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ANTISTRESS EFFECTS OF THE LEAVES AQUEOUS EXTRACT OF *MORINGA OLEIFERA* IN RATS SUBJECTED TO ACUTE AND CHRONIC ANIMAL MODEL OF STRESS

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ABSTRACT

Aims: This study aimed to investigate the antistress properties of the leaves aqueous extract of *Moringa oleifera* in rats subjected to acute and chronic stress models. ***Methods:*** For this purpose, fifty five rats (173-175 g) were divided randomly into eleven groups of five rats each. Six groups were used to evaluate the effect of extract in acute stress model, and five remaining groups were used for the evaluation of this effect in chronic stress. The antistress effect of *M. oleifera* extract was tested at dose levels of 100, 200 and 400 mg/kg, orally and compared with diazepam (1 mg/kg) as standard. Rats were subjected to acute and chronic animal model of stress for 14 days, after which behaviors scored, gastric lesions, oxidative stress parameters in stomach and serum biochemical parameters were measured. In addition, food and water intake and body weights were determined. ***Results:*** The administration of *M. oleifera* extract had no significant effect on body weight, food and water intake as compared to respective stress control groups ($p>0.05$) in acute and chronic stress model. However, significant modifications of behavioral parameters, gastric lesions, oxidative stress parameters and serum biochemical parameters in extract-treated groups as compare to their respective control groups ($p<0.05$-$p<0.001$) were observed. ***Conclusion***: results suggest that *M. oleifera* extract significantly prevented the dysfunctions induced by acute or chronic stressors.

KEYWORDS: *Moringa Oleifera*, Chronic Stress, Acute Stress, Antistress.

INTRODUCTION

Stress is considered to be any condition (real or perceived) which results in perturbation of body's homeostasis.[1] Generally, it is accepted that Homeostasis is re-establish by a complex repertoire of behavioral and physiological adaptive response of the organism. However, inadequate or excessive and/or prolonged activation of stress systems can disturb normal physiological and behavioral function.[2] This can lead to a range of adverse consequences such as peptic ulcer, chronic fatigue, metabolic disorder, hypertension, osteoporosis, diabetes, immunosuppression, reproductive and growth dysfunctions, behavior dysfunction (anxiety and depression) and reduced life expectancy. These consequences are due to the involvement of central nervous system, endocrine system and metabolic system.[3] Amphetamine, benzodiazepines, caffeine and anabolic steroids are the most widely used drugs by people to combat stress.[4] The incidence of toxicity and dependence had limited the therapeutic usefulness of these drugs. In fact, those drugs have not proved effective against chronic stress and induced adverse effects on immunity and behavior cognition.[5] Hence it is imperative to find out drugs with minimum side effect and effective adaptogenic activity.

Moringa oleifera is a plant of Indian origin but which is now widespread in Asia and Africa. It belongs to the Family Moringaceae with about 13 species.[6] It is commonly referred to as 'tree of life', 'miracle tree' or 'divine plant' due to its numerous nutritive, medicinal and industrial potentials.[7] Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, β-carotene, amino and various phenolic acids.[9] Various therapeutic potentials are also credited to different parts of the plant. It is reported to have antitumor, hepatoprotective, analgesic, antispasmodic, antipyretic, antiulcer, diuretic, hypotensive, hypolipidemic and antimicrobial activities. The leaves are widely consumed as a legume and used in traditional medicine in Africa in general and in Cameroon in particular. They are an excellent source of protein, metabolisable energy, vitamins (A, B, C, E,), minerals, and contain the 10 amino acids essential to man.[9] *M. oleifera* leaves are used in traditional medicine to treat malnutrition, fever, headaches, nerve pain, diabetes and many other diseases.[10] This study is to investigate antistress effects of the aqueous leaves extract of *M. oleifera* in rats subjected to acute and chronic animal model of stress.

