Anti-Inflammatory potential of methanol leaf extract of *Senna italica* (Mill.) using cotton pellet-induced granuloma model in Wistar rats

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Abstract

Senna italica Mill. belonging to the family Fabaceae has been used traditionally to treat various ailments diseases including skin diseases, fever, jaundice, stomachache, burns and inflammation. The aim of the present study was to evaluate the phytochemical profile and the anti-inflammatory activity of the methanol leaf extract of the plant. Preliminary phytochemical screening and oral acute toxicity studies were carried out using standard protocols. Anti-inflammatory activity was investigated in Wistar rats using cotton pellet-induced granuloma model at doses of 250, 500 and 1000 mg/kg. Hematological and biochemical parameters were also evaluated. Phytochemical screening revealed the presence of tannins, saponins, carbohydrate, alkaloids, flavonoids, and steroids. The oral median lethal dose was estimated to be greater than 5000 mg/kg. The extract reduced wet granuloma weight with percentage inhibition of 5.9%, 52.9% and 47.1 % at doses of 250, 500 and 1000 mg/kg respectively compared to the negative control. At same doses, the extract showed 14.3%, 17.1% and 28.6% inhibition in weight of dry cotton pellet. A significant ($p<0.05$) reduction was observed in platelets, platelet lymphocyte ratio, creatinine, aspartate aminotransferase and alkaline phosphatase levels. The result from this study suggests that the methanol leaf extract of *Senna italica* has anti-inflammatory activity supporting the traditional use of the plant in the treatment of inflammation and inflammatory related diseases.

Keywords: *Senna italica*, Inflammation, Cotton pellet - induced granuloma, Phytochemical

Introduction

Inflammation is the protective response of the body against injury and infections which may be caused by bacteria, viruses, fungi, parasites, and antigen challenge (Khan *et al*., 2019). It is a multifaceted biological response of the vascular tissues to injurious stimuli which may eventually result in edema formation, leukocyte infiltration and granuloma formation (Gorzalczany *et al*., 2011). Inflammation which can be classified as acute or chronic is characterized by five principal symptoms including redness (rubor), swelling (oedema), heat (calor), pain (dolor), and loss of function (Takeuchi *et al*., 2010; Bennett *et al*., 2018). Inflammation is a common pathogenesis of many chronic diseases including arthritis, cardiovascular disease, diabetes mellitus, inflammatory bowel diseases, retinitis, multiple sclerosis, and cancer which have become major health concerns worldwide, resulting in increased morbidities and mortalities yearly (Furman *et al*., 2019; Pahwa *et al*., 2021). Unfortunately, nonsteroidal anti-inflammatory drugs (NSAIDs), steroidal drugs, and immuno-suppressant drugs, used in the relief of these inflammatory diseases are often associated with severe adverse side effects, such as gastrointestinal bleeding, peptic ulcer, epigastric distress, and iatrogenic Cushing's syndrome (da Silva *et al*., 2014; Meshram *et al*., 2015). Thus, there is an increasing necessity to develop novel anti-inflammatory drugs with less side effects than the agents currently used in the treatment of inflammation. Medicinal plants could therefore provide an excellent source for the discovery of lead compounds which could help in the development of novel, more efficacious, and safer anti-inflammatory agents.

Senna italica Mill. with synonyms *Cassia italica* and *Acacia obovata* belongs to the family Fabaceae (Schmelzer and Gurib-Fakim, 2008). The plant is widely distributed in African countries, Iran, Iraq, Pakistan and from India to Sri lanka due to its ability to grow and flourish in diverse climates and soil types (Okeyo and Bosch, 2007). *Senna italica* Mill. is a perennial herb or small shrub and grows about 50 to 75 cm in height with herbaceous branches from woodystock. Flowers are yellow, nectar less and bisexual (Maeazzi *et al*., 2007).

