GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Anti-inflammatory efficacy of M*angifera indica* stem-bark, *carica papaya* and *eucalyptus* leaf extracts on formalin-induced inflammation in rats

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GSC Biological and Pharmaceutical Sciences, 2025, 33(02), 178-195

Publication history: Received on 27 September 2025; revised on 03 November 2025; accepted on 06 November 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.33.2.0392

Abstract

Inflammation is part of the body's defense mechanism and can be acute or chronic. Inflammatory disease prevalence is on the increase in Africa and anti-inflammatory efficacious alternatives are continually sought for. Effects of herbal remedies consisting of ethanol extracts of *Mangifera indica* Stem-bark (MSB), *Carica papaya* (PL) and *Eucalyptus* (EU) leaf extracts were evaluated on formalin-induced inflammation in albino rats using standard procedures. Forty-five (45) rats were divided into nine (9) groups of five (5) rats each. Induction was by subcutaneous injection of 0.1ml (2.5% v/v in normal saline) formaldehyde solution (formalin) via the right hind paw of all groups except normal control, group seven (7), on 1st & 3rd day of experiment. Groups 1-3 were treated with 200mg/kg, 400mg/kg, and 600mg/kg combined ethanol extracts MSB+PL respectively. Groups 4-6 received 200mg/kg, 400mg/kg, and 600mg/kg combined extracts of MSB+EU respectively. Group 8(Standard) received 5mg/kg piroxicam and group 9(negative control) received 5ml/kg normal saline for 10 days. Paw thickness and body weights taken on days 3, 7&10. MSB+EU combination was most efficacious and effect was dose-dependent. Tumor necrosis factor alpha (TNFα) and ESR (erythrocyte sedimentation rate) did not significantly differ between the groups. White blood cells (WBC), Malondialdehyde (MDA) were highest in untreated group and platelet count lowest. There were no significant changes in liver marker enzymes except in MSB+EU 600mg/kg group, which also corroborated histological findings. The study therefore justifies the anti-inflammatory use of both extract combinations and suggests MSB+EU maybe most effective, however, questions are raised on its effect on liver integrity. Further studies on human clinical parameters and anti-inflammatory properties of these extract over an extended period is suggested to further confirm their potential role in anti-inflammation as well as determination of anti-inflammatory effect of these extracts on immunological pro-inflammatory molecules (IL-6, IL-IB, TNF-a).

Keywords: *Mangifera indica; Carica papaya; Eucalyptus*; Inflammation; Hematology; Antioxidant; TNF-

1. Introduction

Inflammation is part of the body's defense mechanism and can be acute or chronic. Acute inflammation is the initial response and is characterized by the increased movement of plasma and innate immune system cells, such as neutrophils and macrophages, from the blood into the injured tissues. Cardinal signs of inflammation—edema, hyperalgesia, and erythema—develop immediately following subcutaneous injection of inflammatory agent, resulting from action of proinflammatory agents—bradykinin, histamine, tachykinins, complement and reactive oxygen and nitrogen species. Upon the presence of the inflammatory agent, cell membranes induce the activation of phospholipase A2 followed by release of arachidonic acid and inflammatory mediators such as cytokines, serotonin, histamine, prostaglandin and leukotrienes that increase vascular permeability, thus facilitating the migration of leukocytes to the site of inflammation (Sarkhel, 2016).

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Herbal medicine are gaining popularity both in developing and developed countries because of their natural origin and less side effects, many traditional medicines in use are derived from medicinal plants, minerals and organic matter (Grover, et al., 2002). Plants are rich sources of many different bioactive phyto-compounds, including phenolic components, anthocyanins, carotenoids, vitamin E, and vitamin C, which exhibit good antioxidant and health enhancement properties (Liu, 2003, Usman, et al., 2001).

This study is aimed at assessing the anti-inflamatory efficacy and hepatotoxicity of mangifera indica stem-bark (msb), carica papaya (pl) and eucalyptus (EU) leaf extracts on formalin-induced inflammation in rats.

2. Materials and methods

2.1. Sample collection and preparation

The leaves of Carica papaya, and Eucalyptus were gotten from Ihube town in Okigwe L.G.A of Imo State and Magnifera indica stem bark was gotten from Federal University of Technology Owerri Imo State at N5023'33.6876" longitude and E6059'10.5504 latitude. These samples were washed thoroughly with clean tap water, and then air-dried under room temperature, the dried samples were grinded to fine powder and then stored in a clean bottle.

2.2. Preparation of extract

Three hundred grams (300g) powdered samples was placed in a stoppered container and 1200mls of the solvent (ethanol) was added. They were allowed to stand at room temperature for 48 hours, with frequent agitation. The extract was filtered with a fine cloth and then re-filtered using Whatman filter. The filtrate was poured in to a clean round bottom flash at a volume that will not allow the filtrate to siphon into the extraction chamber. The temperature is adjusted in accordance with the boiling point of ethanol (78.4°C). The solvent evaporates and drips into the extraction chamber where is collected. The active component left behind in the flask is dried in water bath to completely evaporate the remaining solvent and then preserved in tightly corked labeled bottles and stored in a refrigerator until required.

2.3. Experimental animals

Fifty male adult albino rats (110-120g) were obtained from Awka. The animals were housed in plastic cages and allowed to acclimatize for 7days, under normal room temperature (25 ± 20C) and natural light cycle and maintained on standard pellets (Vital Feeds, Jos, Nigeria) and water ad libitum throughout the study period. Animals handling and use was done in compliance with the National Institute of Health Guide for care and use of laboratory Animals.

2.4. Induction of Animals

Non-immunological (Udegbunam, et al., 2014) inflammation was induced according to the method described by (Amraoui et al., 2019). Inflammation was induced in rats by subcutaneous injection of 0.1ml (2.5% v/v in normal saline) formaldehyde solution (formalin) into the sub-planter region of right hind paw of albino rats on first and third day of the experiment. Prior to induction, the mean body weights were calculated and different concentrations of the extract were prepared based on the mean body weight according to OECD'S guideline on volume selection; (Barret, et al., 1991).

$$Required\ dose = \frac{\text{weight of animal (g)}}{1000\ (\text{g})} \times standard\ dose\ (mg)$$

$$stock\ solution = \frac{\text{required dose (mg)}}{1000\ (\text{g})} \times volume\ of\ solvent$$

All the groups receives their treatments respectively, while groups 7 and 9 receives 5mls normal saline and group 8 receives 5mg/kg piroxicam (standard drug), one (1) hour before induction. After induction the rats were observed for 1 hour and their paw size were measured after one hour (day1). The paw thickness and body weight of the rats were taken before induction and on day 3, 7 and 10 using vernier caliper and the animals were observed daily for signs/symptoms of inflammation. All the rats had free access to food and water throughout the time of the experiment and treatments were administered orally by intubation.

