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NEUROPHARMACOLOGICAL SCREENING OF ANTIDEPRESSANT AND ANTISTRESS ACTIVITY OF *ACACIA NILOTICA* LINN LEAVES EXTRACT.

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Abstract:

Objective: *The current study was designed to explore Neuropharmacological screening of antidepressant and antistress activity of leaves extract Acacia Nilotica in albino wistar rat.*

Materials and Methods: *Behavioral procedures of depression and Stress were assessed in rats. A methanolic extract of Acacia Nilotica leaves with dose ranges of 100, 200, and 400 mg/kg was given once a daily for 7 days via oral route and the efficacy was matched by those elicited by lorazepam (1 mg/kg, p.o.), imipramine (10 mg/kg, p.o.) and Withania somnifera (100 mg/kg, p.o.) for antidepressant, and antistress studies, respectively. Standard drugs were given 1 time, 30 min preceding the behavioral trials.* ***Results:*** *One-way analysis of variance followed by Newman–Keuls multiple comparison test was employed to analyze the results. P < 0.05 was considered statistically significant as compared to control. AN at 400 mg/kg also induced an antidepressant activity similar to imipramine, in the behavioral despair, learned helplessness test, and tail suspension among selected doses of the AN. More over AN at 400 mg/kg produced a significant antistress effect comparable to Withania somnifera in water immersion-restraint stress by decreasing ulcer index, adrenal gland weight, and by normalizing the plasma levels of corticosterone, glucose, cholesterol, and triglyceride levels when related to stress control.*

Conclusion: *The study shows that among the different AN dose, AN at 400mg/kg possesses significant antidepressant and anti-stress effects has therapeutic beneficial for the management of psychological ailments.*

Keywords: *Antidepressant, Antistress, Acacia Nilotica, Ulcer index, Withania somnifera*

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INTRODUCTION:

Depression, and stress are the most predominant psychological diseases globally. The estimated range of their occurrence among adolescents across the globe is from 5% to 70%.[1] Implausibly, women are more affected by this unwellness rather than men.[2] Currently, antianxiety, antidepressants, and antistress drugs are the only choice of treatment for these maladies. Regardless of therapeutic benefits, these drugs also cause unusual adverse effects which ensue patient's non compliance. The drugs derived from natural origin barely have side effects and moreover, are economically cheaper.

Plants are considered as a wealthy source of safe and effective medicines for a longtime. India has a great history of herbal medicine practice such as Ayurveda, Unani, Siddha, Homeopathy and Naturopathy. Even today, many rural and tribal populations in India are relying up on the natural plant wealth for healing and mitigation of certain diseases. It was already evidenced that traditional herbal medicine could provide a novel treatment for affective and other central nervous system (CNS) disorders.[3]

Acacia Nilotica or Gum Arabic tree, also known as Babul, Kikar (in Hindi), is an evergreen tree with moderate sized within the *Fabaceae* family. Natural habitat includes the tropical and the subtropical Himalayas and Khasi and Jaintiahills. The leaves are usually called "prickly acacia" leaves"often used as a cardiac stimulant, wound healing, diarrhea, inflammation etc. The AN leaves have been evidenced and reported to exert antidiabetic,[4] antioxidant,[4] antihyperlipidemic,[5]and gastroprotective [6] activities.

The literature survey suggests that the neuropharmacological effects of AN were not studied at the time of conceptualization of this investigation. Therefore, we proposed to explore the antidepressant and anti-stress activity of the AN leaves extract on rodent models and compared the efficacy to standard drugs.

MATERIALS AND METHODS:

Animals

Adult Wistar albino rats of either sex weighing 180 ± 20 g were housed in polypropylene cages in groups of five (evenly matched with male and female rats) and maintained under conditions of temperature (25°C±1°C) and relative humidity (50% ± 5%), under a standard light/dark cycle of 12 h. The animals were provided with a standard pellet diet ("Nutrimix std-1020" brand, Mfg by Nutrivet Life Sciences kurkumbh, Tal.Daund, Pune, India) and water *ad libitum*. Experiments were performed between 09.00 A.M. and 02.00 P.M. Guidelines of laboratory animal care (NIH publication number#85–23, revised in 1985) were exercised. All studies were carried out in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals, India. The experimental protocol was approved by the Central Animal Ethical Committee (No. AUCP/24-04/03).

