JOURNAL OF PHARMACOLOGICAL SCIENCES AND EXPERIMENTAL THERAPEUTICS
(Official Journal of the Nigerian Society for Pharmacological Sciences and Experimental Therapeutics)
ISSN 3115 - 6487

Methanol Leaf Extract of Dalbergia saxatilis Altered Nuetrophil Migration and Oxidative Stress Markers using Carrageenan-Induced Peritonitis in Albino Mice

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ARTICLE HISTORY

Received 26th August 2025
Revised 10th September 2025
Accepted 11th September 2025

Published 15th September 2025

Keywords:
Dalbergia saxatilis,
Peritonitis,
Antioxidant,
Anti-inflammatory,
Dexamethasone.

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ABSTRACT

Background: Several plants have been studied and their therapeutic properties have been established scientifically leading to the isolation and production of useful drugs. *Dalbergia saxatilis* is a plant that has been used traditionally in the treatment of swellings and some ailments.
Objectives: This study aims to evaluate the effect of the methanol leaf extract of *Dalbergia saxatilis* on Carrageenan-induced peritonitis in albino mice.
Method: The LD_{50} was determined using the OECD, 425 guidelines. Twenty-five (25) albino mice (25) divided into five groups (n=5 per group) were pretreated with distilled water as negative control, dexamethasone as the positive control, and the plant extract (1000 mg/kg, 500 mg/kg and 250 mg/kg) respectively. Peritonitis was induced with 1% carrageenan in saline solution (0.25ml per mouse). Leukocyte migration, Total protein and antioxidant (MDA, SOD, GSH and CAT levels) potential were determined.
Results: The LD_{50} of the extract was estimated to be > 5000 mg/kg. When compared with distilled water as the negative control and dexamethasone as the positive control, doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg of the extract were observed to possess anti-inflammatory potential due to its inhibition of leukocyte migration. It also had anti-oxidant activity due to the observed reduction in the levels of malondialdehyde (MDA) and catalase (CAT) and also an increase in levels of antioxidant enzymes superoxide dismutase (SOD) and glutathione (GSH).
Conclusion: Results from the study suggest that the extract possesses anti-inflammatory and anti-oxidant properties. Further studies should be carried out to determine the mechanism by which the extract exerts anti-inflammatory and antioxidant activity.

INTRODUCTION

Medicinal plants are the main sources of several valuable chemicals and/or drugs. Over 1300 medicinal plants are used globally, and of them, 90% are from wild sources. According to the International Union for Conservation of Nature and the World Wildlife Fund, about 50,000–80,000 of plants are used because of their medicinal values[1].
For centuries, Africans have treated different diseases using medicinal plants[2]. Several of these plants are indigenous to numerous African countries, including Nigeria. For instance, in Zimbabwe, there are over 5,000 plant species growing with about 10% of these plants having medicinal value, while in South Africa, a good number of flowering plants are used in the treatment and management of pain-related inflammatory disorders in both animal and human subjects[2]. In the majority of cases in Africa, traditional medicine is usually the first contact in meeting the primary health care need which is mainly due to its cheaper cost, easy accessibility, cultural and spiritual acceptance, and knowledge of its preparations and use[2].

A good number of pharmaceutical products that are used today are drawn from nature and traditional knowledge, including landmark drugs like: aspirin with its analgesic, antipyretic and antiplatelet properties, artemisinin with antimalarial action and morphine as a narcotic analgesic among others[3]. Numerous herbal medicines have been employed in the treatment of inflammation including but not limited to the commonly used ginger, rosemary, turmeric etc.[4].

Dalbergia are significant timber trees, treasured for their decorative and often fragrant wood, rich in aromatic oils and are infamous for causing allergic reactions owing to the presence of sensitizing quinones in the wood[5]. The plant is usually cultivated and collected in the wild primarily for its medicinal purposes and for its wood[6]. The plant is a member of the Fabaceae family and is used traditionally in the treatment of a variety of conditions from simple cough, swellings, edema, skin lesions and toothaches to bronchial ailments and smallpox[7].