Traditionally, various parts of *Senna italica* are used to cure many diseases including stomach complaints, fever, jaundice, venereal diseases, biliousness, burns, ulcers, constipation, colic, influenza, nausea, vomiting and dysmenorrhea (Schmelzer and Gurib-Fakim, 2008; Vijaya *et al*., 2018). Previous studies have reported that *Senna italica* exhibited a broad spectrum of pharmacological activities such as analgesic and central nervous system depressant activities (Ali *et al*., 1997) antipyretic, analgesic activities (Jain, 1997) antioxidant, antibacterial, and antiproliferative activities (Masoko *et al*., 2009) cytotoxic activity (Mohammed, 2014), antioxidant activity (Jothi *et al*., 2015), hypoglycaemic and antiobesity effects (Malematja *et al*., 2018). Compounds have also been isolated from the plant namely, physcion, emodin, 2-methoxy-emodin-6-O-ßD-glucopyranoside,1-hydroxy-2-acetyl-3-methyl-6hydroxy-8-methoxynaphthalene (tinnevellin), quercetin 3-O αL-rhamnopyranosyl-(1→6)-ß-D-glucopyranoside (rutin) and 1,6,8-trihydroxy-3-methoxy-9,10-dioxo-9,10-dihydroanthracene by bio-guided fractionation (Khala *et al*., 2017). This study evaluated the anti-inflammatory effect of the methanol leaf extract of *Senna italica* using cotton pellet-induced granuloma method in Wistar rats.

Materials and Methods

Plant Collection and Identification

Fresh leaves of *Senna italica* were collected in April 2021 from Nguru local government, Yobe state Nigeria. They were identified and authenticated by Mr. N. Sambo, a botanist with the Herbarium Section of Department of Botany, Faculty of Life Sciences, Ahmadu Bello University Zaria by comparison with an existing specimen with voucher number (ABU03106).

Extraction of Plant Material

The collected leaves of *Senna italica* were gently washed with water and air dried under shade until a constant weight was obtained. The dried leaves were ground into fine powder with the aid of a pestle and mortar and stored in an airtight glass container. Two hundred and fifty-five grams (255 g) of the leaf powder was macerated with 70%v/v methanol under constant agitation. The resulting mixture was then filtered through Whatman no.1 filter paper and the residue were re-extracted. The filtrate was concentrated over a water bath at 45°C to remove excess solvent. The extract obtained was placed in a jar, labelled MLSE (methanol leaf extract of *Senna italica*) and then stored in a desiccator until further use.

Experimental Animals

Adult male and female Wistar rats weighing between 150 – 200g were obtained from Animal House facility of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria. The rats were housed in a well-ventilated room under standard laboratory conditions and were provided with standard pellet diet and water *ad libitum*. All experiments performed on the laboratory animals were in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals by the National Institute of Health (Publication No. 80-23, 2011).

Reagents/Drugs

The chemicals and reagents used were of analytical grades and include tabs Aspirin (manufactured by Biopharm Nigeria Limited Exp.06/2024), Injection-ketamine (manufactured by Embassy Pharmaceutical and Chemical Limited Exp.09/2023), 0.9% normal saline, distilled water, Injection-penicillin (manufactured by Clarion Medicals Limited Exp.09/2023), Injection-streptomycin (Health and Longevity International Limited Exp.09/2023).

Experimental Grouping and Treatment

Animals were divided into five groups of six animals in each group as follows:
Group I: Negative control received 1 ml/kg distilled water
Group II: Treated with MLSE 250 mg/kg
Group III: Treated with MLSE 500 mg/kg
Group IV: Treated with MLSE 1000 mg/kg
Group V: Treated Aspirin 150 mg/kg
Treatments were given once daily for 9 days.

Qualitative Phytochemical Screening

Preliminary phytochemical analysis for the identification of secondary metabolites including alkaloids, flavonoids, tannins, anthraquinones, cardiac glycosides, saponins, and steroids was performed according to the methods described by Trease and Evans (2004).

Acute Toxicity Study

Oral acute toxicity study in female rats was carried out based on the guideline specified by the Organization of Economic Co-operation and Development 423 (OECD, 2001). Six (6) animals divided int two groups were fasted prior to the commencement of the study. From the first group a single rat was administered orally with the extract at a dose of 5000 mg/kg rat and observed for 24 hours. In the absence of mortality, the same dose

was given to the other two rats. Distilled water 10 ml/kg were administered to the second group of animals. The animals were housed separately and observed (for signs of toxicity or mortality) on the day of dosing at 30 min, 1hr, 2hr and 4hr and until for 14 days of observation period.

