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EFFECT OF *MORINGA OLEIFERA* LAM. ROOT ON COLONIC MOTILITY DISORDERS INDUCED BY WATER AVOIDANCE STRESS IN RATS

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Keywords:

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ABSTRACT: Irritable bowel syndrome (IBS) is a chronic condition that affects the lower gastrointestinal tract. It affects the function and behavior of the intestine resulting in abdominal pain, flatulence, loose stools, frequent stools, feeling of being unable to completely empty bowel and nausea. *Moringa oleifera*, also commonly known as drumstick tree, is a highly valued plant with a wide range of medicinal uses. The objective of the study was to evaluate the effect of *Moringa oleifera* Lam roots extract on colonic motility disorders induced by water avoidance stress in rats. Male Wistar rats weighing between (150 g -200 g) were divided into the following five groups (n=6). Group 1 Normal control (saline), Group 2 Disease control animals subjected to water avoidance stress (WAS) for 10 days, Group 3 Animals received standard drug (Ramosteron) and WAS, Group 4 Animals received LMOE (100 mg/kg) and WAS, Group 5: Animals received HMOE (400 mg/kg) and WAS. Animals subjected to WAS, showed a significant increase in fecal pellet count and increased levels of MDA and MPO, decrease in SOD, Catalase, and in the whole gut transit time. Treatment with *Moringa oleifera* significantly reversed changes induced by WAS and restored normal physiology.

INTRODUCTION: Irritable bowel syndrome (IBS) is a major functional gastrointestinal disorder that affects the function and behavior of the intestine. IBS is characterized by bloating, abdominal pain, abnormal bowel movements and Visceral hyperalgesia, affecting about 11.2% of the population across the world ¹. There are three subcategories of IBS, pain associated with diarrhea, pain associated with constipation pain, and diarrhea alternating with constipation.

IBS with constipation comes with stomach pain and discomfort, bloating, abnormally delayed or infrequent bowel movement (IBS-C), IBS with diarrhoea (IBS-D) comes with stomach pain and discomfort, an urgent need to move your bowels, abnormally frequent bowel movements, loose and watery stool. Mixed IBS (IBS-M) alternate constipation and diarrhea ².

The majority of the symptoms are seen in patients at the age of 20-50 years old, but in 50% of patients, symptoms begin before age 35. The disorder is also identified in children, generally appearing in early adolescence ³. The interaction of certain lifestyle factors plays a major role in developing IBS that include psychosocial, psychological, neuro-transmitter, active Infections, and role of diet. Treatment of patients with IBS

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would be patient-oriented, symptom-specific and depends on the three main pathophysiological factors: visceral hypersensitivity, dysmotility and psychosocial disturbances. Drug therapy includes smooth muscle relaxants, anti-diarrheal drugs, tricyclic anti-depressants, selective serotonin reuptake inhibitors. Alternative therapies include herbs like chamomile, ginger, and mint that have been found to be helpful in reducing gastrointestinal pain in IBS patients.

Several new treatment options have been shown to be effective in management of IBS. 5HT₄ agonists (tegaserod and prucalopride), M₃ muscarinic receptor antagonists (zamifenacin and darifenacin), and cholecystikinin (CCK) receptor antagonists (loxiglumide). Tegaserod has been shown to be positive to improve pain and bowel habits in women with constipation-predominant IBS. New therapeutic outcome also targets abnormal visceral sensitivity which includes 5HT₃ antagonists, 5HT₁ agonists (Buspirone), kappaopioid agonists (fedotozine) and α_2 adrenergic agonists (Clonidine). These agents seem to lessen gut sensation in addition to improving motility.

M. oleifera Lam the flowering plant, which is native to parts of Africa and Asia and is the sole genus belonging to the family Moringaceae. The plant is commonly mentioned to as the drumstick tree. *Moringa oleifera* tree is referred to as "miracle tree" due to its medicinal and nutritional properties in world³.

