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EFFECT OF THE METHANOL LEAF EXTRACT OF *DALBERGIA SAXATILIS* HOOK.F ON PLATELET INDICES FOLLOWING COTTON PELLET-INDUCED GRANULOMA IN WISTAR RATS

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ABSTRACT

Dalbergia saxatilis is a member of the Fabaceae family. It is used to treat a variety of ailments including cough, small pox, skin lesions, bronchial ailments, and toothache. The plant is native to Tropical Africa, Senegal, Democratic Republic of Congo, Angola, and also Nigeria. The aim of this study is to evaluate the effect of the methanol leaf extract of *Dalbergia saxatilis* leaves on platelet indices in Wistar rats. Anti-inflammatory activity was evaluated using cotton pellet-induced granuloma method at doses of 250, 500 and 1000mg/kg body weight. Complete blood count was carried out to determine the platelet indices (Platelet to lymphocyte ratio, Mean platelet volume, Platelet distribution width). Histology of the granuloma tissue was also carried out. The methanol leaf extract decreased the dry weight of the cotton pellet granuloma, it significantly ($p < 0.05$, 0.01) reduced the Platelet to lymphocyte ratio (PLR) in the aspirin 150 mg and extract 1000 mg/kg groups; Mean platelet volume (MPV) and Platelet distribution width (PDW) were also reduced but statistically insignificant compared to distilled water group. The extract also reduced hemorrhage and hyperplasia of inflammatory cells in the granuloma tissue in rats, when compared with the distilled water group. The result from this study suggests that the anti-inflammatory effect of the methanol leaf extract of *Dalbergia saxatilis* may be through its effect on platelet indices.

Keywords: Cotton pellet, *Dalbergia saxatilis*, Cotton pellet, Granuloma, inflammatory cells, platelet

INTRODUCTION

Medicinal plants have long been used in traditional medicine and ethnomedicine around the world (Da-cheng, 2019). Early humans treated their illnesses with plants, guided by instinct, taste, and experience; thus, the history of medicinal plants is as long as the history of humans (Singh, 2018).

Dalbergia saxatilis (Hook.f) is a member of the Fabaceae family. It is used to treat a variety of ailments including cough, smallpox, skin lesions, bronchial ailments, and toothache (Saha *et al*, 2013). The plant is native to tropical Africa, Senegal, Democratic Republic of Congo, Angola, and Nigeria. It is typically a vigorous climbing shrub with stems up to 20 meters long, but it sometimes grows as a shrub only 2-3 meters tall (Burkil, 2004). The stem is usually up to 5cm in diameter near the base, but it can occasionally grow to be 15cm. The branches are extremely tortuous, with some modified as woody spine hooks. The plant is collected in the wild, primarily for local medicinal purposes and for its wood. It is occasionally used as a leafy vegetable and is sold in local markets (Achigan-Dako *et al.*, 2009).

Inflammation is a natural response of the body's immune system to injury, infection or damage (Bennett et al., 2018). When

there is inflammation, chemicals from the body's white blood cells enter the blood or tissues to protect the body from invading organisms (Furman *et al*., 2019). This increases blood flow to the site of injury or infection, which causes flushing and warmth. Some of the chemicals cause fluid leakage into the tissues, causing swelling. This protective process has the potential to irritate nerves and cause pain. Over time, a high number of white blood cells and the substances they produce inside the joints cause irritation, swelling of the joint interior, and loss of cartilage (Furman *et al*., 2019). Anti-inflammatory drugs relieve pain by reducing inflammation; they are used to treat symptoms such as pain, stiffness, swelling, and fever (Tong, 2010). Synthetic analgesic and anti-inflammatory drugs have significant side effects such as constipation, nausea and vomiting, sedation, and mental clouding, etc (Saidi *et al*, 2016). These factors make the development of new anti-inflammatory drugs essential, and medicinal plants are preferred due to their lower side effects (Ezeja *et al*, 2011).

