON NOREPINEPHRINE TOXICITY IN MICE

Groups	Treatment	Dose (mg/kg)	Mortality	Mortality dead/used (%)
Group 1	Normal control	35mg/kg p.o.	-	0/6
Group 2	*A. officinarum* + Norepinephrine	400.0 × 2 p.o.+ 4.0, i. p	0.33 ± 0.21	2/6 (33.33%)
Group 3	*A. officinarum* + Norepinephrine	200 × 2 p.o. + 4.0, i. p	0.16 ± 0.16	1/6(16.66%)
Group 4	*A. officinarum* + Norepinephrine	100 × 2 p.o. + 4.0, i. p	-	0/6 (0%)
Group 5	Norepinephrine	4.0, i. p	0.16 ± 0.16	1/6(16.66%)
Group 6	Desipramine + Norepinephrine	30 × 2 i. p + 4.0, i. p	0.66 ± 0.21*	4/6(66.66%)

Results are expressed as mean ± S.E.M., n = 6 in each group. The comparison made with Vehicle group. Data were analyzed by one way ANOVA followed by Dunnett's test. Data of 'mortality' was analyzed by Chi-Square test, the p-value was found to be not significant

Effect of *Alpinia officinarum* on 5-hydroxy tryptophan (5-HTP) 75 mg/kg Induced Head-Twitch Responses in Mice: Intraperitoneal administration of 5HTP (75 mg/kg), 60 min after moclobemide (50 mg/kg, p.o.), induced the characteristic head to twitch response. *Alpinia officinarum* (400 mg/kg, p.o.) on the intraperitoneal administration of 5HTP (75 mg/kg) significantly [F (4, 16) = 5. 7, p<0.0001] potentiate the head twitch response as compared to 5HTP control group. On the administration of *Alpinia officinarum* (100, 200 mg/kg, p.o.) on the intraperitoneal administration of 5HTP (75 mg/kg, i.p.) treatment showed less significant (p<0.01) potentiation of head twitch response as compared to 5HTP control group.

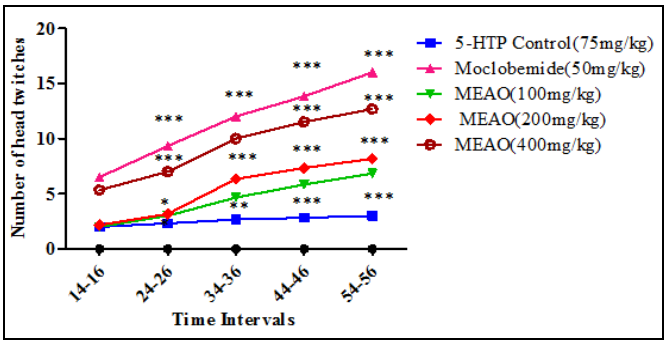

FIG. 4: EFFECT OF *ALPINIA OFFICINARUM* ON 5-HYDROXY TRYPTOPHAN (5-HTP) 75 mg/kg INDUCED HEAD-TWITCH RESPONSES IN MICE. MEAO- Methanolic extract of *Alpinia officinarum*, (5-HTP)- 5-hydroxytryptophan, Data was analyzed with two way ANOVA followed by Bonferroni p tests.* p<0.05 ***P*<0.01, ****P*<0.001 as compared to the normal control group.

DISCUSSION: In the present study antidepressants like potentials of *Alpinia, officinarum* was investigated in two most widely accepted and most commonly used behavioral model *viz*. tail suspension test and forced swim test model in rodents. Porsolt *et al*., (1978) [12] proposed an animal model, forced swim test (FST) for assessing the effect of antidepressant drugs. FST is considered a rapid and simplest test for evaluation of the antidepressant potential of drugs [21]. The tail suspension test (TST) has been described by Steru *et al*., (1985) [9] as a facile means of evaluating potential antidepressants, share a common basis as forced swim test. Both FST and TST models were based on two different mechanisms.

In the forced swim test rat after placing in a non-escapable cylinder of water showed initial 2 min vigorous struggle followed by an adaptation (helplessness or despair) of typical immobile posture with alternate swimming and climbing movements [12, 22, 23]. The helplessness or despair behavior in experimental animals serves as a tool for screening antidepressant-like activity of drugs [12]. In the tail suspension test, animals on hanging by its tail become immobile and float with stretched limb which is an indication of depression [23].

