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Investigation of Antistress Activity of *Operculina Terpethum* Roots using Various Experimental Models

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Research Article

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Abstract

The root part of *Operculina terpethum* has been widely used in *Ayurveda* to treat variety of common and stress related disorders. The antistress effect of a seven-days treatment (100 and 200 mg / kg, p.o.) of the 80% ethanolic extract of *Operculina terpethum* root (OTRE) was evaluated by using the swimming endurance test, anoxic tolerance test, and biochemical changes in the cold-restraint stress test. The immunomodulatory activity was also evaluated for the same doses, and treatment of OTRE using the hem agglutination test. Diazepam 1 mg/kg was used as reference standard for the comparison. Both the doses of OTRE showed anti stress activity in all the tested models. 7 days pretreatment of OTRE (100 and 200 mg / kg, p.o.) treated animals showed a decrease in immobility time and an increase in anoxic tolerance time in swimming endurance and the anoxic tolerance tests, respectively. Pretreatment with OTRE significantly ameliorated the cold restraint stress induced variation in biochemical level such as increase in plasma cholesterol, triglyceride, glucose, total protein, and cortisol. Further, OTRE treatment significantly inhibited cold restraint stress induced alteration in brain noradrenaline, dopamine and serotonin levels. In mice immunized with sheep red blood cells, the treatment groups subjected to restraint stress prevented the humoral immune response to the antigen. The immune stimulating activity of the OTRE was indicated by an increase in the antibody titer in mice pre-immunized with sheep red blood cells and subjected to restraint stress. The findings of the present investigations indicate that the OTRE has significant anti stress activity, which may be due to the immune stimulating property and increased resistance, nonspecifically, against all experimental stress conditions.

Keywords: *Operculina Terpethum*; Antistress Activity; Cold-Restraint Stress Test; Swimming Endurance Test; Anoxic Tolerance Test

Introduction

Stressors have a major influence upon mood, our sense of well-being, behavior and health. Acute stress responses in young, healthy individuals may be adaptive and typically do not impose a health burden. However, if the threat is unremitting, particularly in older or unhealthy individuals, the long-term effects of stressors can damage health. In contrast, if stressors are too strong and too persistent in individuals who are biologically vulnerable because of age, genetic, or constitutional factors, stressors may lead to disease [1]. Adaptogens are stress-response modifiers that increase an organism's nonspecific resistance to stress by increasing its ability to adapt and survive. Current and potential uses of adaptogens are mainly related to stress-induced fatigue and cognitive function, mental illness, and behavioral disorders [2].

Operculina turpethum is classified in *Ayurveda*, as a *rasayana* reputed to promote physical and mental health, augment resistance of the body against disease and diverse adverse environmental factors, revitalize the body in debilitated conditions, and increase longevity. *Operculina turpethum,* commonly known as Trivrit, belonging to family *convolvulaceae* is widely distributed throughout India, China, Sri Lanka and Australia. The plant has a wide range of applications in Ayurvedic formulations. The roots of this plant have been regarded traditionally as strong purgative and used for fevers, anorexia, edema, anaemia, ascites, constipation, hepato-splenomegaly, hepatitis, intoxication, abdominal tumors, ulcers, wounds, worm infestation, pruritus, and other skin disorders [3]. Roots are also traditionally claimed for the treatment of obesity, haemorrhoids, cough, asthma, dyspepsia, flatulence, paralysis, gout, rheumatism, melancholia, scorpion sting and snake bites [4]. The major constituents isolated from the plant are turpethin, Turpethinic acids-A,B,C,D& E3, Stigma -5,22dien-3-o-b-D-glucopyranoside, 3-(4- hydroxy-phenyl)-N-[2-(4-hydroxy phenyl)-ethyl]-acrylamide [5-8].

Several investigations have proposed that this plant possesses nephroprotective, hepatoprotective, anticancer, analgesic, antioxidant, antimicrobial, antiulcer, antidiabetic, antiarthritic, antidiarrheal, anti-inflammatory, neuropharmacological, Immunomodulatory and laxative effects [9-20]. Charak samhita describes use of *rashayana* such as Trivrutta kashya (herbal decoctions) as dietary supplement for the brain and neurological activity [21]. Ashwagandharishta, Saraswatarishta, Saraswata ghrita are wellknown polyherbal formulations containing trivrit used for brain disorders. Hence, the present study is designed to evaluate the antistress effect of 80% ethanolic extract of *O. turpethum* roots using various experimental models.