Inflammation is a natural body defense mechanism for initiating the healing process in the body tissues after the removal of harmful stimuli. If left unchecked, it can develop into chronic inflammatory conditions such as autoimmune diseases such as rheumatoid arthritis, gastrointestinal disorders such as inflammatory bowel disease and even some cancer[8]. The majority of synthetic anti-inflammatory drugs have undesirable side effects such as nausea and vomiting, gastric disturbances like ulcers and constipation among others[9]. These factors highlight the importance of development of novel anti-inflammatory drugs preferably from medicinal plants due to their lower side effects[10].

This study evaluated the effect of the methanol leaf extract of *Dalbergia saxatilis* on Carrageenan-induced peritonitis in albino mice.

MATERIALS AND METHODS

Preparation of plant extract.

The fresh leaves of *Dalbergia saxatilis* were collected from its natural habitat in Giwa Local Government, Kaduna State, Nigeria in May, 2021. Identification and authentication of the plant was carried out by a trained taxonomist, Umar Gallah, in the Department of Botany, Kaduna State University (KASU) and a voucher specimen number (No KASU/BSH/1608) was issued for the plant for future reference. The collected leaves were then air dried under a shade until constant weight was obtained before being crushed into a powder. Subsequently, 500g of the powder was extracted with 1000ml of 70% methanol by maceration for seven (7) days method in accordance with the standard extraction procedure. The extract was evaporated to dryness on a water bath under reduced pressure and controlled temperature. The yield of methanol leaf extract of *Dalbergia saxatilis* was 30.6%. The residue was then kept in an airtight container for subsequent use.

Experimental animals

Twenty-five (25) albino mice of both sexes weighing 20-28g were used in the study. The animals were kept in plastic cages under standard laboratory conditions in the animal house facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria and were allowed access to food and water ad libitum. Mice were allowed to acclimatizea to the laboratory for about a one week prior to the test procedure. The study protocol and procedures were reviewed and approved by the institutional animal ethics committee of ABU, Zaria. Thereafter, the study was conducted in accordance with the guidelines for the use and care of experimental animals, ABUCAUC/2023/144.

Acute toxicity study

Acute toxicity study of *Dalbergia saxatilis* was carried out according to Organization for Economic Cooperation and Development (OECD 425)[12] guidelines for testing of chemicals acute oral toxicity. The animals were observed for the first 30 minutes after dosing, and periodically during the first 24 hours. Thereafter, observation was done every day for 14 days any sign of behavioral changes and symptoms of toxicity such as tremor, convulsion, salivation, lacrimation and respiratory distress, and mortality.

Experimental design for carrageenan-induced peritonitis test.

Carrageenan-induced peritonitis test was carried out as described by [11]. Mice were randomly divided into five groups each containing five mice treated as follows:

Group I: Distilled water (10 ml/kg)
Group II: Dexamethasone (2 mg/kg)
Group III: Methanol leaf extract (1000 mg/kg)
Group IV: Methanol leaf extract (500 mg/kg)
Group V: Methanol leaf extract (250 mg/kg)

All administrations were done via the oral route. The pretreated mice were left for thirty minutes prior to injection with 1% carrageenan in saline solution (0.25 ml per mouse) into the peritoneal cavity. Four (4) hours after carrageenan injection, the animals were sacrificed. Phosphate buffer, 3ml, was injected into the peritoneal space of each mouse before the animals were euthanized and the fluid contents of the peritoneal cavity were collected into plain bottles.

Determination of WBC (leukocytes count)

The number of leukocytes that had migrated to the peritoneum was determined in a Neubauer chamber

and results were expressed as cells × 10^6/mL as described by [11].
The % inhibition was calculated using the relation;
Inhibition (%) = (C-T/C) × 100%
Where; C = Control group, T = Test group

Determination of Total Protein
To determine the total protein, the Bradford protein assay was used. The sample was treated with Coomaise brilliant blue dye which caused a color change from brownish-green to blue, whereby the intensity of the color is directly proportional to the protein concentration. This color shift was measured spectrophotometrically at a wavelength of 595nm. To quantify the protein concentration, the absorbance of the sample was compared to a standard curve of known protein concentrations.