Cotton Pellet-Induced Granuloma Model

The cotton pellet-induced granuloma model was adopted to induce chronic inflammation in experimental animals. The method described by Swingle and Shideman, (1972) was employed. Thirty (30) rats were randomly divided into 5 groups, each containing 6 animals. On day 1, a sterilized cotton pellet weighing 50 mg was surgically inserted into the dorsal part of the rats under anesthesia (ketamine hydrochloride, 25 mg/kg) Rats in the negative control group received distilled water orally (1 mL/kg), the positive group received aspirin orally (150 mg/kg), while the 3 test groups were treated with the extract at doses of 250, 500 and 1000 mg/kg orally, daily, for 9 consecutive days. On the 10th day, the animals were subjected to light ether anesthesia, the wet cotton pellets were removed and weighed to measure the antitransudative effect. These pellets were later dried were dried to constant weight in a hot air oven and weighed again to determine the dry weights of the cotton pellets, which corresponded to the antiproliferative effect. Percentage inhibition was calculated using the formula below.

$$\text{Percentage inhibition} = \frac{\text{Tc} - \text{Tt}}{\text{Tc}} \times 100,$$

where Tc = weight of granuloma tissue of the control group and Tt = weight of granuloma tissue of the treated group (Okoli *et al*., 2007).

Hematological Studies

Blood samples (about 2 mls) were collected by cardiac puncture into EDTA bottles for estimation of Packed Cell Volume (PCV), haemoglobin concentration (Hb), platelets (PLT), white blood cell count (WBC) and differentials, mean corpuscular haemoglobin concentration (MCHC) using an automated haemotological machine (Sysmex-XT-1800, Germany).

Biochemical Studies

Blood samples (about 2 mls) were collected into plain bottles, allowed to clot and centrifuged at 3,500 rpm for 10 minutes. The separated sera were stored at -4^{o}C, and used for evaluation of biochemical parameters. These parameters included; urea, creatinine, alanine aminotranferase (ALT), aspartateaminotranferase (AST), and alkaline phosphatase (ALP).

Statistical Analysis

Data was expressed as Mean ± SEM (n=6). Statistical analysis was carried out using SPSS Version 25. Difference between groups was analyzed by One-way ANOVA followed by Dunnetts post hoc test. $p < 0.05$ was considered statistically significant.

Results

Oral Acute Toxicity Studies

There were no signs or symptoms observed in the animals after oral administration of MLES. The median lethal dose was thus estimated to be greater than 5000 mg/kg.

Phytochemical Screening

Preliminary phytochemical screening of the extract revealed the presence of several phytochemicals as shown in table 1.

Table 1: Phytochemical Constituents Present

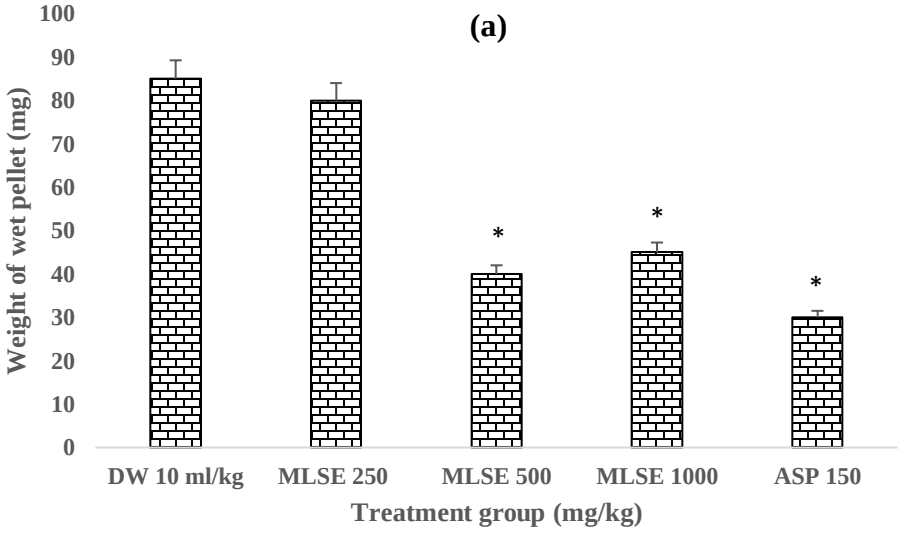
in the Methanol Leaf Extract of *Senna italica*