Several studies on the pharmacology and safety profile of the plant *Dalbergia saxatilis* have been reported. Saponin (DSS) isolated from Dalbergia saxatilis displayed a concentration dependent contractions of the Uterine muscle contractile preparations from rata (Uchendu and Leek, 1999), a triterpenoid glycoside (DSS) from *Dalbergia saxatilis* decreased maternal body weights and inhibited conception in female Wistar rats (Uchendu, 2000), the root extract of *Dalbergia saxatilis* demonstrated anxiolytic and muscle-relaxant activity (Yemitan and Adeyemi, 2003), and protected against generalized seizure in pentylenetetrazol and electroconvulsive models (Yemitan and Adeyemi, 2005). The aqueous root extract of *Dalbergia saxatilis* had a good safety profile after a 90 days administration, it also protected against Pentylenetetrazol, strychnine, and bicuculine induced acute seizure models (Yemitan, 2008). The dried powdered leaves of *Dalbergia saxatilis* drastically reduced the damage to cowpea seeds without affecting the viability, crude 95% ethanol bark extract, aqueous methanol and hexane fractions showed insecticidal activity against adult mosquitoes, the bark extract demonstrated a broad spectrum of antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*, while the leaf extract was active only against *Staphylococcus aureus* (Okwute *et al*., 2009).

The aqueous root extract of *Dalbergia saxatilis* significantly ($p < 0.05$) retarded the development and progression of strychnine kindling, and also significantly ($p < 0.05$) retarded the development of picrotoxin kindling, decreased the scoring from kindling progression and prevented convulsion in fully picrotoxin-kindled mice (Yemitan and Adeyemi, 2013). The methanol leaf extract of *Dalbergia saxatilis* significantly decreased acetic acid-induced writhes, carrageenan induced-inflammation, and Brewer's yeast-induced pyrexia in mice and rats respectively (Hassan *et al*., 2015). The aqueous root extract of *Dalbergia saxatilis* significantly ($p < 0.05$) elevated CAT, SOD, and GSH and significantly decreased ($p < 0.05$) MDA following 90 days treatment in rats (Yemitan and Adeyemi, 2016). Prolonged administration of the methanol leaf extract of *Dalbergia saxatilis* caused significant ($p < 0.05$) reduction in electrolytes concentration, and revealed massive necrosis of the glomerulus with tubular distortion and lymphocyte hyperplasia (Hassan *et al*., 2016). The methanol leaf extract of *Dalbergia saxatilis* significantly reduced the levels of progesterone and estrogen, and the number of follicles and endometrial thickness were

also reduced (histopathology) at 300 and 600 mg/kg (Ukwuani-Kwaja *et al*., 2021). The methanol leaf extract of *Dalbergia saxatilis* significantly increased SOD, GSH, and CAT, and also decreased MDA following cotton pellet-induced granuloma in rats (Hassan *et al*., 2022).

The aim of this study is to evaluate the effect of the methanol leaf extract of *Dalbergia saxatilis* on platelet indices following cotton pellet-induced granuloma method in Wistar rats.

MATERIALS AND METHODS

Drugs, Chemicals, and Equipment

Methanol, chloroform, formalin (Sigma Aldrich, Germany), distilled water, aspirin (Abcam Inc., Cambridge, MA), streptomycin, penicillin (GSK, Bretford, GB), ketamine (Greenco Biologicals PVT Ltd, Kolkata, India), digital weighing balance, mortar and pestle, syringe (1ml), dissecting kit (Dusseldorf, Germany), EDTA and plain bottles (Mokshy Surgicals, Mumbai), cotton. The reagents used were of analytical standard grade.

Experimental Animals

Wistar rats of both sexes weighing 150 - 200 g were used in the present study. Animals were housed in standard cages under standard laboratory conditions in the animal house facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. They were allowed access to feed and water *ad libitum*. The study protocol and procedures were conducted in accordance with the guidelines for the use and care of experimental animals.