Determination of Malondialdehyde (MDA) activity
Malondialdehyde levels in each sample were measured using the thiobarbituric acid reaction[12]. Quantification of the thiobarbituric acid reactive substances was determined at 532 nm by comparing the absorption to the standard curve of MDA equivalents generated by acid-catalyzed hydrolysis of 1, 1, 3, 3-tetramethoxypropane. To measure MDA level, a working solution containing 15% trichloroacetic acid, 0.375% thiobarbituric acid, and 0.25 N hydrochloric acid was prepared. For each sample, 250 μL serum and 500 μL working solution were mixed and placed in boiling water for 10 min. After cooling the samples were centrifuged at 3000 rpm for 10 min. Finally, 200 μL of each supernatant was transferred to microplates and the optical density of samples was measured at 535 nm. The values of MDA were expressed as μmol/L.

Determination of Superoxide dismutase (SOD) activity
The determination of the SOD activity was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazon dye. Briefly, 300 μL of mixed substrate was added to 200 μL of diluted hemolysates. The samples were mixed well and 75 μL xanthine oxidase was added to reactions. The absorbance was measured at 505 nm and the SOD activity was then calculated according to the manufacturer's instruction (Ransod®-Randox Lab, Antrim, UK) and expressed as U/mL.

Determination of Reduced Glutathione Activity
Determination of the level of reduced glutathione (GSH) level was done according to the method described by [13]. In this method thiols react with Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid) or DTNB), cleaving the disulfide bond to give 2-nitro-5-thiobenzoate (TNB^-), which ionizes to the TNB^{2-} dianion in water at neutral and alkaline pH.
To evaluate GSH level in samples, 15 μL of hemolysates was mixed with 260 μL assay buffer (0.1 M sodium phosphate and 1 mM EDTA, pH: 8) and 5 μL Ellman reagents. Samples were incubated for 15 min at room temperature and the TNB^{2-} formation was quantified in a spectrophotometer by measuring the absorbance of visible light at 412 nm. Absorbance values were compared with a standard curve generated from standard curve from known GSH.

Determination of Catalase Activity
Catalase activity was determined spectrophotometrically by the method of[14]. Briefly, 10 μL of sample was incubated with 100 μmol/mL of H_2O_2 in 0.05 mmol/L Tris-HCl buffer pH = 7 for 10 min. The reaction was terminated by rapidly adding 50 μL of 4% ammonium molybdate. Yellow complex of ammonium molybdate and H_2O_2 was measured at 410 nm. One unit of catalase activity was defined as the amount of enzyme required to decompose 1 μmol H_2O_2 per min.

Data analysis
Data were analyzed usingStatistical Package for Social Sciences (SPSS) version 20 software and one-way analysis of variance (ANOVA), followed by Bonferroni post hoc test. Results obtained were presented as tables and graphs (mean ±SEM). Significance was considered at $p \leq 0.05$.

RESULTS
The LD_{50} of the extract was estimated to be > 5000 mg/kg. LD50 was above 5000mg/kg and did not cause mortality in the tested rats (Namadina *et al*., 2019). The methanol leaf extract of *Dalbergia saxatilis* (250mg/kg, 500mg/kg and 1000mg/kg) decreased the total amount of leukocytes in a dose-dependent manner by 19.94%, 29.58% and 31.83% respectively when compared with the negative control group (Table 1). No significant change was observed when the extract groups were compared with dexamethasone.

Table 1: Effect of *Dalbergia saxatilis* on Leukocyte Migration in Mice

Treatment mg/kg	Total Leukocytes x 10^6/mL	% Inhibition	% Lymphocytes	% Neutrophils	% Mixed
Distilled water 10 ml/kg	6.22 ± 0.38	-	44.0 ± 3.53	54.0 ± 2.41	1.8 ± 0.4
Dexamethasone 2	4.46 ± 0.27*	28.30	60.4 ± 2.74**	37.2 ± 2.33**	3.2 ± 0.2*
Extract 250	4.98 ± 0.51	19.94	47.6 ± 2.12	49.8 ± 2.44	2.4 ± 0.3
Extract 500	4.38 ± 0.38*	29.58	58.4 ± 2.67**	39.2 ± 2.78*	3.0 ± 0.5*
Extract 1000	4.24 ± 0.37*	31.83	59.0 ± 2.30**	38.2 ± 2.85**	3.2 ± 0.4*

Values are Mean ± SEM. *represents P < 0.05; and ** represents p < 0.01 when other group are compared with Distilled water group. One way ANOVA followed by Bonferroni post hoc test.