Materials and Methods

Plant Material and Preparation of Extract

The roots of *Operculina terpethum* were obtained from the B. A. College of Agriculture, Anand. and authenticated (Authentification No. BACA/GPB/1357/15) by Dr. D. B. Patel, Professor and Head, Department of Genetic and Plant Breeding, Anand Agricultural University, Anand, Gujarat, India. A herbarium was prepared and deposited in the department of Pharmacognosy, A. R. College of Pharmacy, Vallabh Vidyanagar. The plant material was completely dried under the shade and powdered. The dried powdered material was placed in the Soxhlet thimble and with 80% ethanol in 500ml flat bottom flask and refluxed for 18h at 80˚C for two days. The crude extract was filtered and dried under reduced pressure at 40°C (yield: 12.5 % w / w). Freshly prepared aqueous solution of the dried extract of *Operculina terpethum* roots (OTRE), in a suitable dilution, was administered to the animals in the treatment groups.

Preliminary Phytochemical Screening

The hydroalcoholic extract of the *Operculina terpethum* roots was tested for the presence of carbohydrates, saponins, steroids, terpenoids, flavanoids, alkaloids, tannins and phenolic compounds using the standard procedures [22].

Animals

Healthy adult Swiss albino mice of either sex (25-30 g) were used for the swimming endurance test, anoxic tolerance test, immunological assay and cold restrained stress test. The animals were housed under standard conditions, with a commercial pellet diet and had free access to water. The animals were acclimatized to the laboratory environment for one hour before the experiments. The animals were randomly distributed into groups of six animals each. All experiments were conducted during the light period (08.00 – 16.00 hours). All the protocols were approved (CPCSEA/IAEC/ARCP/2014-15/05) by the Institutional Animal Ethics Committee (IAEC) and conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs

Diazepam (Ranbaxy, India) was used as the standard drug (positive control) in various stress models. All the

chemicals and reagents used for the biochemical studies were commercial grade analytical reagents.

Swimming Endurance Test

The mice were randomly divided into four groups of six animals each. The treatment groups were pretreated with OTRE (100 mg / kg, 200 mg / kg, p.o.) for seven days. The control group was pretreated with normal saline (10 ml / kg, p.o.), while the positive control group received diazepam (1 mg / kg, i.p.) for seven days. The swimming test was carried out on the seventh day, after one hour of oral and 30 minutes of intraperitoneal administration of the drug, using a polypropylene vessel (45 × 40 × 30 cm) with a water level of 20 cm, and the immobility time was recorded for 30 minutes [23].

Anoxic Tolerance Test

The mice were randomly divided into four groups of six animals each. The treatment groups were pretreated with OTRE (100 mg / kg, 200 mg / kg, p.o.) for seven days. The control group was pretreated with normal saline (10 ml / kg), while the positive control group received diazepam (1 mg / kg, i.p.) for seven days. On the seventh day, the mice were subjected to anoxic stress by keeping them in a confined airtight 250 ml glass jar. The time taken for the mice to exhibit the first clonic convulsion was taken as the end point. The animals were removed immediately from the vessel for recovery and resuscitated if needed [23].

Immunological Assay

The mice were randomly divided into five groups of six animals each. All the mice were immunized with sheep red blood cell (SRBC), (0.5 × 10 9 cells / ml / 100 g, i.p.) on day zero. The treatment groups were then pretreated with OTRE (100 mg / kg, 200 mg / kg, p.o.) for seven days. The normal control group and stress control group were pretreated with normal saline (10 ml / kg, p.o.), while the positive control group received diazepam (1 mg / kg, i.p.) for seven days. On the seventh day, after initial immunization with SRBC, the mice were subjected to restraint stress for two hours. After induction of stress, blood was collected and the serum assayed for hemagglutination (highest dilution giving hemagglutination was taken as the antibody titer) [23].

Cold Restraint Stress Test

The mice were randomly divided into five groups of six animals each. The treatment groups were pretreated with OTRE (100 mg / kg, 200 mg / kg, p.o.) for seven days. The control group and stress control groups were pretreated with normal saline (1 ml / kg, p.o.), while the positive Control group received diazepam (1 mg / kg, i.p.) for seven days. A cold restraint stress was given to all the mice, except the control animals, by tying the limbs for two hours at 4°C on the seventh day of treatment [23].