In TST animals subjected to inescapable, aversive situation showed agitation and immobility called searching–waiting for strategies of Steru *et al*., (1982) [24]. Antidepressants reduced the period of immobility [25]. FST and TST are well characterized behavioral model thinks to be predictive of antidepressant activity in human. It is an invention inspired by Porsolt's situation is extensively used, validated models for screening drugs having antidepressant-like activity. Apart this one of the prerequisites for this is the chronic administration of antidepressant drugs treatment is essential for the complete recovery of the patient [25].

In the present study, antidepressant-like activity (duration of immobility) in mice of *Alpinia officinarum* in the forced swim test and tail

suspension test was studied and the results obtained was in line with the results reported by many researchers [26]. **Table 2** and **3** represent the reduction in immobility time in TST and FST. As can be seen from the table. The standard drugs Moclobemide (monoamine oxidase inhibitors) [27], Fluoxetine (selective serotonin reuptake inhibitor) and desipramine (tricyclic antidepressant) were administered for 15 successive days showed a significant decrease in the duration of immobility in tail suspension and forced swim test. *Alpinia officinarum* (400 mg/kg) was identical with that exhibited by standard drugs. However, a dose-dependent effect could be established in case of higher doses, as the immobility period declined at a higher dose of 400 mg/kg.

The first neurochemical theory of depression was monoamine hypothesis which postulated that the major cause of depression is the decrease or deficiency of biogenic amine function, *i.e.* imbalance of available monoamines- dopamine, serotonin and norepinephrine (DA, 5HT and NE) as a result of disturbed synthesis, storage and release of monoamines [28] or subnormal monoamine receptors functioning in the brain [29]. The clinically used antidepressant molecules that have been developed in the past were aimed at increasing extracellular levels of biogenic amines 5 HT, DA and NE within the brain either by blocking the reuptake or by inhibiting the degradation of monoamines by inhibiting monoamine oxidase.

The subsequent development of monoamine oxidase inhibitors was based on a similar approach, namely an indirect elevation of extracellular concentration of the biogenic amines. Researchers reported the monoamines DA, 5HT and NE are involved in the etiopathogenesis of depression [30]. The commercial antidepressant drugs act by increasing the synaptic level of monoamines in the brain [13, 31].

The researcher reported that the monoamine oxidase enzyme inhibition may contribute to the increasing level of brain monoamines. Thus the present study focuses on brain monoamine estimation. The Researchers reported different methods for the estimation of monoamines like Shore and Olin., (1958) [32] reported the butanol extraction method for the assay of norepinephrine (NE) and epinephrine in the brain. Lund, (1949) [33] reported the method for extraction of monoamines but these methods are cumbersome and lack the rapidity and facility of the extraction procedures. Udenfriend-(1962) [34] reported the estimation of catecholamines by spectrophotofluorometric assay, but one of the limitations of this assay was fluorescence of tissue blank. Chang- (1964) [35] used acidified n-butanol, a batch method, with alumina, to isolate catecholamines. Maickel *et al.,* (1968) [36] and Miller *et al*., (1970) [37] they utilized the method of extraction using acidified n-butanol but did not include alumina also uses column or batch process to isolate the NE. Ciarlone (1976) [38] combines the analysis of Maickel *et al*., (1968) [36]; Miller *et al*., (1970) [37] and Chang (1964) [38] and describes a modified procedure of analyzing 5HT, NE, and DA in a single brain sample using the spectro-photofluorometric method.

Brain monoamine levels were estimated using High-performance liquid chromatography [39]. HPLC-ECD [39], Yoshitake *et al*., 2008 [40] describe a highly selective and sensitive column liquid chromatographic method for fluorescence determination of serotonin (5-HT), dopamine (DA), noradrenaline (NA) and their related metabolites is described. Fluorescence spectrophotometer was used for the estimation of brain monoamines [41]. A simple and sensitive spectrophotofluorometric determination of monoamine was used by many researchers in studying the brain monoamines activity. The monoamines play a very important role in the etiopathogenesis of depression.

The spontaneous and experimentally induced deficiencies in monoamines (serotonin, nor-epinephrine, and dopamine) are well documented and implicated in the onset of depression. A much experimental procedure designed to increase monoaminergic activity proved antidepressant properties [42]. In the present study, *Alpinia officinarum* (400 mg/kg) showed a significant increase in the level of monoamine such as (NE, DA, 5-HT) in brain tissue while at a lower dose, 100 and 200 mg/kg showed a less significant increase in monoamines. A major physiological response to stress in rats and mice is the activation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to an increase in circulating glucocorticoids level increases the chances of mood disorder [43].