Phytochemical constituents	Inference
Carbohydrates	+
Steroids	+
Flavonoids	+
Tannins	+
Anthraquinones	-
Saponins	+
Cardiac glycosides	+
Alkaloids	+

Key; + = Present, - = Absent

Effect of Methanol Leaf Extract of *Senna italica* on Cotton Pellet-Induced Granuloma Weight

Administration of MLSE at higher doses produced a significant (p<0.05) reduction in the weight of both the dry and wet granuloma (Figures 1a and 1b). The standard drug (Aspirin, 150 mg/kg) however produced a better reduction in the weight of both wet and dry granuloma.



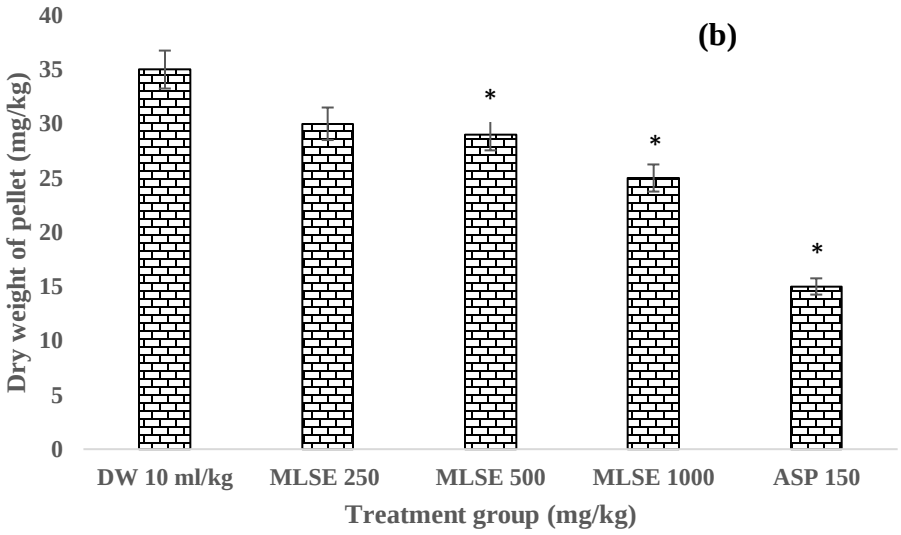


Figure 1: Effects of Methanol Leaf Extract of *Senna italica* Cotton Pellet-induced Granuloma in Rats. (a) Wet weight of cotton pellet; (b) Dry weight of cotton pellet

Values are presented as Mean + SEM; Data analyzed by one way ANOVA followed by Dunnett's Post-hoc test; n=6; * = $p<0.05$ versus control; DW = Distilled Water; MLSE = Methanol Leaf Extract of *Senna italica*; ASP = Aspirin; Route of administration = oral

Evaluation of the Level of Some Haematological Parameters after Oral Administration of the Methanol Leaf Extract of Senna italica

The extract produced a reduction in the levels of lymphocytes compared to the negative control group (distilled water treated group). However, this was not significant. There was a statistically significant ($p<0.05$) decrease in the platelet levels and platelet lymphocyte ratio (Table 2).

Table 2: Effect of Oral Administration of Methanol Leaf Extract of *Senna italica* on Some Hematological Indices of Rats

	Doses (mg/kg) of MLES				
Hematological Indices	**Distilled Water 1 ml/kg**	**250**	**500**	**1,000**	**ASP 150**
GRAN (10^{-3}µL)	2.44 ± 0.28	4.00 ± 0.58	3.60 ± 0.20	3.20 ± 0.24	3.00 ± 0.37
PLT ($\times10^{9}$L)	282.6± 3.54	266.0± 0.68	226.8 ±3.96*	204.20 ± 0.80*	182.4±4.5*
MPV (10^{-3}µL)	8.14±0.51	8.22±0.54	7.74±0.49	7.56±0.01	7.23±0.63
PDW	7.80±0.10	7.90±0.00	7.80±0.10	7.90±0.00	7.90±0.00
PLR	62.85±0.72	61.86±9.63	39.03±4.39*	38.51±4.17*	30.77±1.57*
WBC ($\times10^{9}$L)	4.04 ± 0.12	5.36 ± 0.75	4.28 ± 0.31	4.36 ± 0.20	5.14 ± 0.49
HGB (g/dL)	13.16± 0.57	12.42± 1.14	15.80± 2. 09	12.86 ± 0.34	12.94 ± 0.37
LYMPH (10^{-3}µL)	5.92 ± 0.14	4.72 ± 0.49	5.84 ± 0.11	4.28 ± 0.33	4.58 ± 0.26
RBC ($\times10^{12}$L)	6.02 ± 0.07	5.94±0.02	5.96 ± 0.02	5.82 ± 0.13	5.76 ± 0.19