Plant Material and Preparation of the Extract

Fresh leaves of AN (Family- *Fabaceae*) were gathered in August month from Chittor district region, India, and they were taxonomically identified and authenticated. The leaves were shade dried and powdered with a mechanical grinder and preserved in an air tight vessel. The dried powder material was defatted with petroleum ether and later extracted with distilled water by hot maceration method. The solvent was wholly evaporated, and 9% yield of aqueous extract of AN was acquired. The final yield was kept at 4°C in an air tight vessel till needed. Fresh solutions of AN were prepared in distilled water and used for the investigation.

Drugs

Imipramine (standard antidepressant drug) was a gift sample from Sun Pharma Ltd, India. Lorazepam (reference standard for anti-anxiety effect) was provided by Intas Pharma Ltd, India, as a gift sample. All chemicals used in the present study were of analytical grade and were obtained from Sigma Chemical Co. and Merck. *Withania somnifera*, herbal adaptogen, was used as a standard for antistress activity[7] and was obtained from the Department of Unani college of medicine and research centre, CRIUM, ESI, Erragadda, Telangana, India.

Experimental Protocol

The animals were housed for 1 week in a laboratory room for acclimatization. They were grouped into five, containing six animals each group.

- GroupI: Control (10mL/kg/p.o.)
- GroupII: AN 100mg/kg/p.o.
- GroupIII: AN 200mg/kg/p.o.
- GroupIV: AN 400mg/kg/p.o
- GroupV: Standard drugs (lorazepam 1mg/kg, imipramine 10 mg/kg, and *W. somnifera* 100 mg/kg in the case of antidepressant, and antistress activities, respectively).

In addition, stress control group was included in the anti-stress activity study design to investigate the influence of stress on neurochemical and histopathological changes compared to normal rats.

AN dose was selected on the basis of randomization and was administered in distilled water to Groups (II,

III, and IV) at 100, 200 and 400mg/kg orally, once in a day for 7days. The control group (Group I) received an equal volume of distilled water. Standard drugs were also employed to Group V accordingly in each set of protocol and were dispensed orally to animals 1h before the experiments. On day 7, animals were subjected to behavioral studies.

Behavioral Screening

Elevated plus maze -

The apparatus for elevated plus-maze test contained 2 open arms (50cm×10cm×40cm) and 2 closed arms (50 cm × 10 cm × 40 cm), for rats facing each other with an open roof that extends from a common central platform (10 cm × 10 cm).The maze was kept in a dimly lit room and elevated at a height of 50cm above the ground level. The rats were positioned in the center of the maze independently, facing one of the closed arms. Thereafter, a number of entries and time spent on the open and closed arms were documented throughout the 5 min observation period. An arm entry was defined when four paws of the rats were inside the arm. A neutral "blind" observer made observation. [8]

Open field test-

The open field test apparatus was built of plywood (60cm×60cm×60cm) and was painted black. White marks of 6mm wide divide the floor in to sixteen squares (15cm×15cm). The open field was lit with 16W bulb focusing on to the arena from a height of 100 cm excluding the open field; the whole room was kept dark throughout the experiment. Animals were positioned individually at the center of the test apparatus for 5min, and the following behavioral aspects were observed:[9]

- Ambulation: Assessed in terms of the number of squares traversed by the rat
- Rearings: Measured in terms of the number of times the rat raised on its rear limbs
- Self-grooming: Measured in terms of the number of times The rat cleaned facial region and licked different body parts
- Activity in center: Measured in terms of the number of Central squares traversed by the rat
- Fecal dropping: Measured in terms of the number of fecal Droppings excreted during the observation.

Social interaction test-

The rodents were individually accommodated for 5 days prior investigation. The test device consists of a wooden box (60cm×60cm×35cm) located in a faintly illuminated room.