Stressful situations in mice have reported the significant high concentration of blood glucocorticoids due to dysfunction of negative feedback mechanism [44]. In the present study 15 days pre-treatment of mice with *Alpinia officinarum* and moclobemide (50 mg/kg), fluoxetine (30 mg/kg) and imipramine (30 mg/kg) significantly ($p<0.001$) reduces the stress induced increased corticosterone.

The *Alpinia officinarum* at 100 and 200 mg/kg showed less significant ($p<0.01$) decreased in plasma corticosterone indicate normalization of the HPA axis prove its antidepressant potential. Gamma-amino-butyric acid is the inhibitory neurotransmitters act by inhibiting the release of monoamines and also act by reducing GABAnergic synaptic inhibition, activate the HPA axis leads to the subsequent excessive release of corticosterone [41]. In the present study, *Alpinia officinarum* at a dose of (400 mg/kg) showed significant ($p<0.001$) increase in GABA level while *Alpinia officinarum* at a dose of (100 and 200 mg/kg) showed less significant ($P<0.05$) increase in GABA level.

The researchers reported the MAO activity had been shown to result in higher free radicals generation [45]. Additionally, oxidative stress affects the number of synaptic function which results in impaired neurotransmission, plasma membrane integrity, ATP level and Na^+-K^+-ATPase activity. In this study, mice pre-treated for 15 consecutive days with, moclobemide and fluoxetine showed improvement in the stress parameter in the brain. It is also observed that the activity of Na^+-K^+-ATPase responsible for neuronal transmission is also restored by *Alpinia officinarum* at a dose of (400 mg/kg). *Alpinia officinarum* at a dose of (100 and 200 mg/kg) showed a non-significant increase in enzyme activity. Potentiation of norepinephrine toxicity in mice is used to reveal an adrenergic component of the pharmacological activity of antidepressant. It has been observed that the administration of norepinephrine (4.0 mg/kg, i. p) showed potentiation of lethality which is less as compare to desipramine and norepinephrine-induced lethality. In the present study, *Alpinia officinarum* at a dose of (400 mg/kg) potentiated markedly release of norepinephrine in mice, but *Alpinia officinarum* at a dose of (100 and 200 mg/kg) did not potentiate NE toxicity in mice.

So, results suggest that *Alpinia officinarum* at a dose of (400 mg/kg) may be an adrenergic component. Few antidepressant drug act by increasing monoamine level DA, NE and 5HT, is one of the most important mechanisms of action of antidepressant. 5HTP is a precursor for the synthesis of serotonin, administration of 5HTP reported to increase the serotonergic transmission leads to induce head twitches [46]. In the present study head, twitches induced by 5HTP was significantly ($P<0.001$) potentiated by *Alpinia officinarum* at a dose of (400 mg/kg), proved its antidepressant potential [47].

CONCLUSION It is concluded that both *Alpinia officinarum* at a dose of (400 mg/kg), showed antidepressant activity similar to moclobemide (50 mg/kg) and imipramine (30 mg/kg) in different models in mice. The mechanism of action of antidepressant activity appears to be primarily due to increases monoamine and the gamma-amino-butyric acid level in mouse brain. It also acts by normalized Na-K-ATPase activity on the plasma membrane and contributes to the normal regulation of the HPA axis evidenced by significant ($P<0.001$) decreased in plasma corticosterone level. The potentiation of norepinephrine and serotonin was also contributed to antidepressant activity.

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REFERENCES:

1. Tushar BS, Sarma GC and Rangan L: Ethnomedical uses of Zingiberaceous plants of Northeast India. J Ethnopharmacol 2010; 132: 286-296.
2. Carlini EA: Screening farmacológico de plants brasileiras. Rev Bras Biol 1972; 32: 265-274.
3. Hasimun P, Sulaeman A, Sutrisno E and Putra HM: Potential diuretic activity from Zingiberaceae family and its effect on the levels of potassium and sodium ions in the urine. World Journal of Pharmacy and Pharmaceutical Sciences 2018; 7(3): 226-236
4. Lim TK: Edible medicinal and non-medicinal plants: modified stems, roots, bulbs 2002; 12. London: Springer 178.
5. Bown D: Encyclopedia of herbs and their uses. London: Dorling Kindersley 1995: 424.