Data = Mean ± SEM; Statistical tool = ANOVA followed by Dunnett's Post-hoc test; * = $p<0.05$ statistically significant difference compared to control; GRAN = Granulocyte; PLT = Platelet; MPV= Mean Platelet Volume; PDW = Platelet Distribution Width; PLR = Platelet Lymphocyte Ratio; WBC = White Blood Cell; HGB = Hemoglobin; LYMPH = Lymphocyte; RBC = Red Blood Cell; ASP = Aspirin; MLES = Methanol Leaf Extract of *Senna italica*; n = 6

Effect of Methanol Leaf Extract of *Senna italica* on Urea and Creatinine Levels in Rats

Administration of the extract at 500 and 1000 mg/kg doses significantly ($p<0.05$) decreased creatinine levels compared to the negative control. However only the standard drug (aspirin 150 mg) was able to reduce urea levels significntly ($p<0.05$) compared to the extract treated and negative control groups (Figure 2).

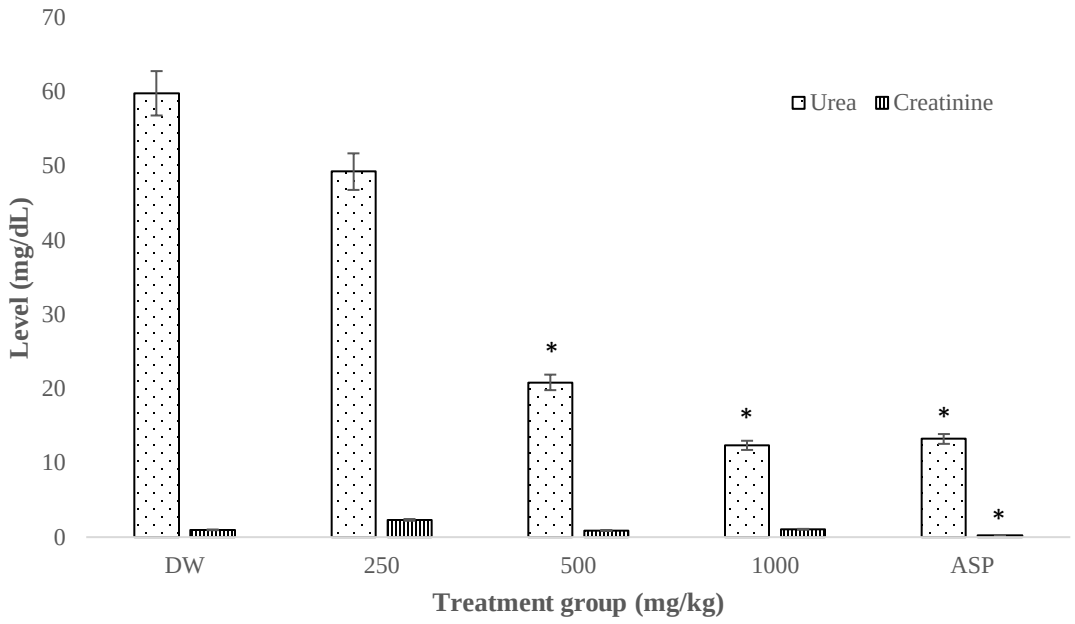


Figure 2: Effect of Methanol Leaf Extract of *Senna italica* on Urea and Creatinine Levels in Rats

Data = Mean ± SEM; Statistical tool = ANOVA followed by Dunnett's Post-hoc test; * = $p<0.05$ statistically significant difference compared to control; Na^+ = Sodium ion; K^+ = Potassium ion; Cl^- = Chloride ion; HCO_3^- = Bicarbonate ion; Cr = Creatinine; MLSE = Methanol Leaf Extract of *Senna italica*; n = 6