Aqueous and alcoholic extract of the *Moringa* leaves contains Niazirin and Niazirin-nitrile glycosides, 4(4-O-acetyl- α -L-rhamnosyloxy) benzyl isothiocyanate, Niaziminin A, and Niaziminin B, three mustard oil glycosides, niaziminin, a thiocarbonate, 4-(α -L-rhamnosyloxy)-benzylglucosinolate, quercetin-3-O-glucoside and quercetin-3-O-(6-Malonyl-glicoside), Niazimicin. Pyrrole alkaloid (pyrrolemarumine 400-O- α -L-rhamnosyloxy) and 40-hydroxyphenylethanamide (marumosi A and B) 4 α , γ -tocopherol. The Aqueous and hydroalcoholic extract of the seeds carries active constituents like Methionine, cysteine, 4(α -rhamnosyloxy) benzylglucosinolate, Moringine, benzylglucosinolate, niazimicin, niazirin⁴. The alcoholic extract of the root contains

Moringine, moringinine, spirachimn, 1, 3-dibenzyl urea, α -phellandrene, p-cymene, Deoxy-niazimicin, 4(α -L-rhamnosyloxy) benzylglucosinolate. The present study was undertaken to evaluate the effect of *Moringa oleifera* Lam. root extract on colonic motility disorders induced by chronic water avoidance stress in rats.

MATERIALS AND METHODS:

Chemicals: Evans blue marker and Tris buffer (Research - Lab fine chem industries), Ethylene diamine tetra acetic acid, Ammonium molybdate, Trichloro acetic acid, Thiobarbituric acid Hydrogen peroxide and Pyrogallol (S D Fine - Chem Limited Mumbai).

Experimental Animals: Male Wistar rats were procured from Sainath agency, Hyderabad, and were acclimatized to laboratory conditions (25 \pm 3 $^{\circ}$ C of temperature, 12-h light/dark cycle), food, and water was given *ad libitum*. The rats were randomly divided into 5 experimental groups (n=6). All the experimental procedures were carried out in accordance with the committee for the purpose of control and supervision of experiments on animal (320/CPCSEA dated 03-01-2001) guidelines. The study was reviewed and approved by the Institutional Animal Ethics Committee (GPRCP/IAEC/10/18/02/PCL/AE-3-Rats-F-30) of G. Pulla Reddy College of Pharmacy, Mehdiapatnam, Hyderabad, Telangana state, India.

Preparation of *Moringa oleifera* root extract:

The roots of *Moringa oleifera* were collected, completely dried in the sunlight, and powdered. Root powder was extracted exhaustively with 50% methanol by a process called maceration for 2 days at room temperature with frequent shaking. The crude (hydroalcoholic) extract which is obtained, was filtered and dried under reduced pressure at 40 $^{\circ}$ C (Yield - 9.3% w/w of dried plant material). Aqueous solution of dried extract (MO) is diluted in suitable dilution with distilled water and administered in test animals⁵.

Experimental Study Design: Female Wistar rats weighing between (150-200 g) were divided into the following five groups (n=6).

Group 1: Normal control animals received saline as a vehicle.

Group 2: Disease control animals were subjected to water avoidance stress for 10 days.

Group 3: Animals subjected to water avoidance stress for 10 days and received standard drug Ramosetron (3 µg/kg).

Group 4: Animals subjected to water avoidance stress for 10 days and received low dose of *Moringa oleifera* Lam root extract (LMOE) (100 mg/kg p.o.).

Group 5: Animals subjected to water avoidance stress for 10 days and received high dose of *Moringa oleifera* Lam root extract (HMOE) (400 mg/kg p.o.).

All the animals were treated for a period of 10 days. During the induction of colonic motility disorder fecal pellet output was measured in all the rats. After the treatment schedule, rats were then injected with 1ml of Evan's blue marker for the estimation of whole gut transit time. At the end of the experiment, rats were sacrificed, and colon was isolated for recording contractions of colonic smooth muscle and estimation of biochemical parameters.