6. Lee J, Kim KA, Jeong S, Lee S, Park HJ and Kim NJ: Anti-inflammatory, anti-nociceptive, and anti-psychiatric effects by the rhizomes of *Alpinia officinarum* on complete Freund's adjuvant-induced arthritis in rats. J Ethnopharmacol 2009; 126: 258-64. [PubMed]
7. Xie ZS. Xu XJ. Xie CY. Huang JY. Yang M and Yang DP: Volatile components of rhizoma *Alpinia officinarum* using three different extraction methods combined with gas chromatography-mass spectrometry. J Pharm Anal 2013; 3: 215-20.
8. Chen KX: Quantitative structure-activity relationship analysis of aryl alkanol piperazine derivatives with antidepressant activities. European Journal of Medicinal Chemistry 2009; 44: 4367-4375.
9. Steru L, Chermat R, Thierry B and Simon P: Tail suspension test: A new method for screening antidepressants in mice. Psychopharmacology 1985; 85: 367-370.
10. Bonting SL: Sodium–potassium-activated adenosine triphosphatase and cation transport. In Membranes and Ion Transport, vol. 1 (ed. E. E. Bittar), 1970; 257-363. New York: Wiley Interscience.
11. Bonting SL: Na-K activated adenosine triphosphatase and active cation transport in the lens. Investigative Ophthalmology and Visual Science 1965; 4: 723-738.
12. Porsolt RD, Anton G, Blavet N and Jalfre M: Behavioral despair in rats: a new model sensitive to antidepressant treatments. Euro Journal of Pharmacology 1978; 47: 379-391.
13. Porsolt RD, Bertin A, Balavet N, Daniel M and Jalfre M: Immobility induced by forcing swimming rats: effect of agents which modify central catecholamine and serotonin activity. European Journal of Pharmacology 1979; 57: 201-210.
14. Abel EL-Bilitzke PJ: A possible alarm substance in the forced swimming test. Physiol Behav 1990; 48: 233-9.
15. Bartosj PM: Colorimetric and fluorimetric determination of steroids. Pure Appl Chem 1979; 51: 21-57.
16. Brownlee G and Spriggs TLB: Estimation of dopamine, noradrenaline, adrenaline, and 5-hydroxytryptamine from single rat brains. Journal of Pharmacy and Pharmacology 1965; 17: 429-433.
17. Maynert EW, Klingman GI and Kaji HK: Tolerance to morphine. II. Lack of effects on brain 5-hydroxytryptamine and gamma-aminobutyric acid. J Pharmacol Exp Ther 1962; 135: 296-9.
18. Dhingra D and Valecha R: Behavioral and neuroendocrine effects of aqueous extract of Boerhaaviadiffusa Linn. in mice using tail suspension and forced swim tests - A preliminary study. Indian Journal of Experimental Biology 2014; 52: 53-59.
19. Vogel HG: Drug discovery and evaluation: pharmacological assay. 2nd ed. Springer-Verlag Berlin Heidelberg New York 2002: 558-603.
20. Sanchez CC, Bonkanka CX, Prado B and Rabanal RM: Antidepressant activity of some *Hypericum reflexum* L. fil. Extracts in the forced swimming test in mice. Journal of Ethnopharmacology 2007; 112: 115-121.
21. Kulkarni SK and. Parale MP: Despair behavior: a tool in experimental psychopharmacology A review. Methods Find Exp Clin Pharmacol 1986; 8: 741-4.
22. Borsini F and Meli A: Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology (Berl) 1988; 94: 147-60.
23. Redrobe JP and Bourin M: Evidence of the activity of lithium on 5-HT1B receptors in the mouse forced swimming test: comparison with carbamazepine and sodium valproate. Psychopharmacology 1999; 141: 370-7.
24. Mezadri TJ, Batista GM, Portes AC, Marino-Neto J, Oliveira C and Lino D: Repeated rat-forced swim test: Reducing the number of animals to evaluate gradual effects of antidepressants. Journal of Neuroscience Methods 2011; 195: 200-205.
25. Stéru L, Thierry B, Chermat R and Simon P: Animal modeling of depression: an ethological approach and its relevance to psychopharmacology. Presented at the 13th CINP Congress, Jerusalem, Israel 1982; 20-25.
26. Cryan JF, Mombereau C and Vassout A: The tail suspension test as a model for assessing antidepressant activity: a review of pharmacological and genetic studies in mice. Neuroscience & Biobehavioral Reviews 2005; 29: 571-625.
27. Mute VM and Bodhankar SL: Potentiation of pressor effect of tyramine by newly synthesized compound 2[(n-benzylacetamido) mercapto] benzimidazole (VS 25), a putative inhibitor of monoamine oxidase–A enzyme in rats. Der Pharmacia Lettre 2014; 6(6): 165-174
28. Zafir A, Ara A and Banu N: *In-vivo* antioxidant status: A putative target of the antidepressant action. Progress in Neuro-Psychopharmacology & Biological Psychiatry 2009; 33: 220-228.
29. Kharade SM, Gumate DS and Naikwade NS: A review: the hypothesis of depression and role of antidepressant drugs. IJPPS. 2010; 2: 3-6.
30. Takano HT: Cognitive Function and Monoamine Neurotransmission in Schizophrenia: Evidence From Positron Emission Tomography Studies. Frontiers in psychiatry 2018; 9: 228.
31. Moret C and Briley M: The importance of norepinephrine in depression. Neuropsychiatric disease and treatment. 2011; 7(Suppl 1): 9.
32. Mute VM and Bodhankar SL: Antidepressant-like the effect of newly synthesized compound 2[(n- benzylacetamido) mercapto] benzimidazole (VS-25) and its possible mechanism by inhibition of monoamine oxidase enzyme in mice. Int J Pharm Pharm Sci 2014; 7: 407-410.
33. Shore PA and Olin J: Identification and chemical assay of NE in the brain and other tissues. J. Pharmacol 1958; 122: 295-300.
34. Lund A: Fluorimetric determination of adrenaline in blood-III. A new sensitive and specific method. Acta. Pharmacol. Toxicol. 1949; 5: 231-247.
35. Udenfriend S: Fluorescence Assay in Biology and Medicine. Academic Press, New York 1962; 233.
36. Chang CC: A sensitive method for the spectrophoto-fluorometric assay of catecholamines. Int J Neuro-Pharmacol 1964; 3: 643-649.
37. Maickel RP, Cox RH, Saillant J and Miller FP: A method for the determination of serotonin, and norepinephrine in discrete areas of rat brain. Inc. I. Neuropharmacol 1968; 7: 215-281.
38. Miller FP., Cox RH, Snodgrass NR and Maickel RP: Comparative effects of p-chlorophenyialanine, p-chloro-amphetamine and p-chloro-N methylamphetamine on rat brain norepinephrine, % hydroxytryptamine, and 5-hydroxyindole -3-acetic acid. Biochem Pharmacol 1970; 19: 435-442.
39. Ciarlone AE: Modification of a spectrophotofluorometric method of analyzing serotonin, norepinephrine, and dopamine in one brain sample. Microchemical Journal 1976; 21: 349-354.
40. Lakshmana MK and Raju TR: An isocratic assay for norepinephrine, dopamine, and 5-hydroxytryptamine using