Effect of Methanol Leaf Extract of *Senna italica* on Some Liver Function Indices in Rats

There was a statistically significant ($p<0.05$) decrease in ALP and AST levels in both the extract and aspirin (positive control) treated groups compared to the distilled water treated group. There was also a reduction in ALT levels at all doses including the standard drug (Aspirin 150 mg/kg) but this was not significant

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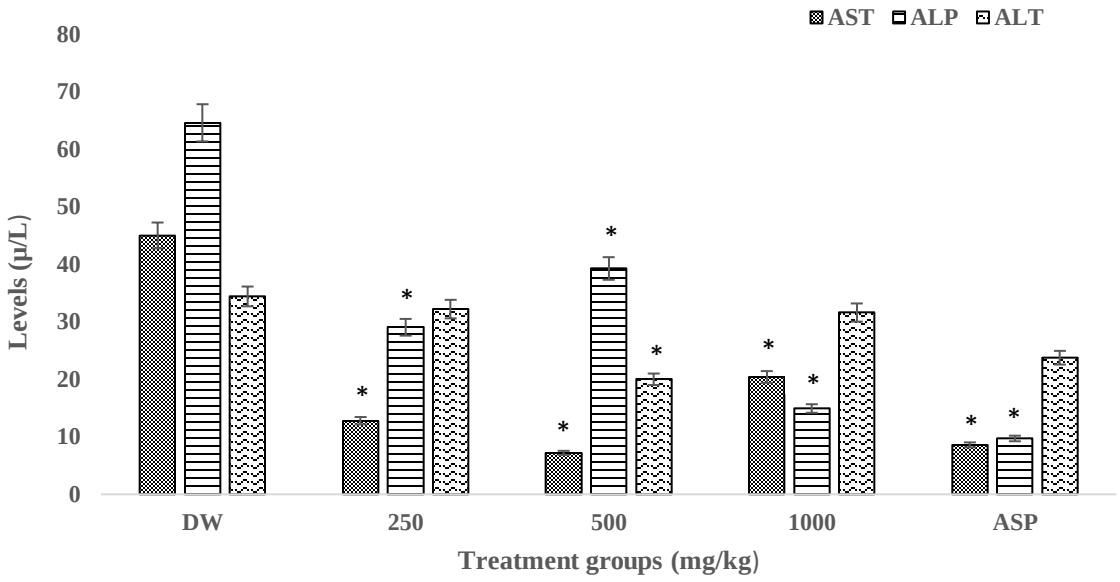


Figure 3: Effect of Methanol Leaf Extract of *Senna italica* on Some Liver Function Indices in Rats

Data = Mean ± SEM; Statistical tool = ANOVA followed by Dunnett's Post-hoc test; * = $p<0.05$ statistically significant difference compared to control; DW = Distilled water; AST = Aspartate Aminotransferase; ALP = Alkaline Phosphatase; ALT = Alanine Aminotransferase; n = 6

Discussion

Senna is an important genus of flowering plants, comprising nearly of 350 species, and widely distributed in tropical and subtropical zones of the world (Khala *et al*., 2017). From literature the leaves, pods, and ripe seeds of *Senna italica* are used traditionally to treat inflammation, stomach aches, fever, jaundice, venereal diseases, and bilious crises, as well as an abortifacient and against intestinal worms (Vijaya *et al*., 2018). The present study sought to validate the folkoric use of the plant in the treatment of inflammation.

Oral administration of MLES did not produce any signs or symptoms of toxicity in the rats used in the study. The oral median lethal dose was thus estimated to be greater than 5000 mg/kg. Acute toxicity studies are designed to determine the dose that will produce mortality and/or serious toxicological effects when given once or over repeated administration. The absence of any change in the general behavior, body weight and mortality of the rats after oral administration of MLES suggests that the plant is considerably safe (Lorke, 1983).