After two hours, the animals were sacrificed by decapitation and the blood was collected in EDTA-coated propylene tubes. The blood samples were centrifuged (3000 rpm for 20 minutes at 4°C) and the plasma were separated out and stored at 20°C for biochemical and hormonal assays. These samples were used to analyze cholesterol, triglyceride, glucose, total proteins and cortisol level [24-28].

Brains were isolated from 3 mice from each group. Whole brain of each mouse was weighed without thawing and immediately homogenized in 5ml ice cold acidified butanol. The homogenate was centrifuge at 500 rpm at 40C for 10 min and the supernatant was collected. Norepinephrine (NE), Dopamine (DA) and serotonin (5-HT) neurotransmitters were estimated by flourimetric method of Jacobowitz and Richardson [29].

Norepinephrine (NE) Estimation

2ml of supernatant was taken in centrifuge tube containing 1.5ml of phosphate buffer. Tube was vortexed for 20sec. NE was extracted in phosphate buffer. After centrifugation at 3000rpm, 1ml of phosphate buffer extract was taken in a test tube. Now 0.25 of ice-cold versene (4g EDTA, 10N NaOH in 100ml of distilled water) was added to the phosphate buffer extract, vortexed briefly followed by addition of 0.2ml of iodine solution (4.8g potassium iodide and 0.25g of iodine in 100ml distilled water), 0.25ml of fresh alkaline sodium sulfite (2.5g of Na_2SO_3 in 100ml of 4N NaOH), and 0.3ml of 5N acetic acid. The cocktail was kept in boiled water for 5min and then ice for 1min. Fluorescence for NE was measured at 385 and 485nm. All values were expressed as ng/g wet tissue.

Dopamine (DA) Estimation

The assay represents a miniaturization of the trihydroxide method. To 0.02ml of HCl phase, 0.05ml 0.4M and 0.01ml EDTA/Sodium acetate buffer (pH 6.9) was added, followed by 0.01ml iodine solution minutes by addition of 0.01ml Na2SO3 in 5m NaOH. Acetic acid was added 1.5 minutes later. The solution was heated to 100ºC for 6 minutes. When the sample would again reach the room temperature, excitation and emission spectra were read. Fluorescence was measured at 330-375nm and the values were expressed in ng/g wet tissue.

Serotonin (5-HT) Estimation

2ml of supernatant was taken in glass stoppered centrifuge tube containing 5ml of heptane and 0.5ml of 0.1N HCl. Tube was vortexed for 20sec, 5-HT was extracted in 0.1N HCl. After centrifugation at 3000rpm 0.3ml HCl extract was taken in test-tube. Now 0.2ml of orthophaldehyde (OPT) solution (50mg/100ml of absolute methanol) was added to 0.1N HCl extract, followed by addition of 1.5ml of concentrated HCl (10N). The mixture was vortexed in boiling water for 10min and cooled under tap water. Fluorescence for 5-HT was measured at 360 and 470nm. All values were expressed as ng/g wet tissue.

Statistical Analysis

Results were expressed as mean(s)±SEM. The statistical significance of the difference between groups for the various treatments were determined by one-way analysis of variance (ANOVA) followed by Tukey's multiple range test. $P<0.05$ was considered statistically significant as compared to control.

Results

Preliminary Phytochemical Screening

Phytochemical screening revealed the presence of carbohydrates, saponins, steroids, alkaloids, flavanoids, tannins and phenolic compounds in the 80% ethanolic extract of OTRE.

Swimming Endurance Test

As shown in Table 1, seven days pretreatment with OTRE (100 mg / kg and 200 mg / kg, p. o.) produced dose dependent and significant increase in the swimming time as compared to the control group. Similarly, positive control diazepam (1mg / kg, i.p.) also showed statistically significant increase in the swimming time as compared to the control.

Groups	Treatments	Dose	Swimming time (min)
I	Control	10 ml / kg, p.o.	12.98 ± 1.31
II	Diazepam	1 mg/kg, i.p.	21.01 ± 1.33*
III	OTRE	100 mg/kg, p.o.	15.51 ± 1.86*
IV	OTRE	200 mg/kg, p.o.	24.11 ± 1.23*

Table 1: Effect of OTRE in Swimming endurance test in mice.
Expressed as mean ± SEM (n=6) and one way analysis of variance (ANOVA) followed by Tukey's test. *p<0.05 when compared with control.