METHODS

Plant material and preparation of aqueous leaf extract of *M. oleifera*

The fresh leaves of *M. oleifera* were collected harvested from the North Region of Cameroon in December 2014 and identified in the National Herbarium (Yaoundé) where a voucher specimen No. 49178/HNC exist. The leaves were cleaned immediately after harvest, cut into small pieces, and dried in the shade for about 2 weeks. The dried material was ground into a powder using an electrical homogenizer (Zaiba®).The aqueous extract was prepared as described by Thilza *et al.*[11] 100g of ground plant material was macerated in 1.5 l of boiled distilled water for one hour. The mixture was filtered through Whatman filter paper N^{o} 3 and the filtrate obtained was evaporated to dryness using a rotator evaporator at 45°C. The extract obtained (22.9% yield) was stored at 4°C. Extract solution was prepared in distilled water each time prior to experimentation.

Phytochemical Tests: Phytochemical tests for the major metabolites of the extract were performed using standard protocols.[12] The aqueous extract of *M. oleifera* was screened for the presence of biologically active compounds such as tannins, alkaloids, saponins, flavonoids, anthocyanins, phenols, quinones, coumarins, sterols, triterpenoids, glycosids and proteins. Based on the intensity of coloration, the lather or the precipitate formed during the test, secondary metabolite proportions were characterized as present (++) or weakly present (+) when the test result was positive, and absent (−) when the test result was negative.

Animals: Male albino rats of Wistar strain weighing 173-175 g were obtained from the Animal house of the National Institute of Youth and Sports in Yaoundé. They were placed in plastic cages in a room under standard Laboratory conditions (temperature 20 to 30°C, relative air humidity 45 to 55%, and 12/12 h light/dark cycle). The rats were fed with a basal diet and water adlibitum. The feed was a standard rat chow (National Veterinary Laboratory (LANAVET), Cameroon) composed of carbohydrates (52%), protein (22%), fat (6.5%), water (12%), ash (6%), and fiber (4.5%). The authorization for the use of laboratory animals in this study was obtained from the Cameroun National Ethics committee (Reg. No FWA-IRB00001954). The use, handling and care of animals were done in adherence to the European Convention (Strasbourg, 18.III.1986) for the protection of vertebrate animals used for experimental and other purposes (ETS-123), with particular attention to Part III, articles 7, 8 and 9. The animals were transferred to the laboratory at least 1 hour before the start of the experiment. The experiments were performed during the day (11:00-17:00hr).

Animals grouping and experimental model for antistress activity

After two weeks of acclimatization, fifty five rats were divided randomly into eleven groups of five rats each. Six groups were used to evaluate the effect of extract in acute stress model, and five remaining groups were used for the evaluation of chronic stress.

Preparation of animals for the acute stress: group 1: a non-stress control group (NSCG) in which rats were kept undisturbed, group 2: a stress control group (SCG) which received the vehicle (distilled water) only, group 3: positive control group (PCG) which receive diazepam (1mg/kg), three treatment groups (group 4, 5 and 6) which received 100, 200 and 400 mg/kg, respectively of *M. oleifera* extract orally once a day for 14 days.

Preparation of animals for the chronic stress: group 1: a stress control group (SCG) which received the vehicle (distilled water) only, group 2: positive control group (PCG) which receive diazepam (1mg/kg), three treatment groups (group 3, 4 and 5) which received 100, 200 and 400 mg/kg, respectively of *M. oleifera* extract orally once a day for 14 days.

The quantity of food and water consumed by each group of rats, as well as body weights were measured every two days during 2 weeks, between 11:00 AM and 12:00 AM before extract administration.

Induction of acute stress: Acute stress was induced by using the method earlier described by Takagi and Okabe[13] with some modifications. After the final extract treatment and following 48 hours of food (but not water) deprivation, test rats were given the extract (100, 200 and 400 mg/kg) by oral route while stress control and positive control rats received respectively the vehicle and diazepam (1mg/kg). Thirty minutes later, the rats were placed in small individual wire cages (4.5×4.5×18 cm) and immersed in cold water (20±1°C), up to the level of the xiphoid for 6 hours. The rats were then removed from the cages, dried with a paper towel, and were prepared for the open field test.