The level of total protein was significantly ($p < 0.05$) reduced in Dexamethasone group and all doses of the extract tested (250, 500, and 1000 mg/kg) when compared to Distilled water group, no significant change was observed when the extract groups were compared with dexamethasone.
(Figure 1).

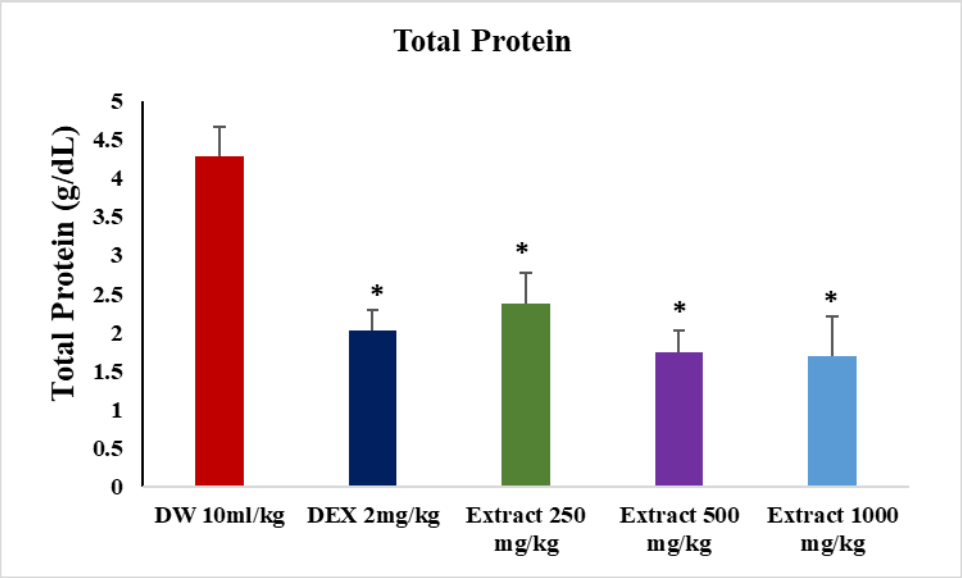

Figure 1: Effect of the Methanol Leaf Extract of *Dalbergia saxatilis* on Total Protein.
Values are mean ± SEM, n = 5. * represents $p < 0.05$ level of significance when other groups are compared with Distilled water group. One-way ANOVA followed by Bonferroni post hoc test. DW = Distilled Water, DEX = Dexamethasone

MDA levels were significantly ($p < 0.05$) reduced in all the groups treated with the extract (250, 500, 1000 mg/kg) when compared with Distilled water group, no significant change was observed when the extract groups were compared with dexamethasone.
(Fig 2).