Anoxic Tolerance Test

The time taken for the mice to exhibit clonic convulsions was taken as the end point in the anoxic tolerance test. Seven days pretreatment with OTRE (100 mg / kg and 200 mg / kg, p. o.) significantly increased the time taken for clonic convulsions as compared to the control animals. Similarly, diazepam treatment also produced significant delay in clonic convulsions (Table 2).

Groups	Treatments	Dose	Latency for convulsions (min)
I	Control	10 ml / kg, p.o.	11.5 ± 1.3
II	Diazepam	1 mg/kg, i.p.	26.16 ± 1.73*
III	OTRE	100 mg/kg, p.o.	21.66 ± 2.43*
IV	OTRE	200 mg/kg, p.o.	25.16 ± 3.86*

Table 2: Effect of OTRE on latency for convulsions in mice in Anoxic tolerance test.
Expressed as mean ± SEM (n=6) and one way analysis of variance (ANOVA) followed by Tukey's test. *p<0.05 when compared with control.

Immunological Assay

Stress control animals had an immunosuppressive effect, as indicated by a decrease in antibody titers. Seven days of pretreatment with OTRE (100 mg / kg and 200 mg / kg) showed significant and dose-dependent inhibition of stress-induced reduction in antibody titers. Similarly, diazepam (1 mg / kg, i.p.) treated animals also had significant protection against stress-induced reduction of antibody titers (Table 3).

Groups	Treatments	Dose	Antibody Titre
I	Control	10 ml / kg, p.o.	6.56 ± 0.5
II	Stress Control	10 ml / kg, p.o.	3.86 ± 0.48*
II	Diazepam	1 mg/kg, i.p.	8.03 ± 0.33#
III	OTRE	100 mg/kg, p.o.	6.3 ± 0.73#
IV	OTRE	200 mg/kg, p.o.	7.67 ± 0.26#

Table 3: Effect of OTRE on Antibody titre in Immunological assay.
Expressed as mean ± SEM (n=3) and one way analysis of variance (ANOVA) followed by Tukey's test. *p<0.05 when compared with control. #p<0.05 when compared with stress control group.

Biochemical Investigations

Cold-restraint stress adversely affected the blood concentration of various biochemical parameters. The results of the biochemical parameters are summarized in Table 4. The induction of cold-restraint stress led to a rise in serum cholesterol, triglycerides, glucose, total proteins, and cortisol levels in stress control animals. Seven days pretreatment with OTRE (100 mg / kg and 200 mg / kg) reduced all the biochemical parameters significantly and dose dependently, as compared to the stress control animals (Table 4).

Groups	Treatments	Dose	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Glucose (mg/dl)	Total Protein (g/dl)	Cortisol (µg/dl)
I	Control	10 ml / kg, p.o.	55 ± 8.25	141.33 ± 14.73	94 ± 4.49	3.53 ± 0.6	7.33 ± 0.4
II	Stress Control	10 ml / kg, p.o.	90 ± 9.27*	187.66 ± 11.56*	164 ± 13.73*	4.13 ± 0.33*	10.66 ± 0.46*
II	Diazepam	1 mg/kg, i.p.	50.33 ± 9.24#	106 ± 11.26#	102.33 ± 9.66#	3.6 ± 0.46	3.33 ± 0.28#
III	OTRE	100 mg/kg, p.o.	68.0 ± 9.44#	130.66 ± 9.02#	154.33 ± 16.27	2.76 ± 0.11#	6.16 ± 0.35#
IV	OTRE	200 mg/kg, p.o.	47.35 ± 5.89#	113 ± 12.75#	109.33 ± 15.55#	4.6 ± 0.47#	5.33 ± 0.33#

Table 4: Effect of OTRE on Biochemical parameters in Cold restraint stress test.
Expressed as mean ± SEM (n=3) and one way analysis of variance (ANOVA) followed by Tukey's test. *p<0.05 when compared with control. #p<0.05 when compared with stress control group.

Measurement of Neurotransmitters

Results of Norepinephrine (NE), Dopamine (DA) and Serotonin (5-HT) estimation are shown in Table 5. There was significant reduction in brain NE level observed in stress control animals as compared to control group. As shown in Table 5, there was significant increase in brain NE concentration with 7 days OTRE (100 mg / kg and 200 mg / kg, p.o.) treatment as compared to stress control. Treatment with Diazepam (1 mg/kg, i.p.) also showed significant rise in brain NE level as compared to stress control.