Induction of chronic stress: Chronic stress was induced by using the method described earlier by Burzing *et al.*[14] with some modifications. One hour after treatments rats of the extract-treated, normal and positive control groups were subjected to the weight loaded force swimming every alternate day for a period of 14 days. Each animal was supplied with a constant load (corresponding to 5% of the body weight) tagged to the tail, and placed individually in a swimming pool (90cm×45cm×45cm), filled with water to a depth of 35 cm,

and maintained at 25 ± 1°C. Exhaustion was determined by observing loss of coordinated movements and failure to return to the surface within 10sec. The rats were then removed from the pool, dried with a paper towel, and returned to their original cages. The pool water was replaced after each session. Briefly after the last swimming session and following 48 hours of food (but not water) deprivation, test rats were given the extract (100, 200 and 400 mg/kg) by oral route while stress control and positive control rats received respectively the vehicle and diazepam (1mg/kg). Thirty minutes later, the rats were placed in small individual wire cages (4.5×4.5×18 cm) and immersed in cold water (20±1°C), up to the level of the xiphoid for 6 hours. The rats were then removed from the cages, dried with a paper towel, and were prepared for the open field test.

Open field test: One hour after cold restraint stress, animals of all groups were submitted to open field test as described by Belzung.[15] The rats were individually placed at the center of a clean open field apparatus (40×40×15 cm, divided into 25 squares), and the activity of each was video-recorded for 5 minutes and analyzed later. The latency period (time in the center square), defecation, crossing (number of squares crossed indicating spontaneous locomotor activity), grooming and rearing (exploratory activity) for individual rat were scored. The arena was cleaned after each test using a 70% v/v ethanol. Animals were then sacrificed using ether blood samples were taken into sterile tubes and serum was prepared (centrifuging at 3000 rpm for 10 min) for the analysis of blood glucose, triglycerides concentration and blood urea nitrogen. The stomachs were removed for the assessment of lesion formation and mucus production. The sum of length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the inhibition percentage was calculated by the formula: Inhibition (%) = [(UI control - UI treated)/ UI control] ×100. Stomach samples were also preserved frozen awaiting measurement of antioxidant parameters.

Mucus production assessment: The mucus covering of each stomach was gently scraped using a glass slide and the mucus weighed carefully using a sensitive digital electronic balance. The same experimenter performed this exercise each time.

Measurement of antioxidant status in stomach: Gastric tissue sample (1g of each) obtained from the experimental animals were homogenized in 4 ml of Tris/HCl. The tissue homogenates were centrifuged at 4000g for 15 min at 4°C and the supernatants were assessed for the antioxidant status. Lipid peroxidation (the level of thiobarbituric acid reactive substances in terms of Malondialdehyde (MDA)) was measured as described previously[16],

and total glutathione (GPx) content was measured according to the method of Ellman.[17] Catalase (CAT) activity was measured according to the method of Sinha.[18] Superoxide dismutase (SOD) activity was measured using the pyrogallol autoxidation method.[19]

Measurement of Serum Biochemical Parameters: The serum levels of glucose were estimated using a glucometer (Reader Accu-CHEK®Active). Levels of BUN were measured using a colorimetric and enzymatic method (Bioassay System, CA Kit) and triglyceride concentrations were measured using a commercial kit for measurement of triglycerides in serum or plasma (Enzymatic Trinder Method).

Statistical analysis

Statistical analysis was done by one-way analysis of variance (ANOVA) followed by the Dunnett's test for multiple comparisons and P values less than 0.05 were considered as significant. The results are expressed as mean ± standard error of mean (SEM).