2.5. Biochemical analysis

The blood samples were collected into EDTA and heparinized bottles by ocular puncture, the animals killed by cervical dislocation and the liver organs were collected in plain bottles, preserved using 10% formalin and then sent to the laboratory for analysis, blood samples were centrifuged for 10mins at 3000rpm and the serum was use to examine

aspartate amino transferase (AST) and alanine amino transferase (ALT) according to the method of Reitman and Frankel method, (1957), Alkaline phosphates (ALP) was assessed according to Kochmar, J.F and moss, D.W method, (1986) and Total Protein using Biuret method by Tietz, (1995). Randox kit was used.

Heamatological parameters were carried out using microheamatocrit centrifuge (UNICO, CMH30, USA), Determination of Lipid profile was carried out using enzymatic end-point method (Unit mmol/L) (Y Kayamori, et al., 1979), Malondialdehyde (MDA) using Ohkawa & Ohishi method (1979).

Erythrocyte Sedimentation Rate (ESR) was carried out using westergren technique (Bharathi, et al., 2011), Rat Tumor Necrosis Factor α (TNF-α) was carried out using enzyme-linked immunosorbent assay method (ELISA) (Engvail, 1972).

2.6. Histopathology examination (Slaoul and Fiette, 2011)

The liver of rats were fixed in 10% neutral buffered formalin solution for 24 hours and cleared in xylene solution followed by embedding on the paraffin block. The tissues were then cut using rotary microtome into 4µm thick, mounted on glass slides, stained with hematoxylin and eosin, and examined under a high powered microscope at magnification ×400.

2.7. Statistical analysis

The data obtained were analyzed using using students package for social sciences (SPSS) version 20 computer software Analysis of Variance (ANOVA). Values for $p \leq 0.05$ were considered statistically significant.

3. Results

3.1. Hematological Results

The packed cell volume (PCV) of inflammation-induced rats treated with different concentrations of combined ethanol extracts of mango stem bark with pawpaw leaves and mango stem bark with eucalyptus leaves revealed that there were no significant difference in PCV levels between most of the various groups and the normal control animals except in the lower -dose (200mg/kg MSB+PL and 200mg/kg MSB+EU) groups. The lower-dose test groups (200mg/kg MSB + PL and 200mg/kg MSB + EU) were significantly lower than that of the normal control group, but very similar to that of the negative control group.

The result of the hemoglobin revealed no significant difference in the level of Hb concentration of the test groups compared to the normal control group, except in the lower dose groups (200mg/kg MSB/PL and MSB/EU) which were significantly lower than the normal control group but similar to the negative control as shown in figure.1a below.

The result of the red blood cell (RBC) revealed no significant differences between the high dose (600mg/kg) concentrations of the extracts compared to the normal control. However, the low doses 200mg/kg MSB+EU and both 200 & 400mg/kg MSB+PL significantly decreased compared to the normal control. This level of decrease is very similar to that of the standard but significantly different from that of the negative control which was most decreased.

The WBC was significantly elevated in all the groups compared to the normal control, except in the high dose (600mg/kg body wt.) of both combinations. The result seen in the high dose combinations is very similar to that of the standard. The negative control and the 200mg/kg body wt. extract combinations have the highest levels of elevation. Results are shown in Figure 1b below

The platelet result showed significant increase in all the groups compared to the normal control. The groups fed with higher doses are similar to the standard control, while the groups treated with low doses are comparable to the negative control result as shown in Figure1c below

The erythrocyte sedimentation rate (ESR) of the inflamed rats treated with different concentrations of combined ethanol extracts of mango stem bark with pawpaw leaves and mango stem bark with eucalyptus leaves showed that there was clearly no significant difference seen in the ESR of all the animals treated with various concentrations of the combined extracts between groups. Hence the extract did not produce any significant change in the ESR of the test groups even at higher doses as shown in Figure 1d.

Legend:

- MSB= mango stem bark
- PL= pawpaw leaves
- EU= eucalyptus
- NS= normal saline

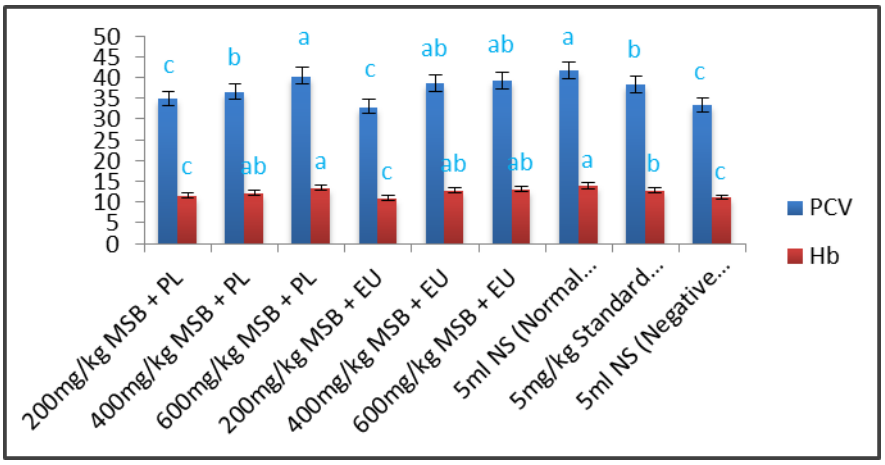

Figure 1a The Packed Cell Volume (PCV) And Hemoglobin (Hb) Level of Inflamed Rats Treated With Different Concentrations of Combined Ethanol Extracts Of Mango Stem Bark With Pawpaw Leaves And Mango Stem Bark With Eucalyptus Leaves Respectively. Bars are mean ± standard deviation. Bars bearing different alphabet are statistically significant ($p<0.05$)

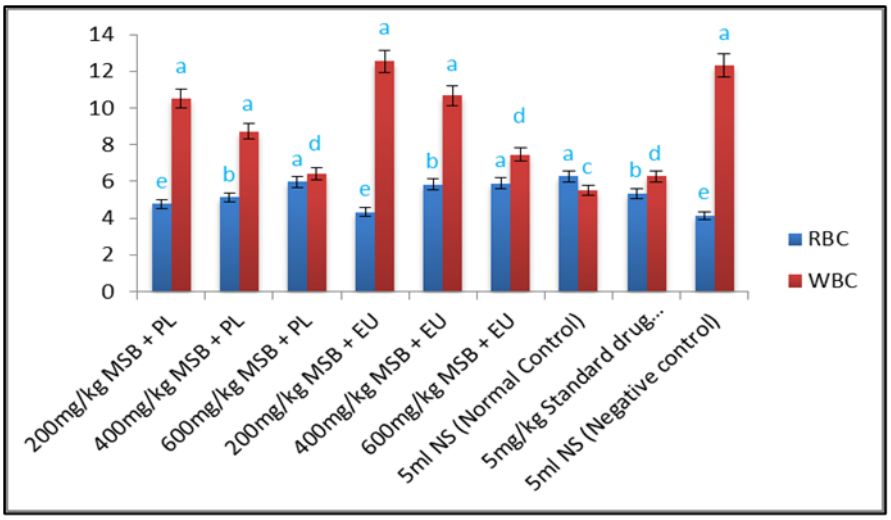

Figure 1b The Red Blood Cell (RBC) And White Blood Cell (WBC) Level Of Inflamed Rats Treated With Different Concentrations of Combined Ethanol Extracts Of Mango Stem Bark With Pawpaw Leaves And Mango Stem Bark With Eucalyptus Leaves Respectively. Bars are mean ± standard deviation. Bars bearing different alphabet letter(s) are statistically significant ($p<0.05$)