There was rise in brain dopamine level observed in stress control animals as compared to control group. 7 days OTRE (100 mg / kg and 200 mg / kg, p.o.) pretreatment produced significant reduction in brain dopamine concentration as compared to control. Also, treatment with Diazepam (1 mg/kg, i.p.) showed significant reduction in brain dopamine level.

Groups	Treatments	Dose	Amount of Noradrenaline (ng/g)	Amount of Dopamine (ng/g)	Amount of Serotonin (ng/g)
I	Control	10 ml / kg, p.o.	387.33 ± 32.44	429.66 ± 24.67	450 ± 23.17
II	Stress Control	10 ml / kg, p.o.	253.66 ± 27.12*	587.66 ± 13.67*	426 ± 24.19*
II	Diazepam	1 mg/kg, i.p.	443 ± 29.0#	373.33 ± 25.95#	577.33 ± 27.58#
III	OTRE	100 mg/kg, p.o.	484.33 ± 30.02#	384 ± 42.15#	645.33 ± 26.63#
IV	OTRE	200 mg/kg, p.o.	590.66 ± 77.6#	317 ± 26.41#	731.66 ± 23.34#

Table 5: Effect of OTRE on brain neurotransmitter level in Cold restraint stress test.
Expressed as mean ± SEM (n=3) and one way analysis of variance (ANOVA) followed by Tukey's test. *p<0.05 when compared with control. #p<0.05 when compared with stress control group

There was significant reduction in brain 5-HT level observed in stress control animals as compared to control group. Significant rise in brain 5-HT level was shown with 7 days OTRE (100 mg / kg and 200 mg / kg, p.o.) treatment as compared to control.

Discussion

Stress is the major contributor to psychiatric pathologies and has been found to alter the neurotransmitter and biochemical profiles and the oxidation status in the central nervous system. In *Ayruveda*, several plants used as tonic have been investigated for its adaptogenic property. In the present study, the antistress activity of the 80% ethanolic extract of *Operculina terpethum* roots (100 mg / kg, 200 mg / kg) has been evaluated using various acute stress experimental models.

The swimming endurance test and anoxic tolerance test are known physical stress models for the evaluation of antistress activity. In the swimming endurance test, the mice are forced to swim in a restricted space from which they cannot escape. This induces a characteristic behavior of immobility. It has been well-demonstrated that drugs with antistress activity increase swimming endurance (or reduce immobility time) and latency of post-anoxic convulsions [30-32]. The results of the swimming endurance test and anoxic tolerance test indicate clearly that the 80% ethanolic extract of *O. terpethum* roots (100 mg / kg, 200 mg / kg) have the properties, whereby, they increase the physical endurance as well as the overall performance in mice.

There has long been an interest in the role of stress in the production of human diseased states, as at least some of them are linked to suppression of the immune response. Both humoral and cell-mediated immune responses are affected, indicating that stress may have an adverse effect on normal immune surveillance [33]. Control stress was shown to suppress experimental paradigms of humoral and cell mediated immune responses. When mice were sensitized with SRBC, the humoral immune response was clearly suppressed in restraint stress control group. OTRE treatment (100 mg / kg, 200 mg / kg, p.o.) prevented the anticipatory fall in antibody titres comparable to diazepam control group. Also, Ayurveda records that *Rasayanas* have the ability of protecting the body against external factors that induce disease. This implied resistance against disease may represent the modern concept of immunity [34]. Thus, OTRE as a rasayana have been shown immunomodulatory action, improving nonspecific immune reactivity [33].

Activation of HPA axis during stress is a well-known phenomenon. In response to stress, the adrenocorticotropic hormone is released, which acts on the adrenal cortex to stimulate the synthesis and release of cortisol. Increase in plasma cortisol influences the mobilization of stored fat and carbohydrate reserves, which in turn increases the blood glucose level, total proteins, cholesterol and triglycerides [35]. Pretreatment with the OTRE significantly ameliorated the stress induced variations in these biochemical levels indicating anti stress activity.