On the 6th day, the rodents were individually positioned in the wooden box and offered two 7.5min familiarization sessions at 2 h time period. On the 7th day, based on sex and weight, rodents were paired and retained in the apparatus for 7.5min. The total time devoted by the rodent pair in "social interaction," included sniffing, biting, grooming, boxing, kicking, and crawling under or over the partner, was documented during 7.5min time, by a neutral blind observer.[10]

Antidepressant activity-

Behavioral despair test

Each animal was restrained in a cylinder (45cm×20cm) filled with water at a height of 38 cm (25°C ± 2°C) so that it could not contact with the bottom of the cylinder with its rear paws or mount over the edge of the cylinder. Dual swim sessions were performed, an early 15 min pretest followed by a 5 min test after 24h of drug administration. During the test session, the immobility period characterized by absolute cessation of swimming and performing necessary movement's essential to place its head beyond the aquatic level was observed. [11]

Tail suspension test

Each rat in the group was hanged by the tail (50 cm above the floor) with an adhesive tape to a cord in an upside-down position so that its nostrils touch the water surface in a vessel. After the early escape-oriented actions, the rat rapidly turns out to be immobile, and the immobility period (the absence of initiating movements and includes passive swaying) was recorded during 5 min observation period.[12]

Learned helplessness test

Rats were exposed to a shock of 0.7 mA for 10 s every minute for 1h. The gadget was a box with dimensions of 30cm×45cm×30cm having grid floor. At an altitude of 20 cm above the base, a platform (7.5 cm × 7.5 cm) was introduced from one side wall to permit a jump-up escape reaction.The platform was not provided for the whole period of training. After the proper treatment, the rats were allowed for acquisition of a jump-up response. At the beginning of a trial, the platform was provided in the box, and a current of 0.7mA was introduced. The shock was ceased in 10s if the rat couldn't escape on to the platform within the specified period. If an escape retort ensued, the rat was permitted to stand on the platform for the length of 10s, and then reverted to the base.[13]

Anti-stress activity-

Water immersion-restraint stress

The rats were deprived of food for 24 h before the stress application. Rats were restrained in iron net and submerged upright to the level of the xiphoid in a water bath maintained at 20°C,for3.5h.[14] At the end of the experiment, 5mL of blood was collected in to

centrifuge tubes containing heparin (10µL, 1000 IU/mL) by cardiac puncture after anesthetizing animals with diethyl ether. All animals were sacrificed by cervical dislocation. Then, their stomachs were isolated and expanded slightly 10 min after injecting 15% formalin. Later, the stomachs were incised alongside the superior curvature and ulcer scoring was done using dissecting microscope bearing square grid-eyepiece. The isolated stomachs were held in 15% formalin solution and then forwarded for histopathological scrutiny. Adrenal glands were collected and their weights were recorded as well.

Biochemical Parameters

Collection of samples

For biochemical estimation, blood was collected in heparin spilt Eppendorf tubes by cardiac puncture before sacrificing the rats. The collected blood was centrifuged at 4°C and 4000rpm, for 15min, to separate plasma. Thus, separated plasma was stored in−80°C till the day of biochemical estimation. Animals were sacrificed and adrenal glands were gathered, weighed, and preserved at −80°C.

Estimation of plasma corticosterone

Rat plasma corticosterone levels were measured by high- performance liquid chromatography (HPLC)/ultraviolet (Waters, USA) according to Woodward and Emery[15] with slight changes. Dexamethasone was used as an internal standard. Five hundred microliters of plasma comprising a 50 µL of dexamethasone was extracted with 5mL of dichloromethane. The dichloromethane extract was allowed to dry and dissolved in 100 µL of the mobile phase. Twenty microliters of the sample was inserted in to the HPLC for quantification purpose. Mobile phase contained methanol and water in the ratio of 70:30. The flow rate was 1.0mL/min and analytical column used was Waters Spherisorb®C 18 (250mm×4.6mm,5µm). Plasma corticosterone was identified at 250nm wavelength by photodiode array detector (Model2998, Waters, USA).The chromatogram was documented and evaluated by "Empower" software.