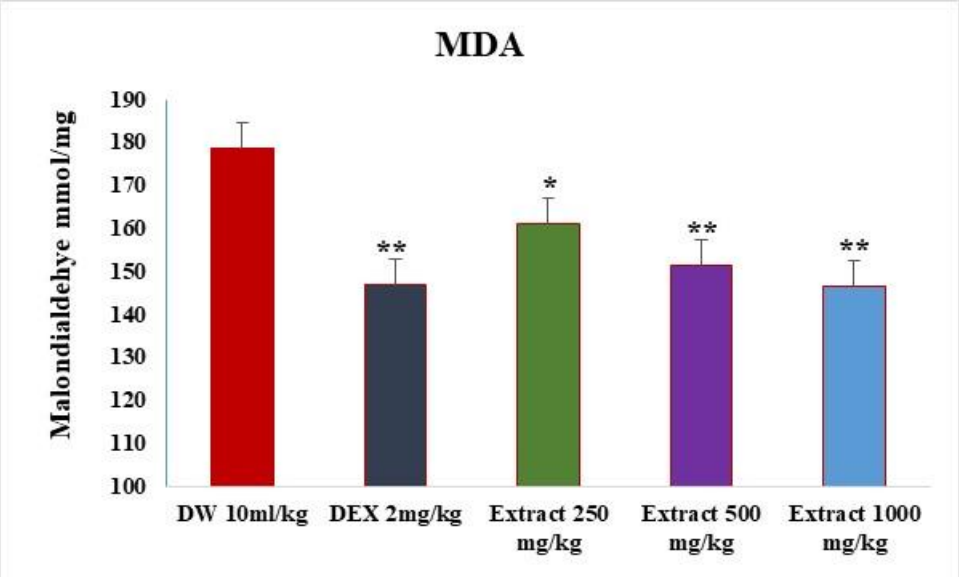

Figure 4.2: Effect of methanol leaf extract of *Dalbergia saxatilis* on Malondialdehyde (MDA)
Values are mean ± SEM, n = 5. *Represents $p < 0.05$ levels of significance ** represents $p < 0.01$ level of significance when other groups are compared with distilled water group. One-way ANOVA followed by Bonferroni post hoc test. DW = Distilled Water, DEX = Dexamethasone

SOD levels were significantly ($p < 0.05$) increased dose dependently in all the groups treated with the extract (250, 500, 1000 mg/kg) when compared with distilled water group (Fig 3).

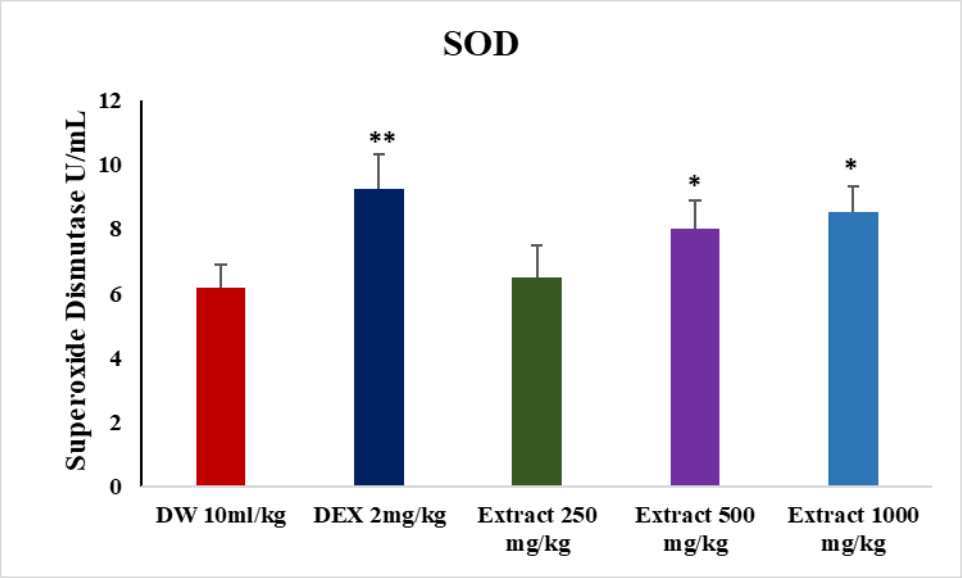

Figure 3: Effect of methanol leaf extract of *Dalbergia saxatilis* on Superoxide Dismutase (SOD)
Values are mean ± SEM, n = 5. * represents $p < 0.05$ and ** represents $p < 0.01$ levels of significance when other groups are compared with distilled water group. One-way ANOVA followed by Bonferroni post hoc test. DW = Distilled Water, DEX = Dexamethasone

Reduced glutathione levels were significantly ($p < 0.05$) increased dose dependently in all the groups treated with the plant extract (250, 500, 1000 mg/kg) when compared to the control group, no significant change was observed when the extract groups were compared with dexamethasone (Figure 4).