Measurement of Fecal Pellet Output: The Baseline 24 h fecal pellet output of each rat in all the groups was observed for three successive days before WAS treatment. On the day of the experiment, each animal was subjected to WAS for one hour and the fecal pellets expelled during the period of WAS from each animal were counted. After WAS, each rat was returned to their individual standard housing cage, and 24 h fecal pellet output of each rat was collected and observed⁶.

Estimation of Whole Gut Transit Time: During the experimental round, rats were transferred to the individual empty plastic cages, devoid of bedding and were left to acclimatize to the cage for 1 h. Rats were then administered or injected with vehicle (saline) or methanolic extract of *Moringa oleifera* Lam root or standard drug Ramosetron p.o and 1 h later gavaged with 1 ml of pre-warmed Evans blue marker. The time from the end of gavage to the appearance of the first blue fecal pellet was measured in minutes and constituted the whole gut transit time⁷.

Biochemical Analysis:

Estimation of Myeloperoxidase activity (MPO): MPO levels were estimated using the method described by Bradley *et al.*,⁸

Estimation of Malonaldehyde (MDA): MDA levels were estimated using procedure followed by Ohkawa *et al.*,⁹

Estimation of Superoxide Dismutase (SOD): SOD levels were estimated using the procedure explained by Misra *et al.*,¹⁰

Estimation of Catalase: Catalase levels were estimated using the following procedure¹¹.

Statistical Analysis: Data expressed as mean ± SEM. All data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's Kramer multiple comparison test using Graph pad Prism software (7.0 version) for comparison of more than two groups. Differences were considered as statistically significant when $p < 0.05$.

Results:

Effect of *Moringa oleifera* Lam Root on Fecal Pellet Output in Water Avoidance Stress-Induced Colonic Motility Disorder in Rats: Baseline 24 h fecal pellet output was collected after WAS treatment in all animals from each group. Disease control animals showed an extremely significant increase in fecal pellet output *i.e.*, 41.12 ± 2.566 ($p < 0.0001$), when compared with normal control animals *i.e.*, 30.05 ± 0.825 ($p < 0.0001$). Treatment with Ramosetron and HMOE showed an extremely significant decrease in fecal pellet output *i.e.*, 29.49 ± 0.567 ($p < 0.0001$) and 28.79 ± 0.884 ($p < 0.0001$). Whereas treatment with LMOE showed significant decrease in fecal pellet output *i.e.*, 33.95 ± 1.520 ($p < 0.001$), when compared with disease control animals *i.e.*, 41.12 ± 2.566 .

Effect of *Moringa oleifera* Lam Root on Whole Gut Transit Time in Water Avoidance Stress-Induced Colonic Motility Disorder in Rats: Whole gut transit time was estimated by administering 1 ml of Evans blue marker in all animals from each group. Disease control animals showed an extremely significant decrease in transit time *i.e.*, 322.3 ± 6.292 ($p < 0.0001$), when compared with normal control animals, *i.e.*, 423.3 ± 4.410 . Treatment with Ramosetron, LMOE, and HMOE

showed an extremely significant increase in transit time *i.e.* 422.5 ± 3.819 ($p < 0.0001$), 387.5 ± 8.342 ($p < 0.0001$) and 417.5 ± 3.819 ($p < 0.0001$) when compared with disease control animals *i.e.* 322.3 ± 6.292 .

Effect of *Moringa oleifera* Lam Root on MPO Level in Water Avoidance Stress-Induced Colonic Motility Disorder in Rats: MPO levels were analyzed in the tissue samples collected on 11th day from all the animals in each group. Disease control animals showed an extremely significant increase in the level of MPO, *i.e.*, 1.085 ± 0.024 ($p < 0.0001$), when compared with normal control animals, *i.e.*, 0.533 ± 0.025 .