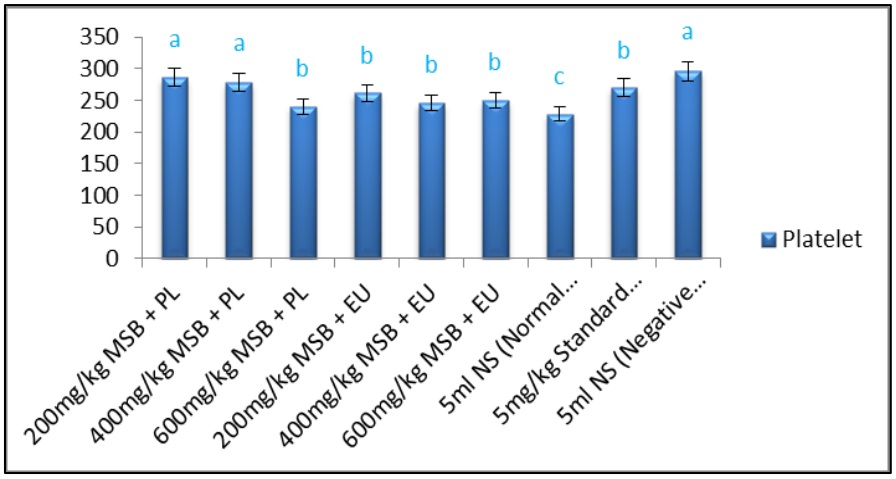

Figure 1c The Platelet Level of Inflamed Rats Treated With Different Concentrations Of Combined Ethanol Extracts of Mango Stem Bark With Pawpaw Leaves And Mango Stem Bark With Eucalyptus Leaves Respectively. Bars are mean ± standard deviation. Bars bearing different alphabet letter(s) are statistically significant ($p<0.05$)

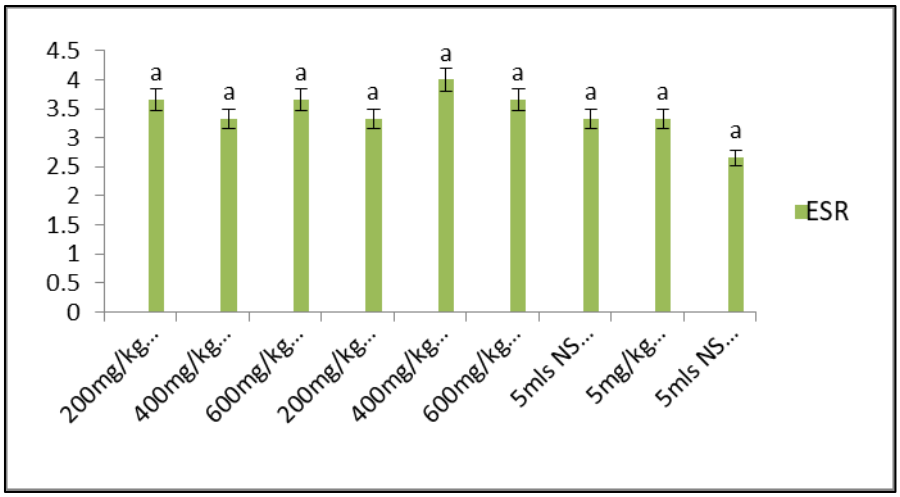

Figure 1d The Erythrocyte Sedimentation Rate (ESR) of inflamed Rats Treated With Different Concentrations of Combined Ethanol Extracts of Mango Stem Bark With Pawpaw Leaves And Mango Stem Bark With Eucalyptus Leaves Respectively. Bars are mean ± standard deviation. Bars bearing different alphabet letter(s) are statistically significant ($p<0.05$)

3.2. Liver Function Test Result

The aspartate amino transferase (AST) activity (IU/L) of inflammation-induced rats treated with different concentrations of combined ethanol extracts of mango stem bark with pawpaw leaves and mango stem bark with eucalyptus leaves revealed no significant change in the serum activity of AST in all the treated groups compared to normal control. However, there was a significant change/decrease in the AST activity of standard control as shown in 2a

The alkaline phosphatase (ALP) activity (IU/L) of inflamed rats treated with different concentrations of combined ethanol extracts of mango stem bark with pawpaw leaves and mango stem bark with eucalyptus leaves revealed elevated activities of ALP in the groups treated with MSB+PL and 600mg/kg MSB+EU compared to the normal control as shown in Figure 2b.

Alanine amino transferase (ALT) activity (IU/L) of inflamed rats treated with different concentrations of combined ethanol extracts of mango stem bark with pawpaw leaves and mango stem bark with eucalyptus leaves revealed that apart from the group fed with 200mg/kg MSB+PL, all other groups are significantly lower in ALT activity compared to the normal control as shown in Figure 2c.

The albumin (ALB) concentration (g/l) of inflamed rats treated with different concentrations of combined ethanol extracts of mango stem bark with pawpaw leaves and mango stem bark with eucalyptus leaves revealed that all the groups showed no significant difference in the ALB concentration compared to the normal control group as shown in Figure 2d

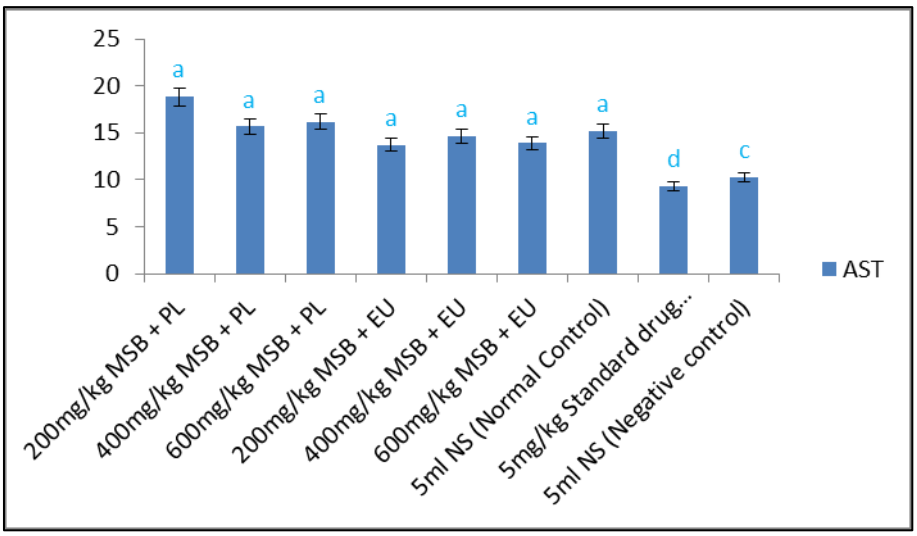

Figure 2a The Aspartate Amino Transferase (AST) Activity (IU/L) of Inflamed Rats Treated With Different Concentrations of Combined Ethanol Extracts of Mango Stem Bark With Pawpaw Leaves And Mango Stem Bark With Eucalyptus Leaves Respectively. Bars are mean ± standard deviation. Bars bearing different alphabet letter(s) are statistically significant ($p<0.05$)