Estimation of plasma triglycerides

Plasma triglyceride level was estimated by autoanalyzer (BioMasteR touch screen biochemistry analyzer, Qualisystems, Fisher Scientific, Mumbai) using the commercially available diagnostic kit (Span Diagnostics Ltd., Surat, India).

Estimation of plasma cholesterol

Plasma cholesterol was estimated by autoanalyzer (BioMasteR touch screen biochemistry analyzer, Qualisystems, Fisher Scientific, Mumbai) using the commercially available diagnostic kit (Span Diagnostics Ltd., Surat, India).

Estimation of plasma glucose

Plasma glucose was estimated by auto analyzer (BioMasteR touch screen biochemistry analyzer, Qualisystems, Fisher Scientific, Mumbai) employing commercially available diagnostic kit (Span Diagnostics Ltd, Surat, India) by glucose oxidase–peroxidase method.

Statistical Analysis

Results of this study were statistically analyzed by GraphPad Prism 5 software. The data were expressed as a mean ± standard error of mean. The data from various groups were statistically analyzed using one-way analysis of variance, followed by *post hoc* Tukey's multiple comparisons test. $P < 0.05$ was considered statistically significant.

RESULTS:

Effect of the *Acacia Nilotica* Extract on Elevated Plus Maze

AN (200 and 400 mg/kg) treated rats exhibited a significant increase in entries made ($P = 0.0021$, $P < 0.001$) and time spent in open arms (P=0.0063, P<0.001) except at 100mg/kg (P>0.05). AN -treated rats exhibited a significant decrease in entries made (P<0.001, P<0.001) and time spent (P=0.0218, P<0.001) in enclosed arms at 200and400mg/kg, respectively. Lorazepam ($P < 0.001$) also produced significant anxiolysis, and its effect was qualitatively comparable to that of AN. The results were summarized in Table 1.

Effect of the *Acacia Nilotica* Extract on Open Field Test

Rats treated with doses (100,200,and400mg/kg) of AN showed a significant increase in open field ambulation (P=0.0221, P<0.001,and$P$<0.001), rearing (P>0.05,P<0.001,and P<0.001), activity in center (P>0.05, P>0.05, and P=0.0041), and self-grooming ($P < 0.001$, $P < 0.001$, and $P < 0.001$) when compared to vehicle-treated rats and produced comparable activity with lorazepam; indicating depressant activity of AN. Simultaneously, a significant decrease in fecal droppings was observed at all doses of AN, except at 200 mg/kg ($P > 0.05$). Lorazepam ($P < 0.001$) also induced significant depressant activity and its effect was also found to be qualitatively comparable to that of AN. The results were summarized in Table 2.

Effect of the *Acacia Nilotica* Extract on Social Interaction Test

Rats treated with AN spent significantly more time in social interaction as compared to control rats in a dose-dependent wise (P<0.001). Similarly, lorazepam-treated group exhibited a significant increase in social

interaction (P<0.001) in rats and its effect was comparable to AN at all doses. The results were shown in Table 3.

Effect of the *Acacia Nilotica* Extract on Behavioral Despair Test

AN at 100mg(P=0.013), 200mg(P<0.001), and 400mg/kg(P<0.001) treated rats caused a significant dose-dependent decrease in immobility time. Imipramine also showed similar activity and effects were qualitatively comparable to that of AN (P<0.001). The results were summarized in Table 4.

Effect of the *Acacia Nilotica* Extract on Tail Suspension Test in Rats

AN at 100(P=0.017), 200(P<0.001) and 400mg/kg (P<0.001) treated rats significantly reduced the immobility time dose- dependently, and AN at 400 mg/kg (P > 0.05) produced the equivalent effect as that of imipramine, a well-established antidepressant drug, which showed a significant reduction in immobility time. The results were summarized in Table 4.