RESULTS AND DISCUSSION

The present study was undertaken to evaluate the antistress effects of the aqueous leaves extract of *M. oleifera* in rats. To achieve this objective, rats were subjected to acute and chronic animal model of stress for 14 days, after which behaviors scored, gastric lesions, oxidative stress parameters in stomach and serum biochemical parameters were evaluated. The preliminary phytochemical screening carried out on the aqueous extract of *M. oleifera* revealed the presence of many phytoconstituents. These included, phenols, tannins, sugars and amino acids (+++), proteins, glycosides and flavonoids (++), alkaloids, acids, Oils, sterols and triterpenoids (+), coumarins, quinines, anthocyanins, saponins and resins (-) were absent. The administration of *M. oleifera* extract had no significant effect on body weight (Figure 1), food and water intake (Table 1) as compared to respective SCG ($p>0.05$) in acute and chronic stress model. Similar results have been obtained in previous research which tested antifatigue and anti-oxydant properties of *M. oleifera* aqueous extract.[9]

Table. 1: Effect of *M. oleifera* extract on food (g/group/week) and water (ml/group/week) intake in rats.

Acute stress				
Treatment	**Food intake (ml/group/week)**		**Water intake (ml/group/week)**	
	Week 1	**Week 2**	**Week 1**	**Week 2**
NSCG	184.87±5.52	186.04±6.24	162.15±5.42	178.64±8.22
SCG	187.72±4.36	188.33±9.50	159.12±7.16	185.33±3.51
PCG	193.67±6.66	201.33±8.50	164.24±6.24	194.67±3.51
M. oleifera (100 mg/kg)	190.33±4.93	193.67±6.03	168.77±6.24	187±7.81
M. oleifera (200 mg/kg)	194.67±4.73	200.67±9.61	167.67±5.86	193.41±7.22
M. oleifera (400 mg/kg)	194.67±8.14	193.67±6.35	162.67±7.50	188.33±9.50
Chronic Stress				
Treatment	**Food intake (ml/group/week)**		**Water intake (ml/group/week)**	
	Week 1	**Week 2**	**Week 1**	**Week 2**
SCG	176.29±6.68	179.00±7.94	165.34±7.50	174.18±7.64
PCG	177.31±2.31	183.18± 4.36	172.10±4.36	178.14±4.00
M. oleifera (100 mg/kg)	179.33±3.79	180.44±7.06	160.37±6.81	174.00±9.64
M. oleifera (200 mg/kg)	180.67±5.51	184.11±6.24	162.33±8.08	179.41±7.50
M. oleifera (400 mg/kg)	178.52±8.90	175.15±3.46	179.17±10.69	183.01±11.04

Each value represents the mean ± SEM, n = 5. NSCG= Non-Stress Control Group, SCG= Stress Control Group, PCG= Positive Control Group treated with Diazepam (1mg/kg).