Treatment with Ramosetron, LMOE and HMOE showed extremely significant decrease in levels of MPO *i.e.*, 0.551 ± 0.044 ($p < 0.0001$), 0.705 ± 0.030 ($p < 0.0001$) and 0.490 ± 0.034 ($p < 0.0001$) when compared with disease control animals *i.e.*, 1.085 ± 0.024 **Table 1**.

Effect of *Moringa oleifera* Lam Root on MDA Level in Water Avoidance Stress-Induced Colonic Motility Disorder in Rats: MDA levels were analyzed in the tissue samples collected on 11th day from all the animals in each group. Disease control animals showed an extremely significant increase in the level of MDA, *i.e.*, 4.072 ± 0.143 ($p < 0.0001$), when compared with normal control animals, *i.e.*, 1.762 ± 0.066 .

Treatment with Ramosetron, LMOE and HMOE showed extremely significant decrease in levels of MDA *i.e.*, 1.617 ± 0.117 ($p < 0.0001$), 2.393 ± 0.107 ($p < 0.0001$) and 1.940 ± 0.055 ($p < 0.0001$) when compared with disease control animals *i.e.*, 4.072 ± 0.143 **Table 1**.

Effect of *Moringa oleifera* Lam Root on SOD Level in Water Avoidance Stress-Induced Colonic Motility Disorder in Rats: SOD levels were analyzed in the tissue samples collected on 11th day from all the animals in each group. Disease control animals showed an extremely significant decrease in levels of SOD, *i.e.*, 0.933 ± 0.140 ($p < 0.0001$), when compared with normal control animals, *i.e.*, 2.230 ± 0.233 . Treatment with Ramosetron and HMOE showed an extremely significant increase in levels of SOD *i.e.*, 2.255 ± 0.122 ($p < 0.0001$) and 2.228 ± 0.158 ($p < 0.0001$).

Whereas treatment with LMOE showed significant increase in SOD levels *i.e.*, 1.958 ± 0.128 ($p < 0.001$) when compared with disease control animals *i.e.*, 0.933 ± 0.140 **Table 1**.

Effect of *Moringa oleifera* Lam Root on Catalase Level in Water Avoidance Stress-Induced Colonic Motility Disorder in Rats: Catalase levels were analyzed in the tissue samples collected on 11th day from all the animals in each group. Disease control animals showed an extremely significant decrease in level of catalase, *i.e.*, 0.480 ± 0.046 ($p < 0.0001$), when compared with normal control animals *i.e.*, 2.248 ± 0.057 . Treatment with Ramosetron, LMOE and HMOE showed extremely significant increase in levels of catalase *i.e.*, 2.470 ± 0.196 ($p < 0.0001$), 1.590 ± 0.106 ($p < 0.0001$) and 2.105 ± 0.078 ($p < 0.0001$) when compared with disease control animals *i.e.*, 0.480 ± 0.046 **Table 1**.

DISCUSSION: Irritable bowel syndrome a chronic functional gastrointestinal disorder which affects mostly the lower gastrointestinal tract. Understanding the pathophysiological mechanisms of IBS made in recent years has helped in developing newer therapy outcomes and improve patient's quality of life. IBS increases the chances of various diseases like inflammatory bowel disease and psychiatric disorders like fibromyalgia, anxiety, and depression¹².