Effect of the *Acacia Nilotica* Extract on Learned Helplessness Test

The escape failure significantly decreased in rats treated with the dose of AN 400mg/kg(P<0.001). In addition, the number of avoidance response also significantly increased, respectively, with the same dose of AN (P < 0.001). Imipramine (P <0.001) also showed significant reduction of learned helplessness and its effect was qualitatively comparable to that of AN at 400 mg/kg. The results were summarized in Table 4.

Effect of the *Acacia Nilotica* Extract on the Ulcer Index in Water-immersion Restraint Stress Test

Stress increases the ulcer index (P < 0.001) and treatment with AN at 200 mg/kg (P < 0.001) and 400 mg/kg (P < 0.001) significantly decreases the ulcer index through its antistress effect. *Withania. somnifera* at 100 mg/kg significantly decreased the ulcer index (P < 0.001). The results were shown in Table 5.

Effect of the AN on the Weight of the Adrenal Gland and Plasma Corticosterone Levels

Stress increased the weight of the adrenal gland (P<0.001) and plasma corticosterone levels (P < 0.001) and treatment with AN significantly decreased both weight of the adrenal gland (P < 0.001) at all doses, but reduced plasma corticosterone levels at 200 and 400mg/kg (P<0.001), comparable to the *Withania. somnifera* significant reduction. The results were shown in Table 5.

Effect of the *Acacia Nilotica* Extract on Plasma Glucose

Stress increased the plasma glucose levels (P<0.001), whereas AN -treated rats showed the significant decrease in plasma. Glucose levels at all doses (P<0.001), comparable to the effect of *W.somnifera* (P<0.001). The results were shown in Table5.

Effect of the *Acacia Nilotica* Extract on Plasma Cholesterol

Stress increased the plasma cholesterol levels (P < 0.001), whereas AN significantly decreased plasma cholesterol levels at 100(P=0.013), 200(P<0.001), and 400mg/kg (P<0.001),

Comparable to the effect of *W.somnifera* (P<0.001).Theresults were shown in Table 5.

Effect of the Acacia Nilotica Extract on Plasma Triglycerides

Stress increased the plasma triglyceride levels (P < 0.001), whereas AN -treated rats showed a significant decrease in plasma triglyceride levels at 200 (P = 0.018) and 400 mg/kg (P<0.001), comparable to the effect of W.somnifera(P<0.001).The results were shown in Table 5.

Table1: Effect of the Acacia Nilotica extract on elevated plus-maze test in rats

Treatment	Time spent on (s)		Entries on	
	Enclosed arms	Open arms	Enclosed arms	Open arms
Control	235.36±3.56	47.42 ± 1.73	2.96 ± 0.07	2.33±0.33
AN (100mg/kg)	226.19±4.32	41.33 ± 1.11	2.92 ± 0.08*	2.42±0.22
AN (200mg/kg)	201.93±3.53*	61.47± 2.33*,$	2.38 ± 0.04*,$	3.47±0.20*,$
AN (400mg/kg)	188.69±3.49*,$	80.56± 3.04*,$,#	1.95 ± 0.02*,$,#	4.77±0.14*,$,#
Lorazepam (1mg/kg)	80.05±2.53*,$,#,¥	95.02± 2.97*,$,#,¥	2.04 ± 0.03*,$,#	5.01±0.24*,$,#

Values are expressed as mean ±SEM (n=6).*P<0.05 significant as compared to vehicle,$$P$<0.05 significant as compared to AN (100mg/kg),#P<0.05 significant as compared to AN (200 mg/kg), ¥P<0.05 significant as compared to AN (400 mg/kg), statistical test employed is one-way ANOVA followed by Tukey's multiple comparisons test. AN: *Acacia Nilotica*, SEM: Standard error of mean, ANOVA: Analysis of variance