Estimation of noradrenaline, dopamine and serotonin level could help in elucidating the mechanism of action behind the significant antistress activity shown by OTRE. Acute stressful conditions activate monoaminergic system leading to an increase in the extracellular levels of noradrenaline, dopamine and serotonin in cortex and hippocampus regions of brain [36,37]. The increased levels of noradrenaline are short lived and are generally terminated thereby decreasing in response. Some studies reported increase level of brain noradrenaline in response to various stressors [38]. While, some studies reported reduction in brain noradrenaline level due to stress [39,40]. The stressor such as immobilization induced noradrenaline synthesis, positively correlated with activity of hypothalamic pituitary adrenal axis (HPA) [41]. It is possible that level of noradrenaline decreases because of increased utilization of this neurotransmitter in brain tissue during stress [40]. In the present study, brain noradrenaline level was decrease in stress control mice. Pretreatment with OTRE prevented this stress induced reduction in brain noradrenaline level. Pretreatment with diazepam also significantly increased the brain noradrenaline as compared to stress control animals.

Level of brain dopamine in stress is reported by some workers indicate decrease, increase or no change [42,43]. In the present study, increase of dopamine level in animal subjected to stress was observed and pretreatment with OTRE prevented the elevation of dopamine level.

Brain serotonine level was decreased in stress condition in mice [44]. Pretreatment with OTRE significantly prevented stress induced serotonin alterations in mice. The results of the present study showed that restraint stress, as a model of psychological stress, caused alterations in the central neurotransmission in mice. Treatment with diazepam also reported rise in brain serotonin level in mice [45]. Similar results also observed in the present study. Thus, neurotransmitter

changes results of present study supported by several other studies [46,47].

The present investigation indicates that OTRE has significant antistress activity as shown by its mitigating effects on several acute stress induced biochemical, and immunological perturbations, comparable to that of diazepam, the conventional antistress agent. Thus, OTRE treatment has been shown to prevent the effect of various types of stress non-specifically by various mechanisms and by increasing resistance against various types of stress. Standard drug diazepam used in the present study is reported to possess a nonspecific antistress activity in experimental models.

Conclusion

In conclusion, our results provide evidence that the seven days treatment with the 80% ethanolic extract of *Operculina terpethum* roots shows antistress (adaptogenic) activity in various acute stress models. The observed antistress activity may be due to the prevention of desensitization of both the peripheral and central components of the hypothalamic-pituitary-adrenal axis (HPA) and due to the non-specifically increased resistance produced by the *Operculina terpethum* roots extract. This study provides significant evidence of the medicinal and traditional uses of *Operculina terpethum* root in stress disorders.

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Conflict of Interest: None.

References

1. Schneiderman N, Ironson G, Siegel SD (2005) Stress and health: Psychological, Behavioral, and Biological Determinants. Annu Rev Clin Psychol 1: 607-628.

2. Panossian A (2017) Understanding adaptogenic activity: specificity of the pharmacological action of adaptogens and other phytochemicals. Ann N Y Acad Sci 1401(1): 1-16.

3. Nadkarni KM, Nadkarni AK (2007) Indian Materia Medica, 3rd (Edn.), Bombay: Popular Prakashan 1: 691-694.

4. The Ayurvedic Pharmacopoeia of India (2006) Part I, Vol III, 1st (Edn.), Government of India, Ministry of health and family welfare, department of ISM & H, New Delhi, India 404: 213-214.

5. Sharma V, Singh MANU (2012) Alterations induced by N-Nitrosodimethyl-amine and ethanolic root extract of *Operculina turpethum* in serum lipid profile of male albino mice. Asian J Pharm and Clini Res 5(3): 69-73.

6. Sharma V, Singh M (2013) Isolation and Characterization of Stigma-5,2-dien-3-O-b-D-Glucopyranoside from the ethanolic root extract of *Operculina turpethum*. Int J Adv Res 1(8): 303-312.

7. Ding W, Zeng F, Xu L, Chen Y, Wang Y, et al. (2011) Bioactive Dammarane-Type Saponins from Operculina turpethum. J Nat Prod 74(9): 1868-1874.

8. Kumar SS, Sujatha C, Syamala J, Nagasudha B, Mishra SH (2006) Protective effect of root extract of *Operculina turpethum* Linn. against paracetamol-induced hepatotoxicity in rats. Ind J Pharm Sci 68(1): 32-35.