The water avoidance stress model is one of the most reliable and reproducible for screening of drugs helpful in colonic motility disorder. Water avoidance stress-induced colonic motility disorder in rats can be easily correlated with the human IBS. In this study water avoidance stress-induced model was employed to prove the effectiveness of the drug *Moringa oleifera* Lam. in the treatment of colonic motility disorders. Water avoidance stress induces visceral hypersensitivity and accelerates colonic transit, causes changes in gut motility, and increase anxiety-like behavior¹³. In colonic motility disorder, fecal pellet count is an important physical parameter in which the fecal pellet count of the individual is increased to a greater extent. Induction of colonic motility disorder by water avoidance stress in rats for 10 days resulted in a significant increase in colonic motility. Treatment with MOE has significantly decreased the fecal pellet output. Stress activates the hypothalamic-

pituitary axis (HPA), which stimulates the release of CRF, a hormone responsible for GI motility. Serotonin, secreted by enterochromaffin cells, is an indoleamine that plays an important physiological role in regulation of intestinal motility (Vahedi H *et al.* 2010). Previous reports suggest that animals subjected to chronic stress stimulates the release of fecal pellets due to increasing the concentration of 5-HT in colon, and this effect was observed in our disease control group animals which showed abnormal fecal pellet output. In the treatment group, there was a decrease in the fecal pellet count and this may be due to a decrease in the concentration of colonic 5-HT¹². Whole gut transit time is estimated to check the movement and motility of the bowel. Previous studies suggest that chronic stress results in increased colonic motility and decreased gut transit time⁷. In our study, we have found that treatment with MOE has significantly reversed the condition and increased the gut transit time. In stress-induced colonic motility disorder, the levels of MDA and MPO increases and the level of SOD and catalase decreases. Increased levels of MDA and MPO in stressed animals are the reasons for the development of inflammation, wherein chronic conditions lead to the onset of inflammatory bowel disease, ulcerative colitis, and crohn's disease. It is well known that the involvement of oxidative stress and inflammatory mediators are the major causative factors for the initiation and progression of gastrointestinal diseases like IBS. Free radicals are also known as reactive oxygen species (ROS) are formed by chain reactions initiated by oxygen free radicals.

These ROS can cause injury to the lipid membranes, protein molecules, and DNA. In stressful conditions, the levels of antioxidant enzymes decrease, and levels of reactive oxygen species increase. The damage of mucosal layer in the GI tract due to oxidative stress leads to the initiation and development of IBS. The protective mechanisms involved against this abrasive process is the presence of enzymatic (SOD, Catalase) and non-enzymatic (TNF-alpha) free radical scavengers. These antioxidant enzymes prevent ROS-mediated injury and protect cellular integrity¹. Catalase is one of the most important enzymes in the antioxidant system that reduces oxidative stress¹³. The enzyme acts by degrading the harmful

hydrogen peroxide into oxygen and water, where decreased levels of the enzyme are seen in the IBS condition. Animals exposed to water avoidance stress lead to a decrease in catalase levels due to the formation of harmful free radicals like hydrogen peroxide and hydroxyl molecules. In our study, we found that treatment with MOE caused a significant increase in the levels of colon catalase due to its radical scavenging activity, hence protecting the tissue from oxidative damage³. Superoxide dismutase (SOD) is an important anti-oxidant enzyme in all living cells exposed to oxygen and acts as a biomarker for oxidative stress in IBS. SOD catalyzes the dismutation of superoxide free radical into O₂, thus protecting the cell from oxidative damage. Treatment with MOE showed a significant increase in the levels of SOD in the colon tissue, hence protecting the tissue from oxidative stress under the inflamed conditions. This was in accordance with the previous studies that the increase in SOD levels is due to its antioxidant activity of MOE³. MDA, a marker for lipid peroxidation was elevated due to the accumulation of free radicals by exposing the animals to chronic stress¹⁴. Chronic stress initiates lipid peroxidation of the membrane, leading to the impairment of the membrane structural and functional integrity. Previous reports suggest that lipid peroxidation and changes in antioxidant enzymatic system might play a crucial role in the pathogenesis of IBS. Animals exposed to water avoidance stress caused an increase in the levels of MDA, leading to oxidative damage. In our study, we found that treatment with MOE significantly decreased MDA levels due to a reduction in lipid peroxidation of colonic tissue¹.