Table2: Effect of the Acacia Nilotica extract on open field test in rats

Treatment	Ambulation	Rearings	Self-groomings	Activity in center	Fecal droppings
Control(vehicle)	34.22±2.04	7.77±1.56	2.89±0.17	0.18±0.13	4.08±0.33
AN (100mg/kg)	39.15±1.98*	14.84±2.04	5.04±0.32*	1.97±0.25	3.67±0.06*
AN (200mg/kg)	43.87±1.26*,$	18.42±1.92*	4.87±0.62*,$	1.43±0.16	2.88±0.27
AN (400mg/kg)	57.23±1.21*,$	28.03±1.86*,$,#	7.12±0.29*,$,#	4.02±0.77*	2.07±0.02*,$,#
Lorazepam(1mg/kg)	55.03±1.06*,$,#,¥	28.52±2.39*,$,#	5.72±0.22*,$,#,¥	6.02±0.44*,$	2.22±0.13*,$,#,¥

Values are expressed as mean ±SEM (*n*=6).**P*<0.05 significant as compared to vehicle,$*P*<0.05 significant as compared to AN (100mg/kg),#*P*<0.05 significant as compared to AN (200 mg/kg), ¥*P*<0.05 significant as compared to AN (400 mg/kg), statistical test employed is one-way ANOVA followed by Tukey's multiple comparisons test. AN: *Acacia Nilotica*, SEM: Standard error of mean, ANOVA: Analysis of variance

Table 3: Effect of the Acacia Nilotica extract on social interaction test in rats

Treatment Social interaction time(s)

Control(vehicle): 61.93±1.98
AN (100mg/kg): 94.03±2.07*
AN (200mg/kg): 110.46±2.12*,$
AN (400mg/kg): 152.32±1.85*,$,#
Lorazepam (1mg/kg): 163.5±1.23*,$,#,¥

Values are expressed as mean ±SEM(*n*=6).**P*<0.05significant as compared to vehicle, $*P*<0.05 significant as compared to AN (100 mg/kg); #*P*<0.05 significant as compared to AN (200 mg/kg), ¥*P*<0.05 significant as compared to AN (400 mg/kg), statistical test employed is one-way ANOVA followed by Tukey's multiple comparisons test. AN: *Acacia Nilotica* SEM: Standard error of mean, ANOVA: Analysis of variance

Table 4: Effect of the Acacia Nilotica extract on behavioral despair test, tail suspension test, and learned helplessness test in rats

Immobility time(s) Learned

Groups	Behavioral despair test	Tail suspension test		Escape failure	Avoidance response
Control(vehicle)	107.50±1.92	135.83±4.95		20.33±1.08	8.66±1.08
AN (100mg/kg)	95.83±2.16*	115.11±5.36*		19.50±0.56	10.50±0.56
AN (200mg/kg)	87.33±2.63*	100.17±1.15*		18.16±0.70	11.83±0.70
AN (400mg/kg)	65.50±2.59*,$,#	84.16±4.13*,$		1.33±0.33*,$,#	18.66±0.55*,$,#
Imipramine (10mg/kg)	49.16±2.30*,$,#,¥	81.83±4.60*,$,#		0.66±0.88*,$,#	19.66±1.16*,$,#

Values are expressed as mean±SEM (*n*=6).**P*<0.05 significant as compared to vehicle,$*P*<0.05 significant as compared to AN (100mg/kg);#*P*<0.05 significant as compared to AN (200 mg/kg), ¥*P*<0.05 significant as compared to AN (400 mg/kg), statistical test employed is one-way ANOVA followed by Tukey's multiple comparisons test. AN: *Acacia Nilotica*, SEM: Standard error of mean, ANOVA: Analysis of variance

Table5: Effect of the Acacia Nilotica extract on the ulcer index, adrenal gland weight, plasma corticosterone, glucose, cholesterol, and triglycerides in rats