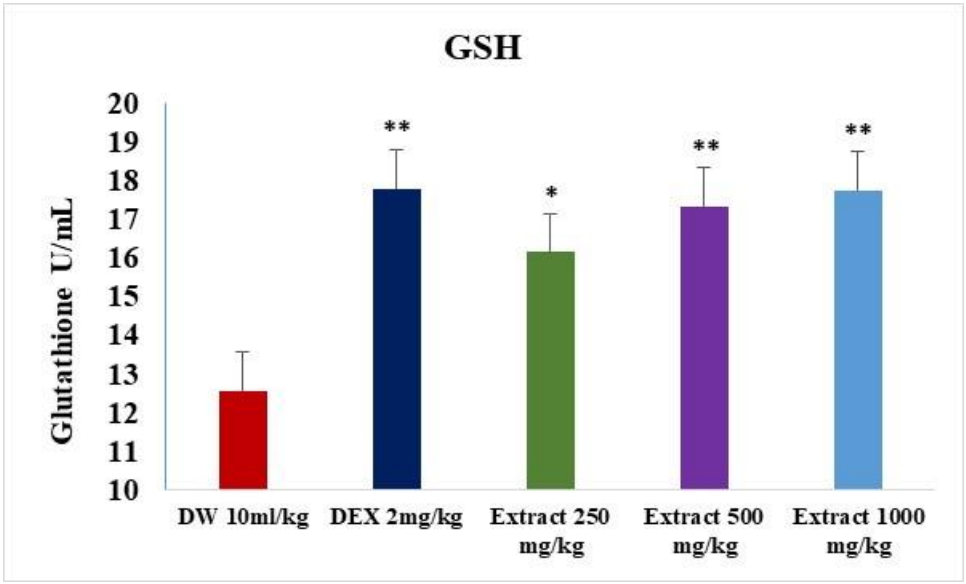

Figure 4: Effect of methanol leaf extract of *Dalbergia saxatilis* on reduced Glutathione (GSH)
Values are mean ± SEM, n = 5, * represents $p < 0.05$; ** represents $p < 0.01$ levels of significance when other groups are compared with distilled water group. One-way ANOVA followed by Bonferroni post hoc test. DW = Distilled Water, DEX = Dexamethasone

Levels of CAT appeared to be significantly ($p < 0.05$) reduced in the groups tested with the extract (500, 1000 mg/kg) when compared with Distilled water group. Those treated with the lowest dose of the extract (250 mg/kg) had levels similar to the group treated with distilled water (Fig 5).

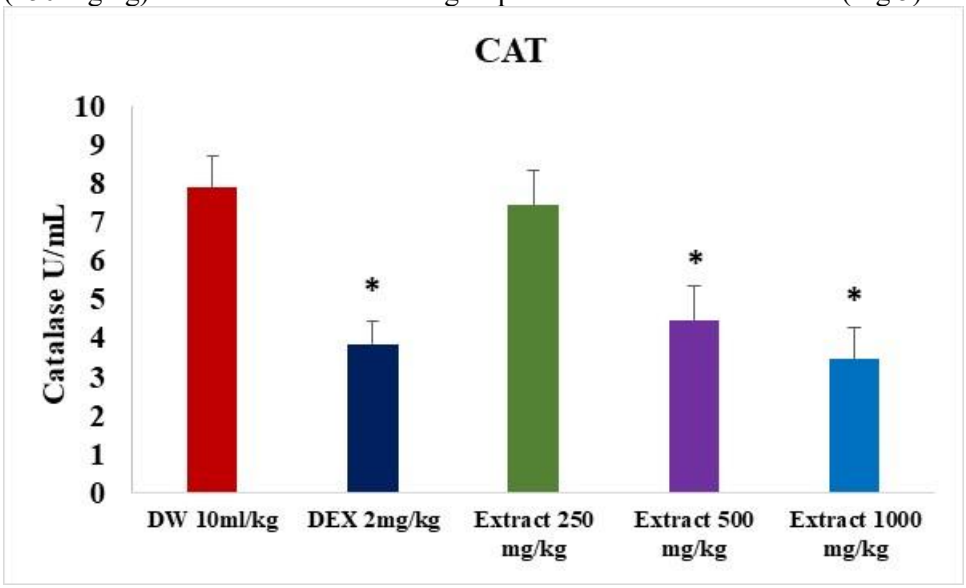

Figure 5: Effect of methanol leaf *Dalbergia saxatilis* on Catalase (CAT)
Values are mean ± SEM, n = 5, * represents $p < 0.05$ levels of significance when other groups are compared with distilled water group. One-way ANOVA followed by Bonferroni post hoc test. DW = Distilled Water, DEX = Dexamethasone

DISCUSSION

Peritonitis serves as an adequate model for determination of leukocytes (neutrophil migration) in inflammation[15]. Leukocytes readily occupy the peritoneum as a result of induction of inflammation by carrageenan; hence, the carrageenan-induced peritonitis model has been widely used. This model makes it possible to assess neutrophil migration and other inflammatory markers[16].