9. Sharma V, Singh M (2014) Enumerating nephroprotective potency of ethanolic root extract of *Operculina turpethum* against N-Nitrosodimethylamine incited renal carcinogenesis in swiss albino mice. Int J Drug Dev & Res 6(4): 54-64.

10. Ezeja MI, Onoja SO, Omeh YN, Chibiko CA (2015) Analgesic and antioxidant activities of the methanolic extract of *Operculina turpethum* leaves in mice. Int J Basic & Clin Pharmacol 4(3): 453-457.

11. Alam MJ, Alam I, Sharmin SA, Rahman MM, Anisuzzaman M, et al. (2010) Micropropagation and antimicrobial activity of *Operculina turpethum* (syn. Ipomoea *turpethum*), an endangered medicinal plant. Plant Omics 3(2): 40.

12. Ignatius V, Narayanan M, Subramanian V, Periyasamy BM (2013) Antiulcer activity of indigenous plant *Operculina turpethum* Linn. Evidence-Based Complement and Alternat Med, pp: 1-7.

13. Pulipaka S, Challa SR, Pingili RB (2012) Comparative antidiabetic activity of methanolic extract of *Operculina turpethum* stem and root against healthy and streptozotocin induced diabetic rats. Int Curr Pharm J 1(9): 272-278.

14. Sharma V, Singh M (2013) *In vitro* antiarthritic and hemolysis preventive: membrane stabilizing efficacy

of ethanolic root extract of *Operculina turpethum*. World J Pharm and Pharm Sci 2(1): 302-312.

15. Shareef H, Rizwani GH, Mandukhail SR, Watanabe N, Gilani AH (2014) Studies on antidiarrhoeal, antispasmodic and bronchodilator activities of *Operculina turpethum* Linn. BMC Complement and Alternat Med 14(1): 479.

16. Bhande RM, Laakshmayya, Kumar P, Mahurkar NK, Setty SR (2006) Pharmacological screening of root of *Operculina turpethum* and its formulations. Acta Pharmaceutica Sciencia 48: 11-17.

17. Dasari P, Chennamaneni SR, Reddy H, Challa SR (2012) Neuropharmacological effects of aqueous and ethanolic extracts from the roots of *Operculina turpethum* in swiss albino mice. J Adv Pharm Sci 2(2): 306-314.

18. TamizhMozhi M, Nagavalli D (2017) Immunomodulatory activity of Formonoetin-7-O-β-D-glucopyranoside isolated from methanolic extract of *Operculina turpethum*. Int J Chem Tech Res 10(9): 356-364.

19. Pulipaka S, Anasuri S, Kumar MR, Mahapatra BB (2018) Phytochemical investigation and antihelmintic activity of *Operculina turpethum* roots. J Pharmacy Research 12(1): 90-94.

20. Onoja SO, Madubuike GK, Ezeja MI, Chukwu C (2015) Investigation of the laxative activity of *Operculina turpethum* extract in mice. Int J Pharm and Clini Res 7(4): 275-279.

21. Sharma H, Chandola HM (2014) Ayurvedic approach to food and dietary supplements for the brain and neurologic health. In book: Bioactive Nutraceuticals and Dietary supplements in Neurological and Brain Disease, prevention and therapy, edited by Ronald Ross Watson, Victor R. Preedy, Academic press, pp: 173-177.

22. Kokate CK (1994) Practical Pharmacognosy. 4th (Edn.), Vallabh Prakashan, New Delhi, India, pp: 107-109.

23. Patel NB, Galani VJ, Patel BG (2011) Antistress activity of *Argyreia speciosa* roots in experimental animals. J Ayu & Integrative Med 2(3): 129-136.

24. Richmond W (1974) Proceedings: The development of an enzymatic technique for the assay of cholesterol in biological fluids. Clin Sci Mol Med 46(1): 6-7.

25. Cole TG, Klotzsch SG, Namara J (1997) Measurement of triglyceride concentration. In: Rifai N, Warnick GR, Dominiczak MH, editors. Handbook of Lipoprotein testing. Washington, AACC Press, pp: 115-126.

26. Richardson T (1977) A modification of the Trinder autoanalyzer method for glucose. Ann Clin Biochem 14(4): 223-226.

27. Peters T (1968) Proposals for standardization of total protein assays. Clin Chem 14(12): 1147-1159.

28. Glick D, Vonredlich D, Levine S (1964) Fluorometric determination of corticosterone and cortisol in 0.02-0.05 milliliters of plasma or submilligram samples of adrenal tissue. Endocrinology 74: 653-655.