Groups	Ulcer Index	Adrenal gland weight (mg/100g)	Plasma corticosterone (ng/mL)	Plasma glucose(mg/dL)	Plasma cholesterol(mg/dL)	Plasma triglycerides(mg/dL)
Control(vehicle)	0	9.1±1.02	98.92±4.55	86.71±4.64	84.66±5.63	62.67±4.47
Stressc ontrol(vehicle)	21.95±1.11*	16.38±1.36*	173.16±8.53*	204.97±8.48*	172.05±10.75*	97.56±5.63*
AN (100mg/kg)	16.67±1.46*	13.54±0.61*,**	154.92±4.67*	144.12±6.41*,**	136.46±4.44*,**	88.86±5.54*
AN (200mg/kg)	17.03±1.17*,**	13.01±0.44*,**	132.71±3.44*,**,$	122.17±5.53*,**	117.54±3.55*,**	76.59±4.52**
AN (400mg/kg)	08.54±1.31*,**,$,#	9.88±0.78**,$,#	109.49±3.52**,$,#	110.34±3.77**,$	101.11±3.34**,$	70.24±2.75**,$
Withaniasomnifera (100mg/kg)	03.51±0.96**,$,#,¥	9.77±0.67**,$,#	103.53±2.77**,$,#	99.88±2.34**,$,#	94.44±3.44**,$,#	65.22±2.45**,$

Values are expressed as mean±SEM (n=6).*P<0.05 significant as compared to vehicle, $$P$<0.05 significant as compared to AN (100mg/kg);#P<0.05significant as compared to AN (200 mg/kg), **P<0.05 significant as compared to Stress control, ¥P<0.05 significant as compared to AN (400 mg/kg), statistical test employed is one-way ANOVA followed by Tukey's multiple comparisons test. AN: *Acacia Nilotica*, SEM: Standard error of mean, ANOVA: Analysis of variance

Discussion

The present results of these experiments show for the first time that aqueous extract of AN leaves possesses antidepressant, and anti-stress effects following long-term administration to rats.

Antidepressant Activity

Both the open field test and elevated plus-maze tests are used to evaluate neurobehavioral screening profiles of rodents under conditions of anxiety.[16]In the open field test, rodents are exposed to an unusual background, where they express fear and anxiety in terms of increased defecation and decreased activities such as rearing, ambulation, and self-grooming along with less activity in the center. Similarly, in elevated plus- maze test, animals upon exposure to an open arm causes an approach conflict that is significantly robust than the response elicited by exposure to an enclosed arm of the maze. Thus, open/enclosed arms entries and time spent in respective arms provide a measure of fear provoked suppression of exploratory action. Generally, classical depressant/anxiety antagonize these behavioral changes,[16] and benzodiazepines are used to validate the depressant activity.[17]

The results obtained from elevatedplus-maze test demonstrate that AN at 200 mg/kg and 400 mg/kg significantly increased the open arm entries and time spent in open arm. Similarly, in the open field test, AN at 400mg/kg significantly increased ambulation, self-grooming, rearing, and increased activity at center as well as significantly decreased fecal droppings compared to lorazepam at 1mg/kg. Furthermore, antianxiety drugs increase the social interaction time in a new environment.[16] AN at 400mg/kg significantly prolonged the social interaction time and an equal effect was recorded with lorazepam at 1 mg/kg. These behavioral changes are indicative of diminished anxiety and confirm the antidepressant activities of AN.

Learned helplessness[13]and behavioral despair test[11]are the two most persuasive and commonly used *in vivo* animal models of depression. In the learned helplessness test, rodents are exposed to in escapable and unavoidable electric shocks in one circumstance and later fail to escape a shock in another circumstance when escape is feasible. If the number of failures to escape is lessened and learned helplessness is decreased, then the drug is assumed to be effective in depression. Likewise, in behavioral despair test, rodents are obliged to swim in a confined area from which they unable to escape and exhibit a distinctive rigidity[11]. In general, antidepressants can abolish or reduce this state of despair.

In the learned helplessness test, AN at 400 mg/kg found to produce a significant decline in a number of escape failures and a significant rise in avoidance response, which is comparable to imipramine antidepressant activity. Similarly, AN produced a dose-dependent reduction in time of immobility in despair test. However, the results obtained in the tail

suspension test provide an additional measure for assessing antidepressant activity and AN at 400mg/kg significantly decreased immobility time in this test also. The results present edhere show that AN given orally is effective in producing significant antidepressant-like effects.