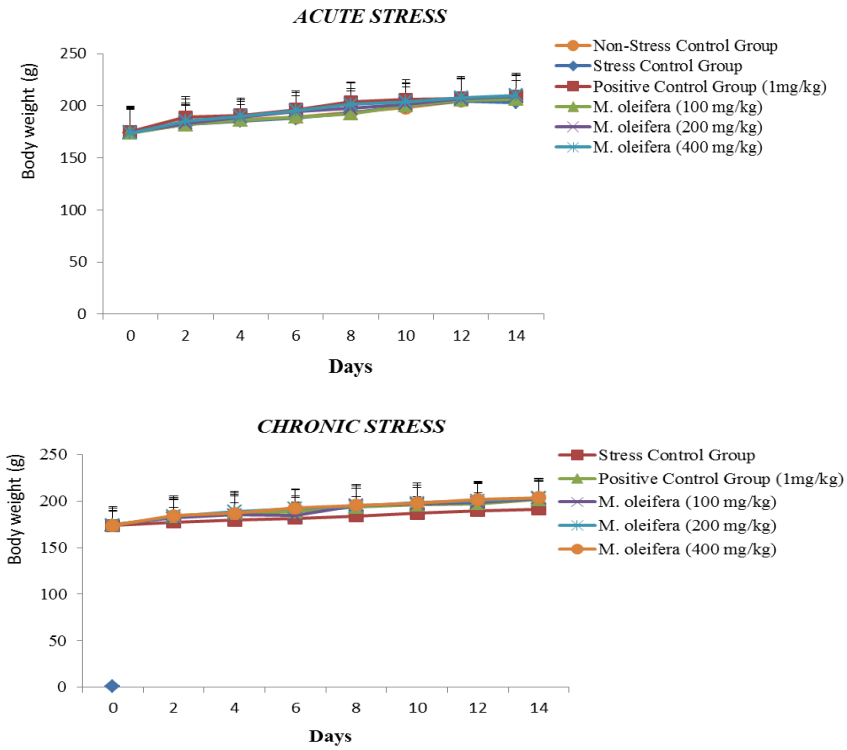

Figure 1: Body weight change of the rats subjected to acute and chronic stress Data are presented as mean ± SEM, n =5.

Open field test were used to evaluate some behavioral parameters (latency period, defecation, crossing, grooming and rearing) in rats subjected to acute and chronic stress model. As shown in table 2, results indicated significant modifications of behavioral parameters in extract-treated groups as compare to their respective control groups ($p<0.05$-$p<0.001$). Defecation, grooming and latency period of the SCG were significantly lower than those of extract-treated groups and PCG in both acute and chronic stress model ($p<0.01$-$p<0.001$). However, significant increase was observed in the number of crossing and rearing of SCG as compare with extract-treated groups and PCG in both acute and chronic stress model ($p<0.05$-$p<0.001$). Those modifications indicate an antistress effect of *M. oleifera* extract in rats subjected to acute or chronic stress model. Similar results were reported by Das *et al.*[20] In fact, those authors postulated that stress is involved in behavioral changes like anxiety because of an involvement of the central nervous system and the endocrine system.

Table. 2: Effect of *M. oleifera* extract on behaviors scored in the rat subjected to acute and chronic stress.

Acute Stress					
Treatment	**Defecation (g)**	**Latency period (sec)**	**Crossing**	**Grooming**	**Rearing**
NSCG	0.24±0.05***	3.36±0.97***	47.36±6.14***	1.54±0.96***	20.38±4.52***
SCG	0.92±0.11	13.80±1.92	14.44±5.73	6.60±1.52	5.21±1.30
PCG	0.23±0.06***	3.24±0.71***	44.80±5.80***	1.60±0.89***	10.40±1.14**
M. oleifera (100 mg/kg)	0.29±0.06***	5.31±1.30***	23.40±5.94	3.03±0.70***	6.82±1.09
M. oleifera (200 mg/kg)	0.22±0.07***	4.60±0.88***	31.40±5.81***	2.11±1.14***	9.20±1.30*
M. oleifera (400 mg/kg)	0.36±0.09***	4.12±1.19***	35.32±4.61***	1.69±0.55***	8.81±1.92
Chronic Stress					
Treatment	**Defecation (g)**	**Latency period (sec)**	**Crossing**	**Grooming**	**Rearing**
SCG	0.48±0.11	11.60±3.36	19.00±7.97	4.05±1.58	6.20±2.17
PCG	0.27±0.06***	3.85±1.64***	33.28±4.38**	2.15±1.0	15.40±1.34***
M. oleifera (100 mg/kg)	0.31±0.06**	5.84±1.64***	24.60±7.24	3.22±1.21	14.80±1.48***
M. oleifera (200 mg/kg)	0.24±0.07***	4.32±2.17***	30.46±5.76*	3.12±0.89	17.40±1.14***
M. oleifera (400 mg/kg)	0.26±0.09***	4.66±1.14***	37.44±6.27***	2.83±1.02	19.80±1.92***

Each value represents the mean ± SEM, $n = 5$. *P<0.05, **$P<$ 0.01, and ***$P<$ 0.001 compared with their respective SCG (Stress Control Group). NSCG= Non-Stress Control Group, PCG= Positive Control Group treated with Diazepam (1mg/kg).