Plant Collection and Preparation

Fresh leaves of *Dalbergia saxatilis* were collected from Giwa Local Government Area of Kaduna State, Nigeria in May, 2021. Identification and authentication were done by a taxonomist Mallam Umar Gallah in the Department of Botany, Kaduna State University. A voucher specimen number of KASU/BSH/1608 was deposited for future reference. The leaves were air dried and size reduced in preparation for extraction. The obtained dried powder was subjected to extraction with 70%$^{v}/_{v}$ methanol using maceration method. The extract was dried under reduced pressure and controlled temperature (40 – 60^{0}C) on a water bath. The residue was kept in an air tight container for subsequent use.

Cotton Pellet-induced Granuloma Method

The rats were grouped into five groups each containing six rats. Pellets each weighing 0.1g were made using gauze that had been sterilized in an autoclave. Chronic inflammation was induced by subcutaneous implantation of a cotton pellet which was put into 0.2 ml distilled water containing streptomycin (0.13 mg) and penicillin (0.1 mg) into a rodent hind limb under ketamine (15 mg/kg) anesthesia (Winter and Porter, 1957). Group I were administered with distilled water (1ml/kg) and served as normal control (negative control), group II were administered with Aspirin (150mg/kg) and served as positive control, group III, IV, and V were administered with methanol leaf extract of *Dalbergia saxatilis* 250, 500, and 1000mg/kg respectively for 9 days. All treatments were given orally. The rats were sacrificed on the 10th day where blood samples were taken for determination of hematological parameters, and tissue at the granuloma sites were taken for histology. The cotton pellets were surgically removed, the wet weight of the cotton pellet was taken immediately and then dried until a constant weight was obtained. The weight of the of the dried cotton pellets were also taken.

Inhibition (%) of granuloma tissue development was calculated using the relation:

Inhibition (%) = [WC−WT/WC] × 100; where WC = weight of granuloma tissue of control group; WT = weight of granuloma tissue of treated group.

Blood and Organ Sampling

Blood samples were taken for complete blood count (CBC) and determination of platelet lymphocyte ratio (PLR), and granuloma tissues were taken for histopathology. Blood was collected from left carotid artery into heparinized tubes, and kept for complete blood count analysis.

Determination of Hematological Parameters

Platelet lymphocyte ration (PLR) is a cost-effective method of evaluating inflammatory response. PLR, MPV, and PDW were determined from complete blood count using an automated analyzer (Sysmex, US). Platelets counts obtained were divided by lymphocyte counts for each sample to obtain the platelet/lymphocyte ratio (Durmus *et al*, 2015).

Histopathological Studies

The harvested granuloma tissue was fixed in 10% neutral buffered formalin (NBF, pH 7.26) for 48 hours. It was then processed and embedded in paraffin. Thick sections (5µm) were prepared and stained with hematoxylin and eosin (H&E). The histopathological analysis was performed using light microscopy.

Statistical Analysis

Data were analyzed using One Way Analysis of Variance (ANOVA) followed by Tukey's post hoc test. Data were expressed as Mean ± Standard Error of the mean (S.E.M) and the differences between means were considered significant at $p < 0.05$. Results were presented as Table and graphs.

RESULTS

Effect Of Methanol Leaf Extract of *Dalbergia saxatilis* on Cotton Pellet-induced Granuloma

The methanol leaf extract of *Dalbergia saxatilis* reduced the weight of cotton pellet granuloma in a dose dependent manner. The extract at different doses of 250, 500, and 1000mg/kg, reduced the weight of cotton pellet granuloma by 2.17, 4.34, and 7.6%, respectively (Table 1).

Table 1: Effect of the Methanol Leaf Extract of *Dalbergia saxatilis* on Cotton Pellet-induced Granuloma

Treatment	Dose	Mean weight of Dry Cotton pellet (mg) ± SEM	% Inhibition
Distilled Water	1ml/kg	153.33 ± 4.22	-
Aspirin	150mg/kg	140 ± 4.90	8.71
MLDS	250mg/kg	150 ± 4.47	2.17
MLDS	500mg/kg	146.67 ± 3.33	4.34
MLDS	1000mg/kg	141.67 ± 5.3	7.6

Values are ± SEM, n=6. MLDS = Methanol leaf extract of *Dalbergia saxatilis*

Effect of the Methanol Leaf Extract of *Dalbergia saxatilis* on Platelet Lymphocyte Ratio (Plr)

The Platelet to lymphocyte ratio (PLR) of the control group was higher than the other groups. The PLR was significantly ($p < 0.05, 0.01$) reduced at 1000mg/kg of the extract and 150 mg/kg of Aspirin when compared with distilled water group (Figure 1).