MPO, an enzyme found in neutrophils, is a marker for neutrophil infiltration, and its activity is linearly related to the inflammatory response. Due to the development of low-grade inflammation in IBS, the enzyme is most active in inflamed mucosa. In the present study, the induction of colonic motility disorder by water avoidance stress increased the colonic MPO levels. The formation of inflammation can be attenuated by preventing neutrophil infiltration into the tissue. Treatment with *Moringa oleifera* significantly decreased the colonic MPO levels. In accordance with the previous reports, a decrease in the levels of MPO

may be due to a decrease in neutrophil infiltration and reduction in inflammatory process¹⁵.

CONCLUSION: The present study was carried out to find the effect of *Moringa oleifera* Lam roots on colonic motility disorders induced by water avoidance stress in rats. From the results, it was found that treatment with *Moringa oleifera* has protected animals from stress-induced colonic

motility disorders. The activity of *Moringa oleifera* may be due to the presence of phytochemical constituents like alkaloids, flavonoids, carbohydrates, tannins, and phenolic compounds. However, further studies are required to isolate and identify the active constant responsible for the activity and also focus on the mechanism of a colonic motility disorder.

TABLE 1: EFFECT OF MORINGA OLEIFERA LAM ROOT ON SOD, MDA, MPO AND CATALASE LEVELS IN WATER AVOIDANCE STRESS-INDUCED COLONIC MOTILITY DISORDER IN RATS

	Normal control	Disease control	Standard	Low dose	High dose
SOD	2.230 ± 0.233	0.933 ± 0.140 ^α	2.255 ± 0.122 ^a	1.958 ± 0.128 ^b	2.228 ± 0.158 ^a
MDA	1.762 ± 0.066	4.072 ± 0.143 ^α	1.617 ± 0.117 ^a	2.393 ± 0.107 ^{β,a}	1.940 ± 0.055 ^a
Catalase	2.248 ± 0.057	0.480 ± 0.046 ^α	2.470 ± 0.196 ^a	1.590 ± 0.106 ^{β,a}	2.105 ± 0.078 ^a
MPO	0.533 ± 0.025	1.085 ± 0.024 ^α	0.551 ± 0.044 ^a	0.705 ± 0.030 ^{β,a}	0.490 ± 0.034 ^a

Data expressed as mean ± SEM. All data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. αp < 0.0001 compared to normal control, βp < 0.001 compared to normal control, ap < 0.0001 compared to disease control, bp < 0.001 compared to disease control.

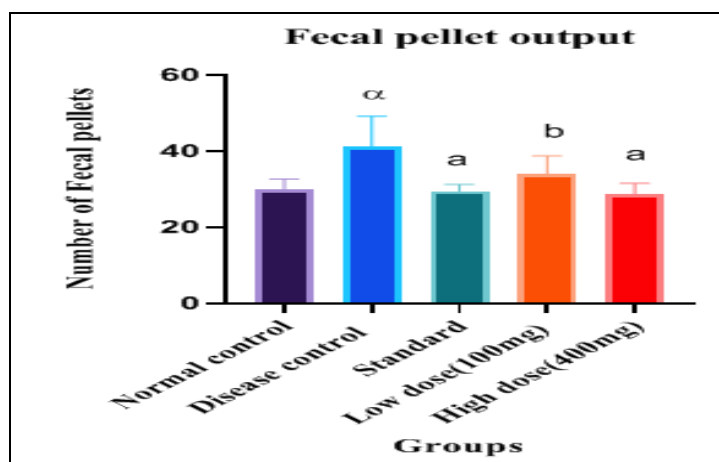


FIG 1: EFFECT OF MORINGA OLEIFERA LAM ROOT ON FECAL PELLET OUTPUT IN WATER AVOIDANCE STRESS-INDUCED COLONIC MOTILITY DISORDER IN RATS. Data expressed as mean ± SEM. All data were analyzed by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. ap < 0.0001 compared to normal control, ap < 0.0001 compared to disease control, bp < 0.001 compared to disease control.