Antistress Activity

Stress is an adaptive physiological response to dysregulation of homeostasis. Stress consists of diverse, intricate neurological, biochemical, and immunological pathways and has been involved in the etiopathogenesis of numerous disease conditions such as anxiety, depression, and cognitive dysfunction.[18] Stress-induced gastric ulcer is a notable example of stress-associated visceral injuries and considered as an indication of stress syndrome.[19]

Water-immersion restraint stress (WIRS) simulates the clinical condition of acute gastric ulcerations, and our results showed that WIRS caused significant gastric ulceration in rats. Furthermore, AN significantly mitigated WIRS-induced ulcer index [Figure 1]. The observed gastroprotective effect of AN extract might be due to its antioxidant activity.[6]

It was reported that stress increases adrenal gland weight and plasma corticosterone levels owing to the stimulation of adrenomedullary response[20] and hypothalamic–pituitary– adrenocortical axis,[18] respectively. Furthermore, elevated corticosterone has a deleterious effect on lipid and glucose metabolism. In our study, stress treatment increased the adrenal gland weight, plasma corticosterone, glucose, cholesterol, and triglyceride levels, whereas prior administration of AN treatment significantly (P<0.05) normalized those parameters. These results indicate that AN effectively suppressed the stress- induced hormonal and metabolic alterations and preserved the homeostatic mechanism.

The present experimental data could not reveal the mode of action through AN -induced antidepressant, and anti-stress actions.The pharmacological effects of AN noticed in this investigation might be credited to plant's phytoconstituents such as linalool, p-cymene, eugenol, cinnamic aldehyde, alpha- and beta-pinene, 3,3,4,5,7-pentahydroxyflavone, kapeferol-3-o-glucopyranoside, kaempferol-3-o-saphoroside, andquercetin-3-o-rutenoside[21] have been reported previously from this plant. Singh *et al*. have isolated the flavonoids such as kaempferol, myricetin, quercetin, kaempferol-3-O- rhamnoside, and quercitrin from the leaves of AN.[22] It has been demonstrated that linalool,[23,24] kaempferol,[25] and quercetin[25] exert significant anxiety/depressant actions in animals, as well as in human beings. It is uncertain whether the reported CNS beneficial actions occur as a result of individual stimulation of neuronal regions either by a single constituent or by the synergistic effect of different phytoconstituents of this plant. Therefore, further neurochemical and pharmacological studies are necessary to establish the mechanism of action of AN.

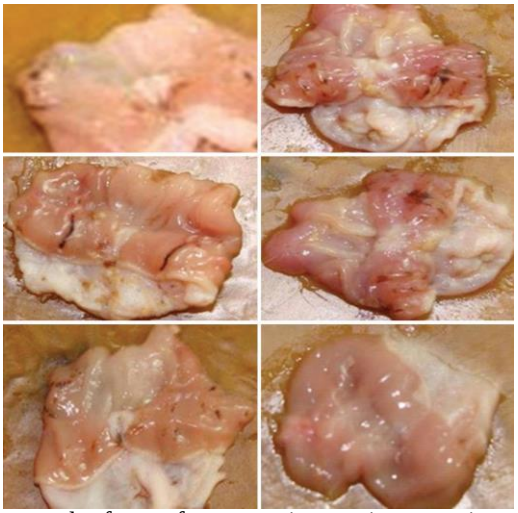

Figure1: Images of stomach of rats after water-immersion restraint stress.

(a) Control rats showing normal mucosa. (b) Water immersion restraint rats showing mucosal ulceration. (c) *Acacia Nilotica* 100mg/kg treated rats showing mucosal ulceration. (d) *Acacia Nilotica* 200 mg/kg treated rats showing focal mucosal ulceration. (e) *Acacia Nilotica* 400mg/kg treated rats showing intact mucosa with mild ulceration. (f) *Withania somnifera* 100 mg/kg treated rats showing normal mucosa

CONCLUSION:

The investigation demonstrated that AN at 400 mg/kg possesses significant antidepressant, and antistress effects and is therapeutically beneficial for the management of psychological ailments.

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