their native fluorescence by High-performance liquid chromatography with fluorescence detection in discrete brain areas of the rat. Analytical Biochemistry 1997; 246: 166-170.

41. Li-Tao Y, Jing L, Bin-Bin L and Cheng-Fu L: Screening of the antidepressant-like effect of the traditional Chinese medicinal formula Si-Ni-San and their possible mechanism of action in mice. Pharmacognosy Res 2013; 5: 36-42.
42. De Benedetto GE, Fico D, Pennetta A, Malitesta C, Nicolardi G, Lofrumento DD, De Nuccio F and La Pesa V: A rapid and simple method for the determination of 3, 4-dihydroxyphenylacetic acid, norepinephrine, dopamine, and serotonin in mouse brain homogenate by HPLC with fluorimetric detection. Journal of Pharmaceutical and Biomedical Analysis 2014; 98: 266-270.
43. Delgado PL: How antidepressants help depression: mechanisms of action and clinical response. J Clin Psychiatry 2004; 65(Suppl 4): 25-30.
44. Leitch MM, Ingram CD, Young AH, McQuade R and Gartside SE: Flattening the corticosterone rhythm attenuates 5-HT1A autoreceptor function in the rat: relevance for depression. Neuropsychopharmacology 2003; 28: 119-125.
45. Johnson SA, Fournier NM and Kalynchuk LE: Effect of different doses of corticosterone depression-like behavior and HPA axis responses to a novel stressor. Behav Brain 2006; 2: 280-8.
46. Reed DJ: Glutathione: toxicological implications. Annual Review of Pharmacology and Toxicology 1990; 30: 603-631.
47. Pandey DK, Devadoss T, Modak N and Mahesh R: Antidepressant & anxiolytic activities of N-(pyridine-3-yl) quinoxaline-2-carboxamide: A novel serotonin type 3 receptor antagonist in behavioral animal models. The Indian Journal of Medical Research 2016; 144(4): 614.

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