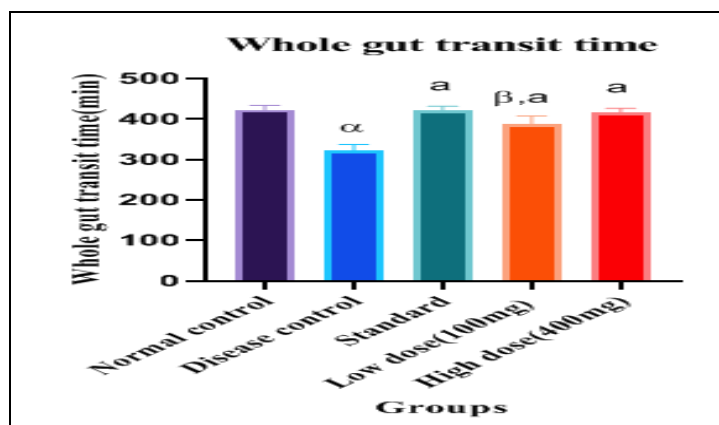


FIG 2: EFFECT OF MORINGA OLEIFERA LAM ROOT ON WHOLE GUT TRANSIT TIME IN WATER AVOIDANCE STRESS-INDUCED COLONIC MOTILITY DISORDER IN RATS. Data expressed as mean ± SEM. All data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. ap < 0.0001 compared to normal control, βp < 0.001 compared to normal control, ap < 0.0001 compared to disease control.

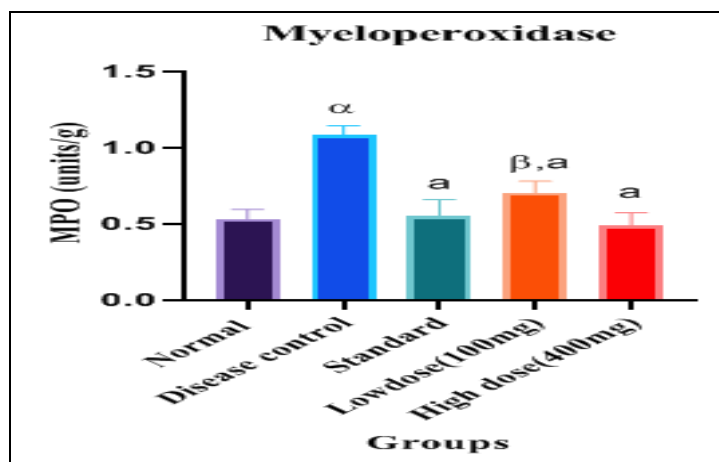


FIG. 3: EFFECT OF MORINGA OLEIFERA LAM ROOT ON MPO LEVEL IN WATER AVOIDANCE STRESS-INDUCED COLONIC MOTILITY DISORDER IN RATS. Data expressed as mean \pm SEM. All data were analyzed by one way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. $\alpha p < 0.0001$ compared to normal control, $\beta p < 0.001$ compared to normal control, $a p < 0.0001$ compared to disease control,