Dalbergia saxatilis plant extract was shown to have anti-inflammatory activity by inhibiting carrageenan-induced neutrophil migration in the experimental model of peritonitis. Both the extract at different doses and dexamethasone as the positive control group reduced the number of leukocytes in the peritoneal fluid. Dexamethasone reduces inflammation by suppressing the migration of neutrophils and decreasing lymphocyte colony

proliferation and capillary membrane permeability[17]. Also, it causes an increase in serum levels of vitamin A compounds, prostaglandins and inhibits some cytokines (IL-1, IL-12, IL-18, TNF, interferon-gamma and granulocyte-macrophage colony-stimulating factor).

The pattern of inhibition of leukocytes by dexamethasone, (with majority being Lymphocytes) was similar to that observed in the inhibition by higher doses of the extract. The lowest dose appeared to have inhibited the lymphocytes and neutrophils to a similar extent. At higher doses, the extract inhibits lymphocytes favorably[18].

During acute inflammation, chemical mediators such as histamine induce vasodilation and vascular permeability, which allows leukocytes and plasma proteins to enter the extravascular tissues[19]. This eventually results in the accumulation of protein-rich fluid (exudate) at the site of injury. The plant extract significantly reduced total protein levels in the peritoneal fluid at the different doses signifying that the plant extract may prevent protein leakage into the extravascular sites.

To evaluate the anti-oxidant activity of the plant extract, the malonaldehyde (MDA), superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) levels were assayed. Malondialdehyde (MDA) is an end product formed through the decomposition of lipid peroxidation products[20]. It is produced from the attack of free radicals on polyunsaturated fatty acids (PUFAs) and serves as a marker of oxidative stress by evaluation of the levels of Thiobarbituric acid (TBARS). MDA levels were reduced in the peritoneal fluid by all doses of the extract, suggesting the extract may have the ability to reduce free reactive oxygen radicals.

SOD and GSH are important enzymes which acts as defenses against oxidative stress in the body. GSH has the ability to minimize oxidative stress by detoxifying the reactive intermediates of drug metabolism and by reducing lipid peroxidation of cellular membranes and other cellular targets[21]. SOD is an enzyme which acts as a good therapeutic agent against several reactive oxygen species-mediated disease[22]. The levels of these antioxidants were significantly increased in the peritoneal fluid by the plant extract as compared to the negative control which indicated that the extract may possess protective effect against oxidative stress and the antioxidant activity of the extract.

CAT is an antioxidant that moderates oxidative stress by destroying hydrogen peroxide intracellularly to produce water[23]. According to previous study carried out on the plant, the antioxidant potential of the methanol leaf extract of *Dalbergia saxatilis* was reflected by an increase in CAT levels[24], however, an opposite effect was observed in this study. The decrease in catalase activity observed in the study may be related to the decrease of leukocyte cells in the peritoneal exudate, since part of this enzyme is produced by the mitochondria of mainly leukocytes whose levels are concurrently reduced by the extract. Dexamethasone increases levels of Malondialdehyde (MDA) and the activity of antioxidants including Glutathione (GSH), Superoxide dismutase (SOD) and also Catalase (CAT) in the kidney tissues after intraperitoneal injection into rats[25]. In this study, the levels of MDA and CAT were decreased by the extract, while the levels of SOD and GSH were increased by the extract.

CONCLUSION

The methanol leaf extract of *Dalbergia saxatilis* reduced carrageenan-induced peritonitis by its significant antioxidant activity due to increased MDA and SOD, and reduced GSH levels. It also has an anti-inflammatory effect due to its action on neutrophil migration and protein content. Further studies should be carried out on the to accurately identify the particular phytochemical possessing the antioxidant and anti-inflammatory activity with its mechanism of action. The results may serve to pave the way for establishing the efficacy of the extract.

Conflict of Interest

Authors declare no conflict of interest.

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