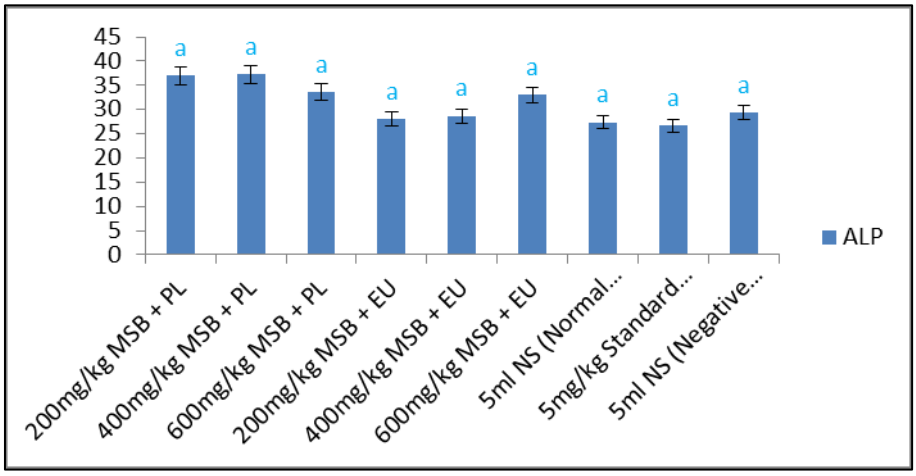

Figure 2b The Alkaline Phosphatase (ALP) Activity (IU/L) of Inflamed Rats Treated With Different Concentrations of Combined Ethanol Extracts Of Mango Stem Bark With Pawpaw Leaves And Mango Stem Bark With Eucalyptus Leaves Respectively. Bars are mean ± standard deviation. Bars bearing different alphabet letter(s) are statistically significant ($p<0.05$)

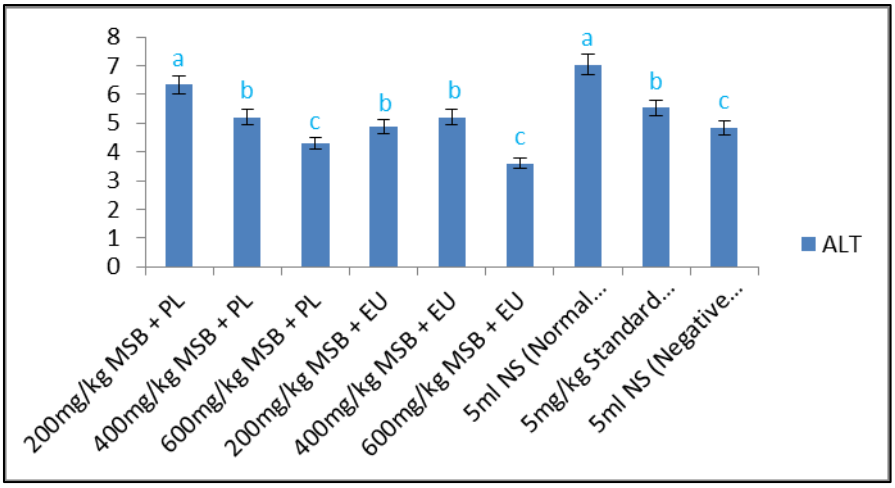

Figure 2c The Alanine Amino Transferase (ALT) Activity (IU/L) of inflamed Rats Treated With Different Concentrations Of Combined Ethanol Extracts Of Mango Stem Bark With Pawpaw Leaves And Mango Stem Bark With Eucalyptus Leaves Respectively. Bars are mean ± standard deviation. Bars bearing different alphabet letter(s) are statistically significant ($p<0.05$)

3.3. Antioxidant Results

The malondialdehyde (MDA) (µmol/Ml) of inflamed rats treated with different concentrations of combined ethanol extracts of mango stem bark with pawpaw leaves and mango stem bark with eucalyptus leaves revealed that Malondialdehyde concentration significantly increased in all the groups, but highest in the untreated inflamed group (group 9) compared to the normal control. The group treated with higher doses is comparable to the standard control, while the group treated with lower doses is similar to the negative control as shown in Figure 3a

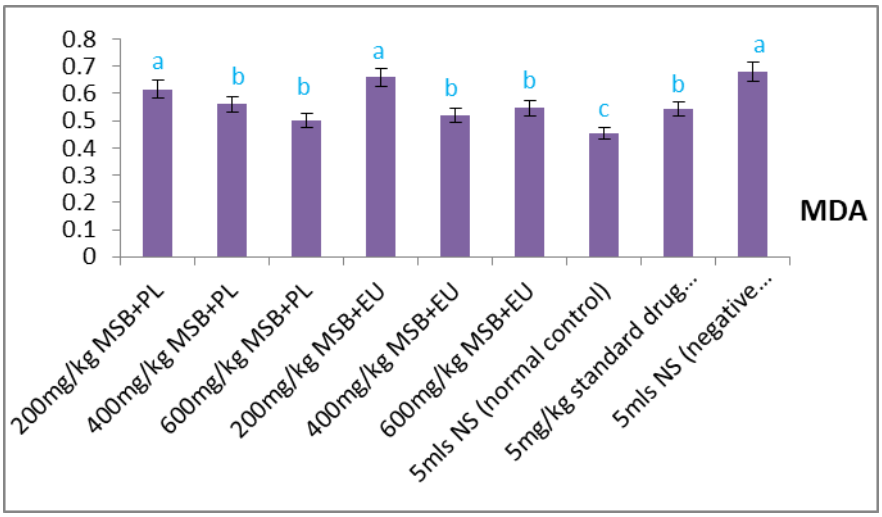

Figure 3a The Malondialdehyde (MDA) (µmol/Ml) Of inflamed Rats Treated With Different Concentrations Of Combined Ethanol Extracts Of Mango Stem Bark With Pawpaw Leaves And Mango Stem Bark With Eucalyptus Leaves Respectively. Bars are mean ± standard deviation. Bars bearing different alphabet letter(s) are statistically significant ($p<0.05$)

3.4. Tumor necrosis factor

Tumor necrosis factor-alpha result of combined ethanol extract of mango stem bark with eucalyptus leaves and mango stem bark with pawpaw leaves result showed that there was no significant difference observed in the TNF-α of all the test groups compared to the test controls as shown In Figure 4.a.