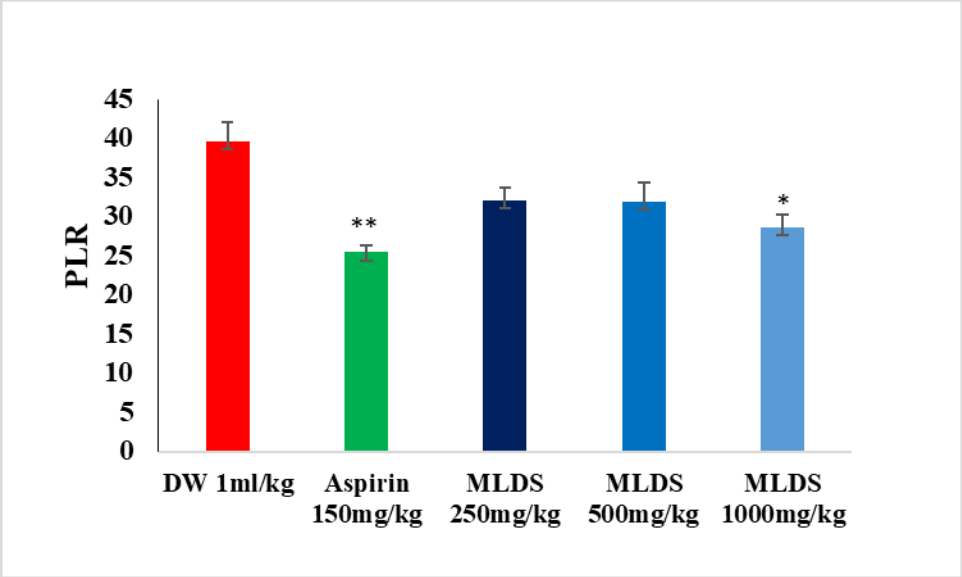

Figure 1: Effect of the methanol leaf extract of *Dalbergia saxatilis* on platelet lymphocyte ratio (PLR). Values are ± SEM, n=6. Results was analysed using one way ANOVA followed by Tukey post hoc test, * and ** represents significance as $P < 0.05$ and $P < 0.01$ respectively. MLDS = Methanol leaf extract of *Dalbergia saxatilis*

Effect of the Methanol Leaf Extract of *Dalbergia saxatilis* On Mean Platelet Volume (MPV)

The Mean platelet volume (MPV) of the control group was higher than the MPV of the other groups. The methanol leaf extract of *Dalbergia saxatilis* reduced the MPV in a dose dependent manner though not statistically significant (Figure 2).

Effect Of the Methanol Leaf Extract of *Dalbergia Saxatilis* on Platelet Distribution Width (PDW)

The Platelet distribution width (PDW) was seen to be higher in the control group than the other groups. The methanol leaf extract of *Dalbergia saxatilis* reduced the PDW in a dose dependent manner. No significance was observed between groups (Figure 3).