Antistress agents have been shown to effectively work by reducing ulceration and increasing gastric mucus.[21] Several mechanisms were developed to explain the protecting effect of those extracts on stomach damages induced by stressors. Literature shows that during stress,

reduction of prostaglandin I2 and prostaglandin E2 production in gastric mucosa were observed.[22] Prostaglandins play important roles in the regulation of gastric mucosal integrity. In fact, a reduction of these compounds leads to decreases in mucus synthesis and mucosal blood flow resulting in the susceptibility of gastric mucosa to gastric acid and noxious factors.[23]

Table 3: Effect of *M. oleifera* extract on gastric ulceration and secretion induced by acute and chronic stress.

Acute Stress					
Treatment	**Ulcerated Surface (mm^2)**	**% of Ulcerated Surface**	**Ulcer Index**	**Inhibition (%)**	**Gastric mucus (mg)**
NSCG	-	-	-	-	270.57±5.33
SCG	48±10.52	7.11±1.56	9.8±0.45	-	257.46±4.57
PCG	6.7±4.09***	1.05±0.23***	3.4±1.14***	65.31	266.58±7.41*
M. oleifera (100 mg/kg)	18.3±6.96***	2.71±1.03***	6.2±1.30***	36.73	259.65±3.08
M. oleifera (200 mg/kg)	11.7±7.02***	1.73±1.04***	4.8±1.48***	51.02	278.02±4.72***
M. oleifera (400 mg/kg)	10.5±5.69***	1.56±0.84***	4.6±1.14***	53.06	284.31±5.88***
Chronic Stress					
Treatment	**Ulcerated Surface (mm^2)**	**% of Ulcerated Surface**	**Ulcer Index**	**Inhibition (%)**	**Gastric mucus (mg)**
SCG	50.60±12.22	7.50±1.81	9.80±0.45	-	237.46±4.57
PCG	7.00± 3.24***	1.04± 0.48***	3.60±0.55± ***	63.26	264.33±8.67***
M. oleifera (100 mg/kg)	11.91±5.22***	1.76± 0.77***	4.80±0.84***	51.02	245.96±6.41
M. oleifera (200 mg/kg)	11.50± 4.36***	1.70± 0.65***	4.83±0.84***	51.02	276.87±7.95***
M. oleifera (400 mg/kg)	10.92± 2.61***	1.61± 0.39***	4.62±0.55***	53.06	278.63±5.64***

Each value represents the mean ± SEM, n = 5. *P<0.05 and ***P< 0.001 compared with SCG (Stress Control Group). NSCG= Non-Stress Control Group, PCG= Positive Control Group treated with Diazepam (1mg/kg).

Results of the effect of *M. oleifera* extract on gastric lesion formation and mucus production in rats subjected to acute and chronic stress model are shown in Table 3. Exposure to stress led to increases in Ulcer Index and decreases in mucus production of SCG compared with those of NSCG (p<0.001). Administration of extract shown, in acute stress model, significant and dose-related reduced of Ulcer Index from 9.8±0.45 in SCG to 6.2±1.30, 4.8±1.48 and 4.6±1.14 in *M. oleifera* 100, 200 and 400 mg/kg respectively (p<0.001). However, treatment with the extracts resulted in significant (p<0.001) and dose-dependent increases in gastric mucus ranging from 257.46±4.57 mg in SCG to 259.65±3.08, 278.02±4.72 and 284.31±5.88 mg in *M. oleifera* 100, 200 and 400 mg/kg respectively. Similar results were obtained in chronic stress model with Ulcer Index reducing from 9.80±0.45 in SCG to 4.80±0.84,