3.5. Weight and paw size

Results on weight (g) of the rats on combined ethanol extract of mango stem bark with eucalyptus leaves and mango stem bark with pawpaw leaves, showed that the weights of the animals in the different treated groups did not significantly differ from that of the control before induction. However after treatment, the weights significantly increased in all the groups compared to the normal control, except in the higher dose-treated groups, which were similar to that of normal control animals. This is shown in figure 3.5a. Results on paw size of the rats on combined ethanol extract of mango stem bark with eucalyptus leaves and mango stem bark with pawpaw leaves showed that there were no significant difference in the paw size of all the test groups and controls one (1) hour after induction.

On the 3rd day, there were significant increase in the paw size of the test groups compared to normal control groups, this increase is comparable to standard and negative controls.

On the 7th and 10th day, the paw sizes of all the test groups were significantly higher than normal control. However, the test groups with high doses were comparable to standard control while the low dose test groups were similar to negative control as shown in table 1

One hour after induction, it was observed that d rats were licking/ chewing the injected paw, partial elevation of the injected paw (edema).

On the 4th and 5th day, it was observed that the rats always cluster together (reduced mobility), could not stand firmly on the injected paw, walking one-sidedly and increased size of the injected paw and was lifted off the floor.

On day 7and 10, it was observed that elevated paw was slightly reduced in some groups.

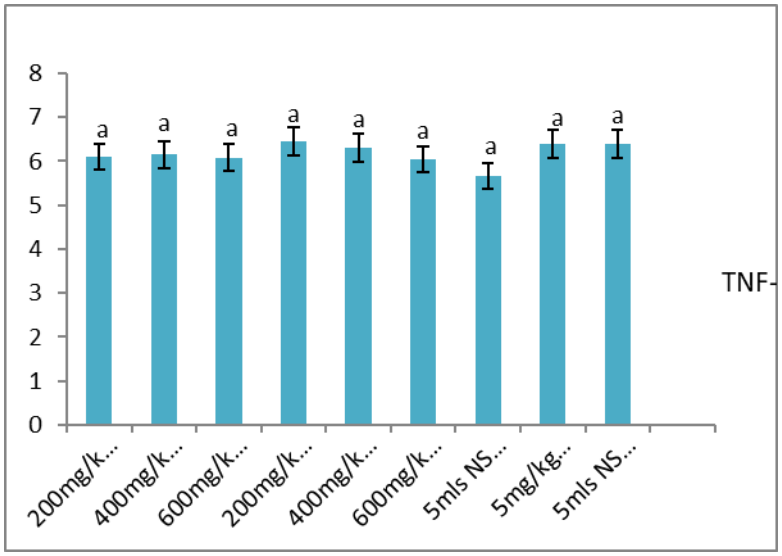

Figure 4a The TNF-α of inflamed rats treated with different concentrations of combined ethanol extracts of Mango stem bark with pawpaw leaves and mango stem bark with Eucalyptus leaves respectively. Bars are mean ± standard deviation. Bars bearing different alphabet letter(s) are statistically significant ($p<0.05$)

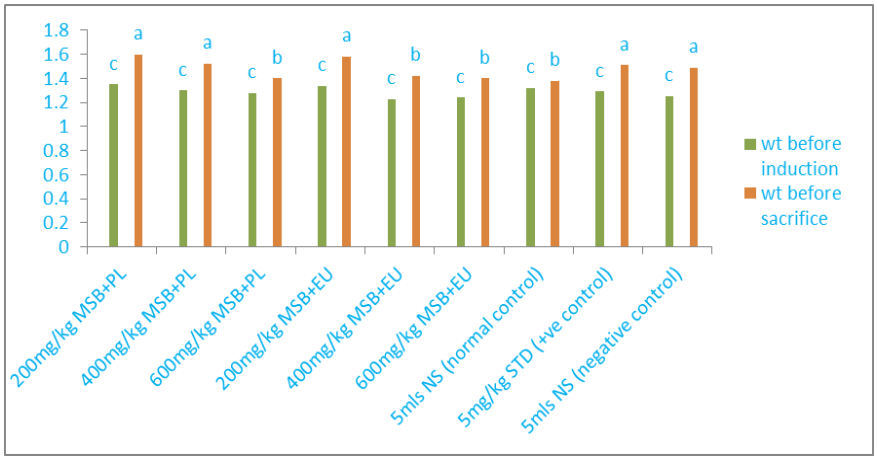

Figure 5a The weight of rats before induction and 10th day before sacrifice. Bars are mean ± standard deviation. Bars bearing different alphabet letter(s) are statistically significant ($p<0.05$)

Table 2 Effect of treatment on paw size of inflamed rats treated with different doses of combined ethanol extract of mango stem bark with Eucalyptus leaves and mango stem bark with pawpaw leaves respectively: Values are mean ± standard deviation. Bars bearing different alphabet letter(s) are statistically significant ($p<0.05$)

Group	**Paw before induction**	**1hour after induction**	**3rd day**	**1hr after induction (3rd day)**	**7th day**	**10th day**
200mg/kg MSB + PL	4.37± 0.15^{a}	6.36± 0.20^{a}	5.65±0.17^{b}	7.55±0.08^{ab}	7.26±0.2^{a}	6.75±0.16^{a}
400mg/kg MSB + PL	4.34± 0.12^{a}	6.57± 0.10^{a}	6.11±0.10^{b}	7.52±0.11^{ab}	6.46±0.11^{ab}	6.29±0.09^{b}
600mg/kg MSB + PL	4.33± 0.07^{a}	6.31± 0.16^{a}	5.72±0.18^{b}	7.48±0.13^{ab}	6.44±0.15^{b}	6.20±0.18^{b}
200mg/kg MSB + EU	4.39± 0.11^{a}	6.27± 0.13^{a}	5.45±0.12^{b}	7.52±0.23^{ab}	6.48±0.19^{ab}	6.45±0.17^{ab}
400mg/kg MSB + EU	4.28± 0.05^{a}	6.65± 0.06^{a}	6.07±0.19^{b}	7.49±0.15^{ab}	6.35±0.13^{b}	6.18±0.22^{b}
600mg/kg MSB + EU	4.37± 0.05^{a}	6.39± 0.13^{a}	5.87±0.18^{b}	7.51±0.18^{ab}	6.38±0.21^{b}	5.95±0.^{17b}
5ml NS (Normal Control)	4.27± 0.21^{b}	4.31± 0.18^{a}	4.44± 0.08^{e}	4.44±0.08^{e}	4.66±0.08^{e}	4.68±0.08^{e}
5mg/kg Std drug (+ive control)	4.45± 0.12^{a}	6.53± 0.13^{a}	6.48± 0.19^{b}	7.31±0.09^{b}	6.30±0.21^{b}	6.02±0.23^{b}
5ml NS (Negative control)	4.24± 0.06^{a}	6.33± 0.18^{a}	7.34±0.10^{a}	8.44±0.18^{a}	7.49±0.08^{a}	7.42±0.11^{a}

Histological Results Mag. X 400 Hematoxyline & Eosine

The histological section of the liver tissue treated with 200mg/kg, 400mg/kg and 600mg/kg Combined Ethanol Extracts of Mango Stem Bark With Pawpaw Leaves (MSB+PL) respectively are shown in plate 3.6.1a to 3.6.1 c) below.