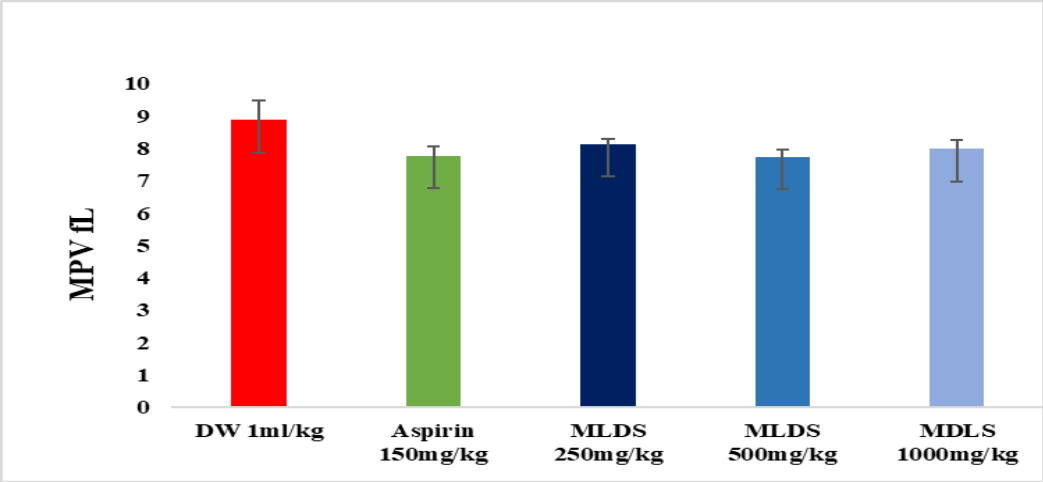

Figure 2: Effect of the Methanol Leaf Extract of *Dalbergia saxatilis* on Mean Platelet Volume (MPV) Values are ± SEM, n=6. MLDS = Methanol leaf extract of *Dalbergia saxatilis*

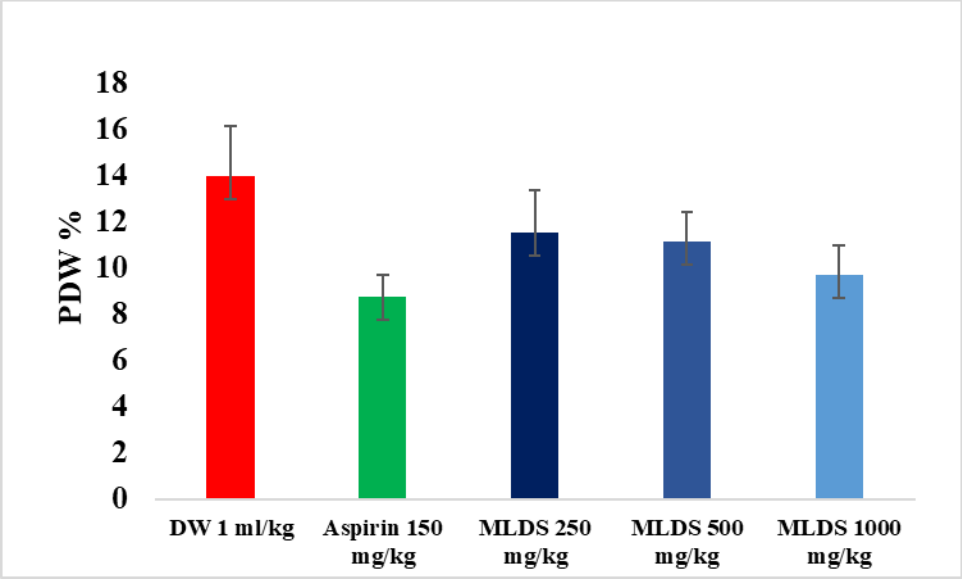

Figure 3: Effect of the Methanol Leaf Extract of *Dalbergia saxatilis* on Platelet Distribution Width (PDW). Values are ± SEM, n=6. MLDS = Methanol leaf extract of *Dalbergia saxatilis*

Effect of the Methanol Leaf Extract of *Dalbergia saxatilis* on Histopathology of Granuloma Tissue in Rats

The methanol leaf extract of *Dalbergia saxatilis* was able to reduce the intensity of hemorrhage and hyperplasia of inflammatory cells caused by cotton pellet-induced granuloma when compared with the distilled water group.