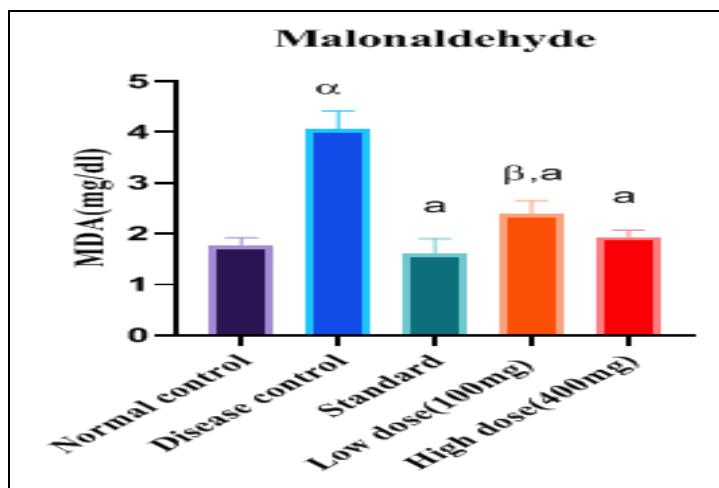


FIG. 4: EFFECT OF MORINGA OLEIFERA LAM ROOT ON MDA LEVEL IN WATER AVOIDANCE STRESS-INDUCED COLONIC MOTILITY DISORDER IN RATS. Data expressed as mean \pm SEM. All data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. $\alpha p < 0.0001$ compared to normal control, $a p < 0.0001$ compared to disease control, $b p < 0.001$ compared to disease control.

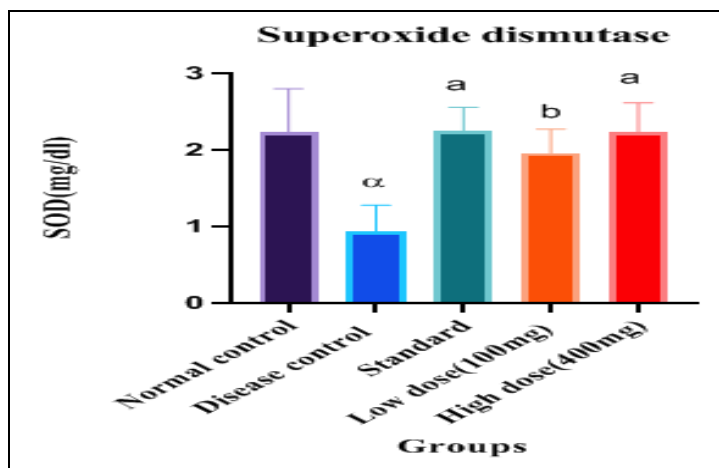


FIG. 5: EFFECT OF MORINGA OLEIFERA LAM ROOT ON SOD LEVEL IN WATER AVOIDANCE STRESS-INDUCED COLONIC MOTILITY DISORDER IN RATS. Data expressed as mean \pm SEM. All data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. $\alpha p < 0.0001$ compared to normal control, $\beta p < 0.001$ compared to normal control, $a p < 0.0001$ compared to disease control, $b p < 0.001$ compared to disease control.

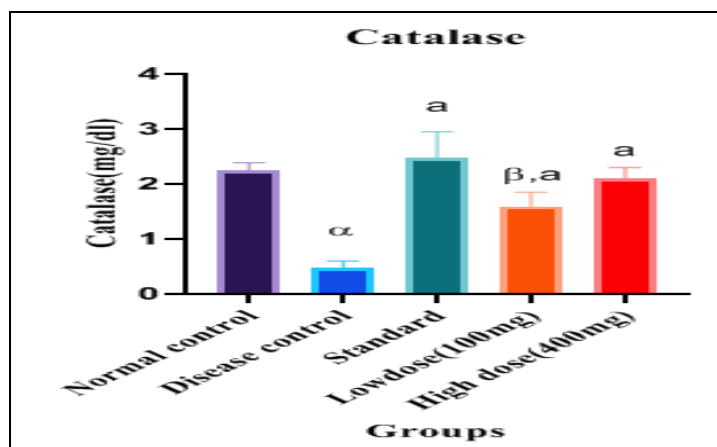


FIG. 6: EFFECT OF MORINGA OLEIFERA LAM ROOT ON CATALASE LEVEL IN WATER AVOIDANCE STRESS-INDUCED COLONIC MOTILITY DISORDER IN RATS IN RATS. Data expressed as mean \pm SEM. All data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. $\alpha p < 0.0001$ compared to normal control, $\beta p < 0.001$ compared to normal control, $\alpha p < 0.0001$ compared to disease control.

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