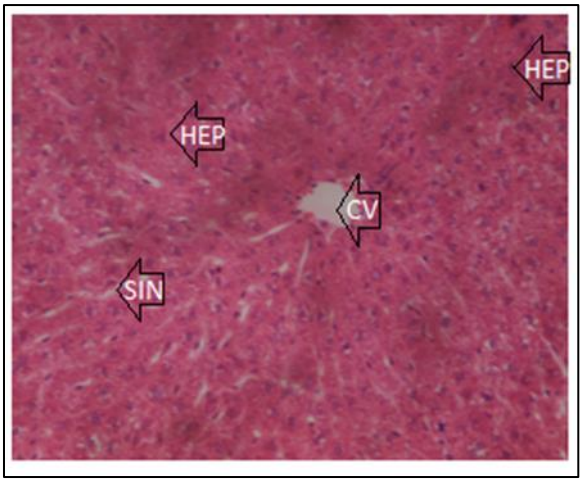

Figure 6 Liver tissue treated with 200mg/kg Combined Ethanol Extracts of MSB+PL. Mag. X 400 Hematoxyline & Eosine: Histologically normal liver, showing; Normal Hepatocyte (Hep), Patent central vein (CV), Sinusoid (sin) containing Von Kupffer cells

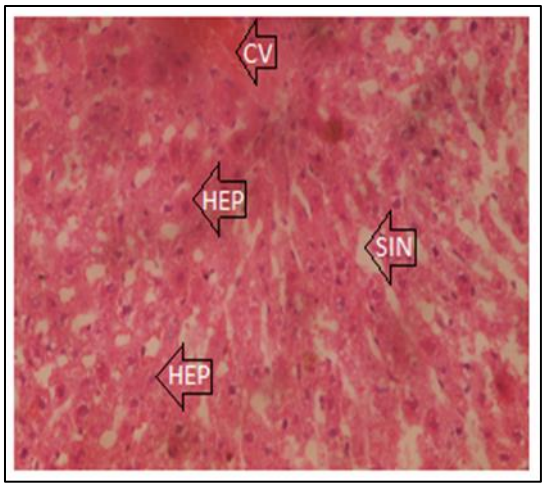

Figure 7 Liver tissue treated with 400mg/kg Combined Ethanol Extracts of MSB+PL. Mag. X 400 Hematoxyline & Eosine: Histologically normal liver, showing; Normal Hepatocyte, congested central vein, sinusoids contain von kupffer cells

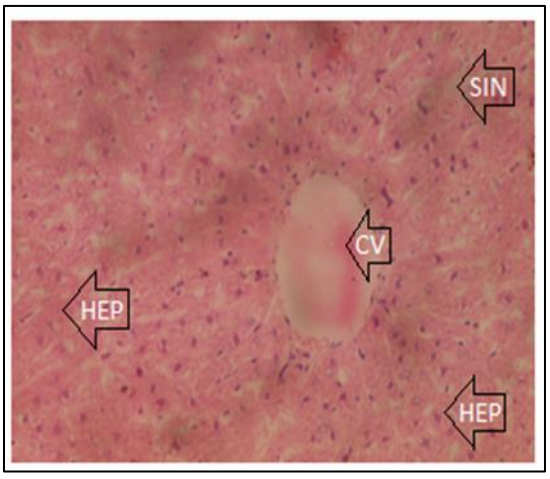

Figure 8 Liver tissue treated with 400mg/kg Combined Ethanol Extracts of MSB+PL. Mag. X 400 Hematoxyline & Eosine: Histologically normal liver showing; normal Hepatocyte, sinusoid and patent central vein

The histological section of the liver tissue treated with 200mg/kg, 400mg/kg and 600mg/kg Combined Ethanol Extracts Of Mango Stem Bark With Eucalyptus leaves (MSB+EU) respectively are shown in plate 3.6.2 (a to c) below

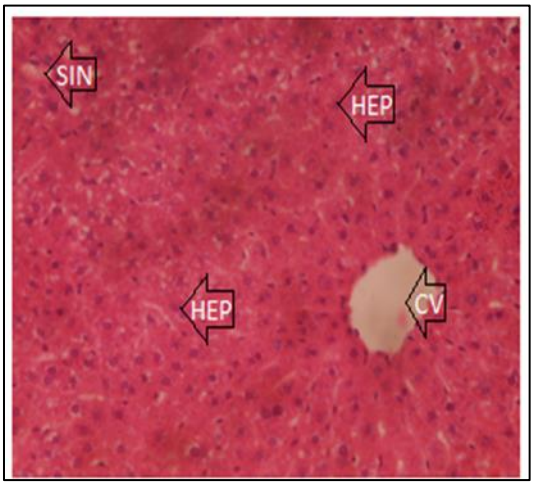

Figure 9 Liver tissue treated with 200mg/kg Combined Ethanol Extracts of MSB+EU. Mag. X 400 Hematoxyline & Eosine: Histologically normal liver, showing; normal hepatocytes, sinusoid and patent central vein

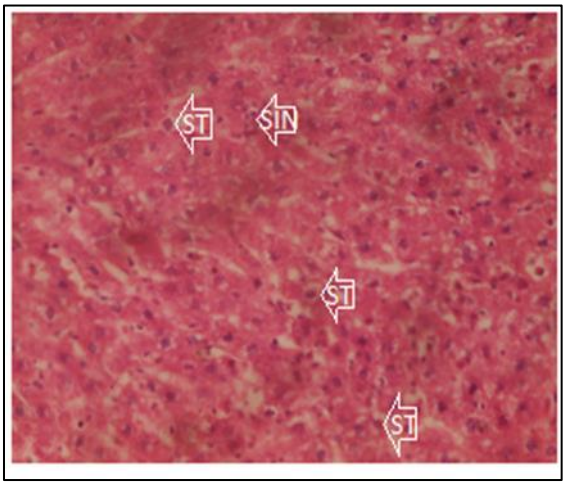

Figure 10 Liver tissue treated with 400mg/kg Combined Ethanol Extracts of MSB+EU. Mag. X 400 Hematoxyline & Eosine: Histologically distorted liver, showing; hepatocytes with microvesicular steatosis (ST), sinusoids containing inflammatory cells