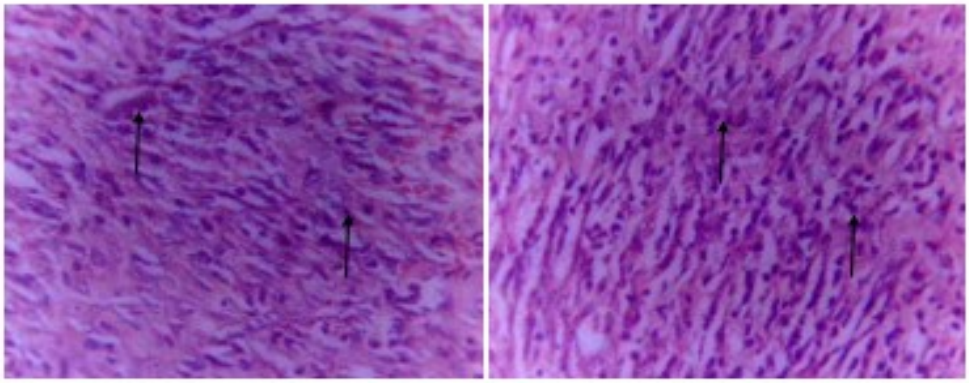

Plate I: Distilled water 1ml/kg group

Plate II: Extract 250 mg/kg group

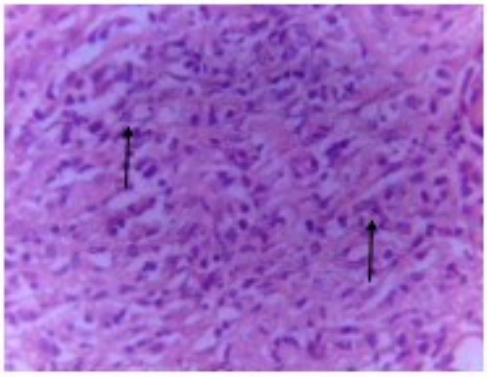

Plate III: Extract 500 mg/kg group

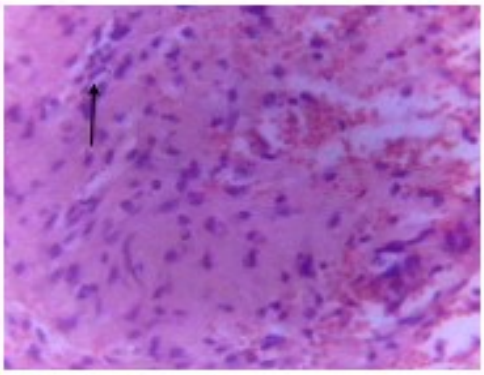

Plate IV: 1000 mg/kg group

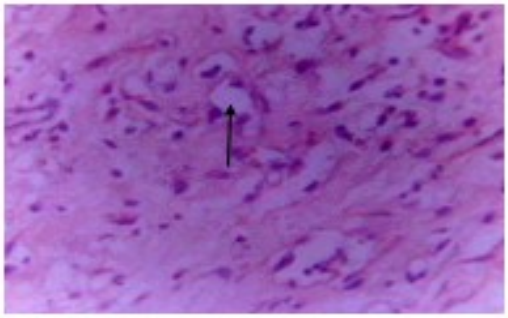

Plate V: Aspirin 150 mg/kg group

Plate I: Distilled water group showing intense hyperplasia of inflammatory cells with fibrocyte (++) collagen (+) and Hemorrhage X 250 magnification. Sections are stained with hematoxylin and eosin.
Plate II: Effect of 250 mg/kg of *Dalbergia saxatilis* showing moderate hemorrhage and moderate hyperplasia of inflammatory cells with fibrocyte (++) and trace collagen X250 magnification. Sections are stained with hematoxylin and eosin.
Plate III: Effect of 500 mg/kg of *Dalbergia saxatilis* showing slight hemorrhage and slight hyperplasia of inflammatory cells with fibrocyte (++) and trace collagen X 250 magnification. Sections are stained with hematoxylin and eosin.
Plate IV: Effect of 1000 mg/kg of *Dalbergia saxatilis* showing slight hemorrhage and collagen deposit (++) and fibrocyte (++) X 250 magnification. Sections are stained with hematoxylin and eosin.
Plate V: Effect of 150 mg/kg of Aspirin showing collagen deposit (+) with slight hemorrhage (H) and fibrocyte (++) X 250 magnification. Sections are stained with hematoxylin and eosin.
(++) = moderate; (+) = mild

DISCUSSION

The plant *Dalbergia saxatilis* has been used for the treatment of variety of ailments including cough, small pox, skin lesions, bronchial ailments, and toothache (Saha *et al*, 2013). (Dzoyem *et al*, 2017). Determination of platelet lymphocyte ratio and other platelet indices are cost effective methods of evaluating inflammatory response (Durmus *et al*., 2015).