4.83±0.84 and 4.62±0.55 in *M. oleifera* 100, 200 and 400 mg/kg respectively. In the same way, the gastric mucus significantly and dose-dependently increased from 237.46±4.57 mg in SCG to 245.96±6.41, 276.87±7.95 and 278.63±5.64 mg in *M. oleifera* 100, 200 and 400 mg/kg respectively. These results indicated that *M. oleifera* extract had a protective effect against acute and chronic stressors by preventing reduction in prostaglandins and mucus content of gastric mucosa. In addition, phenols and flavonoids which were found in significant quantities in the extract, are natural plant substances with well-known preventive antioxidant and antiulcer activities.[24] These compounds most likely inhibit gastric mucosal injury by scavenging stress-generated oxygen metabolites.[25]

Earlier studies have shown that exposure to stress conditions can stimulate numerous pathways leading to an increased production of free radicals.[26] These radicals generate a process, producing lipid peroxidation, protein oxidation, DNA damage, and cell death, and they contribute to the occurrence of pathological conditions. MDA is one of the degradation products in the lipid peroxidation process.[27] Peroxidation is an important indicator of oxidative stress that results from degradation of cell membrane by free radicals. As shown in table 4, MDA concentrations in extract-treated groups and PCG were significantly lower ($p<0.001$) as compared with the SCG in both acute and chronic stress model. This result indicated that *M. oleifera* extract possessed antiperoxidation capacity and prevent generation of gastric ulcer caused by acute and chronic stress. Stress conditions may also impair the antioxidant defense system, leading to oxidative damage, by changing the balance between oxidant and antioxidant factors. Enzymatic antioxidant systems, such as GPx, SOD, and CAT, are important in scavenging free radicals and their metabolites.[28] The improvement in the activities of these defense mechanisms can help to fight against stress. Table 4 shown stomach antioxidant parameters in rats subjected to acute and chronic stress. In both stress model, exposure to stress led to decreases in the activities of SOD, GPx, and CAT in stomach of SCG compared with those of NSCG ($p<0.001$). These effects were significantly ($p<0.001$) attenuated following *M. oleifera* extract and diazepam treatment. These results indicated that antistress effect of *M. oleifera* extract probably occurs through modifying activities of several antioxidant defenses. These results are in accordance with our previous study which demonstrated antioxidant effects of this extract in rats subjected to acute fatigue.[9]

Table. 4: Effect of *M. oleifera* extract on stomach antioxidant parameters in rats subjected to acute and chronic stress.

Acute Stress				
Treatment	**SOD (U/mg protein)**	**GPx (µmol/mg of protein)**	**CAT (U/mg protein)**	**MDA (µmol/cm/g)**
NSCG	8.26±1.12**	3.20±0.41**	4.17±0.31**	7.20±1.48***
SCG	11.53±0.77	1.64±0.11	5.83±0.54	30.08±3.05
PCG	25.49±1.08***	4.42±0.85***	13.77±1.05***	13.89±2.11***
M. oleifera (100 mg/kg)	18.64±2.16***	5.06±1.00***	7.91±0.57***	13.70±1.96***
M. oleifera (200 mg/kg)	20.37±1.17***	3.81±0.32***	9.64±0.83***	16.86±1.35***
M. oleifera (400 mg/kg)	24.50±0.94***	4.51±0.61***	11.46±1.02***	15.92±1.09***
Chronic Stress				
Treatment	**SOD (U/mg protein)**	**GPx (µmol/mg of protein)**	**CAT (U/mg protein)**	**MDA (µmol/cm/g)**
SCG	10.44±0.58	2.32±0.16	4.33±0.19	27.55±4.16
PCG	22.03±2.15***	4.81±0.67***	10.22±0.97***	11.47±1.91***
M. oleifera (100 mg/kg)	18.21±1.69***	3.71±0.46***	6.43±0.81***	11.03±0.98***
M. oleifera (200 mg/kg)	21.71±1.87***	3.94±0.30***	10.44±0.72***	12.14±1.04***
M. oleifera (400 mg/kg)	20.10±1.13***	4.18±0.59***	10.11±0.88***	10.17±0.73***

Each value represents the mean ± SEM, n = 5. **P< 0.01, and***P< 0.001 compared with their respective SCG (Stress Control Group). NSCG= Non-Stress Control Group, PCG= Positive Control Group treated with Diazepam (1mg/kg).