Phytochemical constituents of plants mainly the secondary metabolites have been reported to possess biological and therapeutic properties (Benedec *et al*., 2013). Preliminary phytochemical screening of *Senna italica* revealed the presence of tannins, flavonoids, alkaloids, carbohydrates, sterols, and glycosides. Several phytochemical compounds with a wide various biological activities have been isolated from the genus Senna including quinones (Abegaz *et al*., 1994) anthraquinones (Alamayehu *et al*., 1989),

naphthopyrones (Barbosa *et al*., 2004), triterpenoids (Li *et al*., 2012) and flavonoids (Baez *et al*., 1999). The presence of some these bioactive compounds found in the leaf extract could be responsible for any observed pharmacological activity (Alshehri *et al*., 2022).

Cotton pellet-induced granuloma is an established model for evaluating the transudative and proliferative components of chronic inflammation (Winter and Porter, 1957; Dzoyem *et al*., 2017; Akhtar, 2022). The subcutaneous implantation of a cotton pellet into a rodent results in the formation of a granuloma at the site of the implant, a process which involves mediators of inflammation including cytokines, chemokines, and eicosanoids (Zhang *et al*., 2019). The weight of the wet cotton pellets is related to proliferative material and the weight of the dry pellets is related to the amount of granulomatous tissue (Chitsaza *et al*., 2021). Cotton pellet-induced granuloma tends to mimic chronic inflammation caused by non-microbial and non-degradable pathogens (Sahan *et al*., 2018). Non-steroidal anti-inflammatory drugs have been reported to act by decreasing the size of granuloma resulting from cellular reaction by inhibiting granulocyte infiltration, and thus preventing generation of collagen fibers and suppressing mucopolysaccharides (Bindu *et al*., 2020). In the present study a remarkable increase of both wet and dry weight granuloma tissue in control (distilled water treated) group was observed indicating the proliferation of inflammatory mediators. However, the administration of MLES (at doses of 500 and 1000 mg/kg) reduced wet and dry granuloma weight compared to the negative control indicating suppression of the proliferative phase of inflammatory response. This suggests that the extract has anti-inflammatory activity.

Recent studies have revealed the association between elevated platelets, platelet indices [Platelet Lymphocyte Ratio (PLR), Platelet Distribution Width (PDW) and Mean Plate Volume (MPV)], neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR) and eosinophil-to-lymphocyte (ELR) ratio with inflammation (Pogorzelska *et al*., 2020; Zhou *et al*., 2020; López-Verdugo *et al*., 2020, Targonska *et al*., 2021). These haematological parameters have been identified as sensitive markers of occult inflammation and disease activity for many diseases including systemic lupus erythematosus, rheumatoid arthritis, psoriasis, and oesophageal cancer (López-Verdugo *et al*., 2020; Pornchai and Ratanapha, 2019). In addition, they are used to determine the severity of inflammation and as predictors of poor outcomes in several disease conditions such as in cancers, hypertension, diabetes mellitus and autoinflammatory diseases (Isa *et al*., 2018; Pirozzolo *et al*., 2019; Endo *et al*., 2021). The present study thus evaluated the effect of the extract on some of these previously identified hematological inflammatory biomarkers (platelet level and platelet indices). The ability of MLES to produce a decrease in platelets, PLR and MPV after oral administration suggests that the plant has anti-inflammatory activity.

Inflammation is a major pathogenic mechanism for both acute kidney injury and chronic kidney disease (Andrade-Oliveira *et al*., 2019). Measurements of urea and creatinine levels in the blood are usually performed to evaluate kidney function (Gounden *et al*., 2021). Generally, increases in urea and creatinine levels are associated with kidney dysfunction or damage in the renal filtration mechanism. The ability of the extract at higher doses to cause significant reductions in creatinine levels may be due to the anti-inflammatory activity of the plant.

Elevated liver enzymes have been reported to be associated with several inflammatory disorders. High levels of these enzymes indicate possible liver damage, hepatotoxicity, cardiac infarction, and muscle injury, in which inflammation of liver cells are involved (Valdemar *et al*., 2021). Administration of MLES caused a decrease in the level of liver enzymes - AST, ALP, and ALP which may be linked to the anti-inflammatory activity of the extract.

Conclusion

Based on the results from our study, it may be inferred that the methanol leaf extract of *Senna italica* possesses anti-inflammatory activity confirming the folkloric use of the plant in the management of inflammation and inflammatory related diseases.

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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