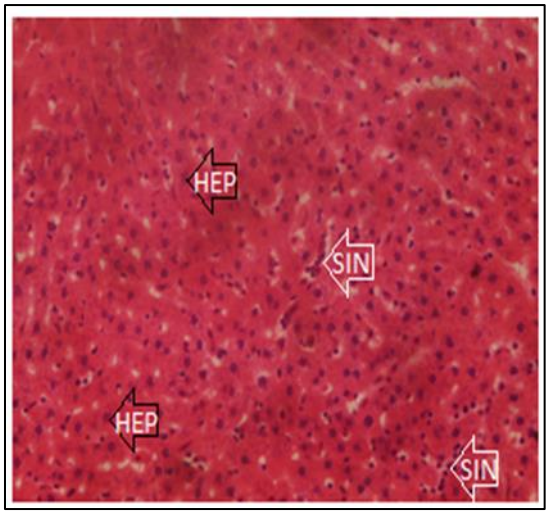

Figure 11 Liver tissue treated with 600mg/kg Combined Ethanol Extracts of MSB+EU. Mag. X 400 Hematoxyline & Eosine: Histologically distorted liver, showing; normal hepatocyte, sinusoids filled with inflammatory cells

The Histological Section of the Liver Tissue of the Normal Control, Standard Control (Positive Control) and Negative Control Respectively Are Shown In Plate 3.6.3 (a to c) Below.

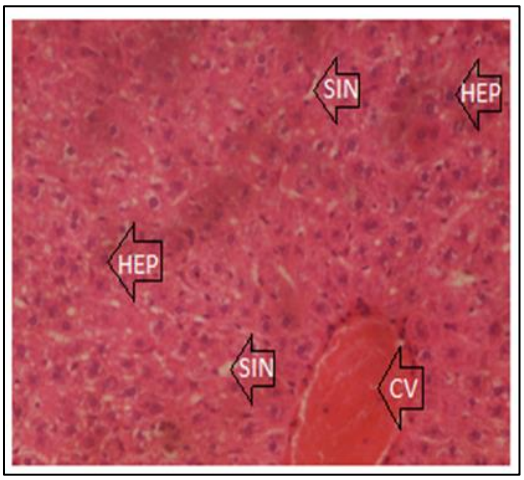

Figure 12 Liver tissue of the normal control. Mag. X 400 Hematoxyline & Eosine: Histologically normal liver, showing; normal hepatocyte, sinusoids containing von kupffer cells

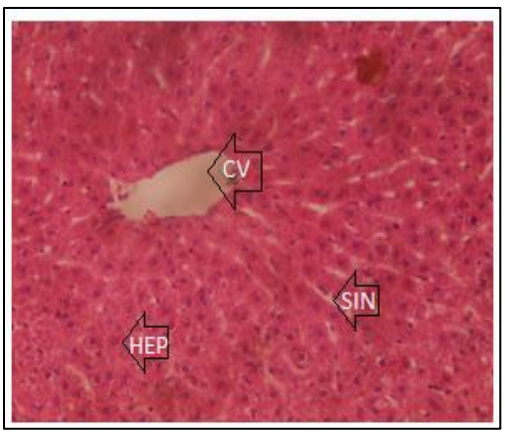

Figure 13 Liver tissue of the standard control. Mag. X 400 Hematoxyline & Eosine: Histologically normal liver, showing; normal hepatocyte, sinusoids and congested central vein

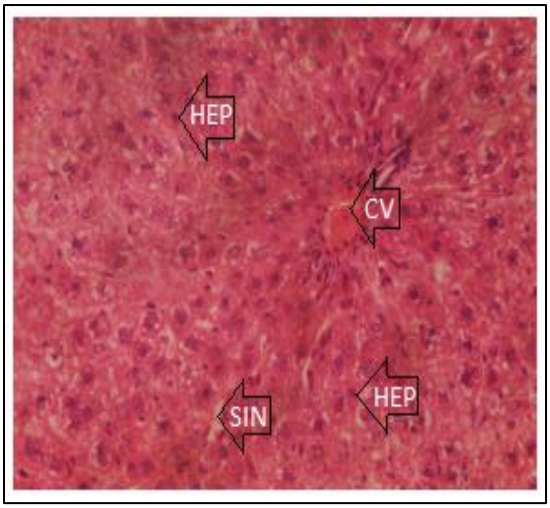

Figure 14 Liver tissue of the negative control. Mag. X 400 Hematoxyline & Eosine: Histologically normal Liver, showing; normal hepatocytes, sinusoids and congested central vein

4. Discussion

Inflammation is a complex biological response of vascular tissues against aggressive agents such as pathogens, irritants, or damaged cells. Acute inflammation is the initial response and is characterized by the increased movement of plasma and innate immune system cells, such as neutrophils and macrophages, from the blood into the injured tissues. The standard signs of inflammation are expressed by increased blood flow, elevated cellular metabolism, vasodilatation, release of soluble mediators, extravasation of fluids and cellular influx, with features such as joint pain, joint inflammation, synovial tissue proliferation which leads to damage and disability of joints (Sarkhel, 2016, Chang, et al., 2015).

The use of plants such as mango, eucalyptus and pawpaw amongst others, for treatment of diseases dates back to the history of human life. The active compounds of these plants have direct or indirect therapeutic effects and are used as medicinal agents. The presence of some bioactive compounds in these plants such as phenolics, Carotenoids, glucosinolate, 8-cineole play anti-inflammatory roles in several chronic pathological disorders associated with inflammatory responses (Masud, 2016, Aravind, et al., 2013, Nagpal, et al., 2010).

Results revealed that the packed cell volume (PCV) and hemoglobin (Hb) level of the negative control (untreated) group decreased significantly as well as the groups treated with 200mg/kg MSB+EU while the PCV and Hb level of other groups showed no significant change compared to the normal and standard (positive) controls. The significant decrease in PCV and Hb level in the negative control and 200mg/kg MSB+EU indicates excessive breakdown of red blood cell which results in anemia, hence suggesting a heightened inflammatory response to the arthritic condition (NseAbasi et al., 2014). This result is in agreement with Anyasor, et al., 2015 who reported a significant reduction in hematocrit in his work on inflammation. The reduction in the PCV and Hb level of the group treated with 200mg/kg MSB+EU may be as a result of low dose of the extract because others treated with same extract at higher doses didn't show any significant reduction in PCV and Hb level.

Packed cell volume (PCV), also known as haematocrit (Ht or Hct) or erythrocyte colume fraction (ECF) is the percentage (%) of red blood cells in the blood and is involved in transport of oxygen and absorbed nutrients. Increased PCV shows a better transportation and hence increased primary and secondary polycythemia (Isaac, et al., 2013). Hb is the iron-containing oxygen transport metalloprotein in the red blood cell with the physiological function of transporting oxygen to tissues for oxidation of ingested food so as to release energy for body function and transportation of carbondioxide out of the body (Isaac, et al., 2013, Omiyale, 2012). PCV, Hb and mean corpuscular hemoglobin are the major indices for evaluating circulatory erythrocyte and are significant in the diagnosis of anemia and as useful indices of bone marrow capacity to produce red blood cells (NsaAbasi, et al., 2014).