Stress situations is also link to plasma cortisol increase which mobilize stored fat and carbohydrate reserves and turn increases blood glucose, total protein, urea, cholesterol and triglyceride levels.[29] In fact, the major neural pathways activated by stressors are the Sympathetic nervous system and Hypothalamic pituitary adrenal (HPA) axis. This activation lead to increase in corticotrophin releasing hormone (CRH) secretion and this stimulates the anterior pituitary to secrete adrenocorticotropin hormone (ACTH) into the systemic circulation.[30] ACTH acts on the adrenal cortex to stimulate the synthesis and release of cortisol. Cortisol influences the mobilization of stores fat and carbohydrate reserves which in turn increased blood glucose level, triglycerides level and urea level.[31] Antistress agents were known to reverse increased cortisol level, blood glucose level, triglyceride level and urea level.[32] Serum biochemical parameters in rats subjected to acute and chronic stress are presented in Table 5. There were significant decreased ($p<0.001$) in blood glucose, urea and triglyceride levels in extract-treated groups and PCG compared to their respective SCG. These results indicated that antistress effect of *M. oleifera* extract probably occurs through regulation of the metabolism of glucose, proteins and lipids.

Table. 5: Effect of *M. oleifera* extract on serum biochemical parameters in rats subjected to acute and chronic stress.

Acute Stress			
Treatment	**Glycemia (mg/dl)**	**Urea (mg/dl)**	**Triglycerides (mg/dl)**
NSCG	76.61±5.42***	14.63±3.11***	52.84±7.12***
SCG	142.26±11.34	43.39±7.22	137.94±13.57
PCG (1mg/kg)	82.06±9.31***	17.57±4.72***	51.13±6.04***
M. oleifera (100 mg/kg)	112.71±13.51***	22.48±5.66***	77.08±7.31***
M. oleifera (200 mg/kg)	91.88±8.66***	18.93±3.81***	80.44±8.65***
M. oleifera (400 mg/kg)	85.55±6.74***	19.02±6.10***	55.78±6.93***
Chronic Stress			
Treatment	**Glycemia (mg/dl)**	**Urea (mg/dl)**	**Triglycerides (mg/dl)**
SCG	156.37±9.74	40.90±6.17	129.34±10.47
PCG (1mg/kg)	88.54±8.27***	18.53±2.36***	38.66±4.49***
M. oleifera (100 mg/kg)	93.28±7.19***	21.82±4.62***	59.43±6.19***
M. oleifera (200 mg/kg)	80.35±8.03***	18.01±2.71***	64.33±7.08***
M. oleifera (400 mg/kg)	79.64±7.55***	15.21±3.51***	65.84±5.10***

Each value represents the mean ± SEM, n = 5. ***P< 0.001 compared with their respective SCG (Stress Control Group). NSCG= Non-Stress Control Group, PCG= Positive Control Group treated with Diazepam (1mg/kg).

CONCLUSION

All results indicated that the administration of leaf aqueous extract of *M. oleifera* could significantly prevented the dysfunctions induced by acute or chronic stressors. The underlying mechanisms responsible for the antistress effect of *M. oleifera* extract involves mainly the modulation of oxidative stress and the regulation of the metabolism of glucose, proteins and lipids. These results suggest that *M. oleifera* extract has significant health benefits due to its antistress activity, which provided scientific evidence for further development of natural products for prevention and treatment of diseases related to stress conditions.

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