29. Jacobowitz DM, Richardson JS (1978) Method for the rapid determination of norepinephrine, dopamine, and serotonin in the same brain region. Pharmacol Biochem Behav 8(5): 515-519.

30. Bishayee A, Chatterje M (1995) Mechanism of antistress activity of *Mikania cardata* root extract in albino mice. Pharm Biol 33(3): 215-221.

31. Yeap SK, Beh BK, Ali NM, Yusof HM, Ho WY, et al. (2015) Antistress and antioxidant effects of virgin coconut oil *in vivo*. Exp Ther Med 9(1): 39-42.

32. Umukoro S, Aladeokin AC (2010) Evaluation of antistress and anticonvulsant activities of leaf extract of *Alchornea cordifolia* in mice. J Ethanopharmacol 127(3): 768-770.

33. Bhattacharya SK, Muruganandam AV (2003) Adaptogenic activity of *Withania somnifera*: An experimental study using a rat model of chronic stress. Pharmacol Biochem Behav 75(3): 547-555.

34. Singh RH, Udupa KN (1993) Clinical and experimental studies on Rasayana drugs and Resayana therapy. New Delhi; Central council for research in Ayurveda and Siddha, Ministry of Health and Family Welfare.

35. Taché Y, Du Ruisseau P, Taché J, Selye H, Collu R (1976) Shift in adenohypophyseal activity during chronic intermittent immobilization of rats. Neuroendocrinology 22(4): 325-336.

36. Nisenbaum LK, Abercrombie ED (1993) Presynaptic alteration associated with enhancement of evoked release and synthesis of nor-epinephrine in

hippocampus of chronically cold stressed rats. Brain Research 608(2): 280-287.

37. Fujino K, Yoshitake T, Inoue O, Ibii N, Kehr J, et al. (2002) Increased serotonin release in mice frontal cortex and hippocampus induced by acute physiological stressors. Neuroscience Letters 320(1): 91-95.

38. Von Eular US (1964) Quantitation of stress by catecholamine analysis. Clinical Pharmacol Ther 5(4): 398-404.

39. Leduc J (1961) Catecholamine production and release in exposure and acclimation to cold. Acta Physiolo Scand 53(suppl.183): 1-101.

40. Zigmond DWJ, Harvey JA (1970) Resistance to central noradrenaline, depletion and decreased mortality in rats chronically exposed to electric foot shcok. J Neurovisc Relat 31(4): 373-81.

41. Pacak K, Palkovits M, Kvetnansky R, Yadid G, Kopin IJ, et al. (1995) Effects of various stressors on in vivo norepinephrine release in the hypothalamic paraventricular nucleus and on the pituitary-adrenocortical axis. Ann N Y Acad Sci 771: 115-130.

42. Blane GD, Harve H, Simoon H, Lisoprowski A, Glowinski J, et al. (1980) Response to stress of mesocorticofrontal dopaminergic neurons in rats after long term isolation. Nature 284: 265-267.

43. Reinhard JF, Bannon MJ, Roth RH (1982) Acceleration by stress of dopamine synthesis and metabolism in prefrontal cortex: antagonismby diazepam. Naunyn Schmiedebergs Arch Pharmacol 318(4): 374-377.

44. Konstandi M, Johnson E, Lang MA, Malamas M, Marselos M (2000) Noradrenaline, dopamine, serotonin: different effects of psychological stress on brain biogenic amines in mice and rats. Pharmacol Res 41(3): 341-346.

45. Fernstrom JD, Shabshelowitz H, Faller DV (1974) Diazepam increases 5-hydroxyindole concentrations in rat brain and spinal cord. Life Sciences 15(9): 1577-1584.

46. Ahmad A, Rasheed N, Chand K, Maurya R, Banu N, et al. (2012) Restraint stress-induced central monoaminergic & oxidative changes in rats & their prevention by novel Ocimum sanctum compounds. Indian J Med Res 135(4): 548-554.

47. Vazhayil BK, Rajagopal SS, Thangavelu T, Swaminathan G, Rajagounder E (2017) Neuroprotective effect of *Clerodendrum serratum* Linn. Leaves extract against acute restraint stress-induced depressive-like behavioral symptoms in adult mice. Indian J Pharmacol 49(1): 34-41.