Hematology is the study of the numbers and morphology of the blood's cellular elements (NseAbasi *et a.l*, 2014). Platelets play an important role in clot formation. Furthermore, new evidence suggests that platelets play important roles in inflammation and immune response (Sonmez and Sonmez, 2017). White blood cells, or leukocytes, are immune system cells that participate in both innate and humoral immune responses. They circulate in the blood and initiate inflammatory and cellular responses in response to injury or pathogens (Tigner *et al.*, 2021). Neutrophils have traditionally been thought of as simple innate immune system foot soldiers with a limited set of pro-inflammatory functions (Kolaczkowska and Kubes, 2013). Neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR), eosinophil to lymphocyte ratio (ELR), and platelet to lymphocyte ratio (PLR) are sensitive markers of occult inflammation derived from a complete blood count (CBC) (Lopez-Verdugo *et al.*, 2020). Patients with a high risk of atherothrombotic illness have a higher mean platelet volume (MPV). As a result, high MPV level could be a risk factor for platelet activation (Park *et al*., 2002). Platelet distribution width (PDW) is a common parameter in routine blood tests that reflects variation in platelet size distribution with a range of 8.3 percent to 56.6 percent (Sachdev *et al.,* 2014).

Platelet to lymphocyte ratio (PLR) is a useful marker that reveals changes in platelet and lymphocyte counts caused by an acute inflammatory and prothrombotic state. Several large observational studies have demonstrated the importance of PLR changes in determining the severity of systemic inflammation (Armen *et al,* 2019). Mean platelet volume (MPV) is a measure of the average size of your platelets, which are a type of blood cell that aids in the prevention of bleeding (Park *et al*., 2002). Platelet distribution width (PDW) is a common parameter in routine blood tests that reflects variation in platelet size distribution with a range of 8.3 percent to 56.6 percent (Sachdev *et al.*, 2014). When platelets are activated in an inflammatory environment, they always undergo morphological changes. As a result, in some inflammatory diseases, PDW can be used as a marker of activated platelet release (Karagoz *et al.,* 2009). From the study, the extract at different doses decreased PLR which shows that the extract may be effective in reducing inflammation. A high PDW indicates that there is a lot of variation in size, which could be linked to specific inflammatory disorders (Ilambirai, 2018). From the study, our extract at different doses reduced the PDW. This suggests that our extract can reduce inflammation by reducing PDW.

Histological studies are used in forensics, autopsies, diagnosis, and education. It is widely used in medicine, particularly in the study of diseased tissues to facilitate treatment (Black, 2012). The most appropriate method for studying drug efficacy against the proliferative phase of inflammation is cotton pellet-induced granuloma formation. The implantation of a cotton pellet under the skin of a rodent results in the formation of a granuloma at

the site of the implant. The accumulation of fluid and proteinaceous material, as well as the infiltration of macrophages, neutrophils, and fibroblasts, and the multiplication of small blood vessels, are the initial events that lead to the formation of the highly vascularized reddish mass known as granulation tissue (Dzoyem *et al*, 2017). This method has been widely used to assess the transudative, exudative, and proliferative phases of inflammation (Swingle and Shideman, 1972). From the study, our extract at different doses reduced hemorrhage and hyperplasia of inflammatory cells, which shows that, the methanol leaf extract of *Dalbergia saxatilis* may be effective on the transudative, exudative, and proliferative phases of inflammation.

CONCLUSION

The findings of the study suggest that the methanol leaf extract of *Dalbergia saxatilis* can reduce inflammation by decreasing the levels of platelet lymphocyte ratio, mean platelet volume, and platelet distribution width using the cotton pellet-induced granuloma method.

Conflict of Interest

Authors declare no conflict of interest.

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