Other groups that show no significant difference in the PCV and Hb level compared to normal and standard control suggest the presence of nutritional content and anti-nociceptive properties of the extracts against pro-inflammatory mediators such as COX-2, INOS, histamine e.t.c. (Garrido, et al., 2009, Ashoush and Gadallah, 2011). The significant

change in the PCV and Hb of the group treated with standard drug (piroxicam) and other groups suggest anti-inflammatory effects which may be through the inhibition of COX-2 and prostaglandin production, hence reducing the levels of TNF-α, IL-6 serum level and iNOS (Marquez, et al., 2010, Kyei, Koffour & Boampong., 2012).

The result of RBC is in line with the report of Agnel and Shobana, 2012 which stated that formalin induction causes significant decrease in RBC, which leads to anaemia. The RBC (erythrocyte) serves as a carrier of hemoglobin, thus involved in the transportation of oxygen and carbondioxide in the body (Isaac, et al., 2013). A reduction in RBC level therefore suggest reduction in the level of oxygen carried to the tissues as well as level of carbondioxide return to the lungs (Isaac, et al., 2013, Ugwuene, 2011).

The WBC was significantly elevated in all the groups compared to the control and was highest in the negative control. This is in agreement with Agnel and Shobana, 2012 which stated that the level of WBC was higher in formalin induced inflammation. The white blood cells (WBC) fight infection, defend the body by phagocytosis against invasion of foreign organisms and to transport, produce and distribute antibodies in immune response. Therefore high WBC suggests the action of WBC in generating antibodies in response to inflammation; stress (NseAbasi, et al., 2014, Isaac, et al., 2013 and Herbert, 2012). Pretreatment with the extracts at higher doses significantly decrease the WBC count, this may indicate the significant recovery from the inflammation process due to the anti-inflammatory properties of the extract, which act by inhibiting the synthesis and release or the action of inflammation mediators such as histamine, serotonin and prostaglandins (Agnel and Shobana, 2012).

Platelet count showed no significant difference among all the groups except in the MSB+PL combination. This may suggest that the MSB+EU combination may be better and very similar in activity to the standard treatment. Platelets are responsible for blood coagulation, therefore rise in platelet count may indicate that the extracts may have a stimulatory effect on thrombopoietin. Increase in platelet count (thrombocytosis) may also be associated with inflammation (Arika, et al., 2016). The significant decrease in the groups treated with 600mg/kg MSB+PL and 400mg/kg MSB+EU from Arika, et al., 2016 report, may be as a result of anti-nociceptive effect from the extract on the inflammation.

The result observed in erythrocyte sedimentation rate (ESR) may be as a result of tissue mediated response elicited by formalin induced acute inflammation which is non-immunologic and as such does not initiate the release of immunological inflammatory mediator proteins; cytokines, TNF-α, C-reactive proteins (CRP), IL-6, IL-1β which cause erythrocyte to stack-up and as such leads to faster settling in chronic inflammations (Udegbunam, et al., 2014). It may also be attributed to the report of Ezeani, et al., 2019 which said that increased disease process (chronic inflammations) is indicated by upsurge in ESR. Chronic infections may play important role in increased erythrocyte aggregation and raised CRP levels play important role in induction and maintenance of increased erythrocyte aggregation (Ezeani, et al., 2019).

The result of the liver markers revealed low AST and ALT levels and this may be related to vitamin B6 (pyridoxal phosphate) deficiency which occurs with liver/kidney or inflammatory conditions (Ueland, Ulvik & Rios 2015; Marghoob Hassan, Marghood & Addelmarouf, 2013). Alkaline phosphate (ALP) is use to assess the integrity of the hepatobiliary system and flow of bile into the small intestine and as such as used to ascertain occurrence of liver injury (Elizabeth, Annette. Stafen and Dina and Pierre, 2014). In this study, groups treated with MSB+PL had elevated activities of ALP, this is in accordance with the result of Abiola, Olori & Farombi, (2021) who stated elevations in liver test markers.

The results of liver markers suggest no signs of general toxicity, hepatoxicity in the treated groups (in terms of altered serum activity of AST, ALP and ALT and concentration serum protein). However, low AST and ALT levels may be related to Vitamin B6 (pyridoxal phosphate) deficiency which occurs with liver/kidney or inflammatory conditions (Ueland, et al., 2015; Marghoob Hasan, et al., 2013).

Kidney function parameters were not assessed in this present study but our team is already working on it. A worrisome aspect of our result is the fact that the 600mg/kg body wt. MSB+EU showed a significant decrease in ALT activity which was corroborated by histological findings. Recall that this high dose MSB+EU had been the dose with the most efficacious results. Hence this finding is like a snag in an already clean slate.

Malondialdehyde was highest in the negative control and low-dose groups (200mg/kg body wt. of extracts) than that of the normal control. This result is in agreement with Udegbunam, et al., 2014 who reported increased MDA level in formalin inflamed group which received normal saline. The significant change of MDA in the tissue is considered a measure of lipid peroxidation which is linked to superoxide radical production. Increased level of MDA seen in negative control suggests profuse production of free radicals due to the presence of formaldehyde in the tissues. The slight

decrease in MDA level of all the groups except 200mg/kg MSB+EU and positive (standard) control indicates that the extracts and the standard drug (piroxicam) played a role in ameliorating the inflammatory process by mopping up the free radicals produced (Udegbunam, et al., 2014). Phenols and polyphenolic compounds such as flavonoids are widely present in plant derived food products and have been shown to possess significant antioxidant activity (Amraoui, et al., 2019).

The result of TNF-α observed may suggest the inflammatory response induced by formalin, which is a non-immunological response which elicits a localized inflammation, followed by cell/tissue mediated response (nociceptive response) (udegbunam, et al., 2014).

From the result, the treated groups did not show any significant change in the body weight on day 10, compared to the negative control; this may indicate their ability to feed even in the face of the inflammatory distress. Significant increase in paw size is a physical indicator of the inflammation in the onset of the disease. This swelling developed as a result of cellular damage which influenced the production of endogenous inflammatory mediators such as histamine, serotonin, prostaglandins and bradykinin (Patel, et al., 2011). The increase in paw size and redness developed over a 48 hour period in the feet of experimental animals injected with formalin and they were often relatively immobile due to the severity of paw swelling. The extract showed a marked reduction in paw size volume be inhibiting the release of endogenous inflammatory mediators, hence showing its anti-nociceptive/anti-inflammatory effect in formalin induced arthritis. This may be attributed to phytochemical components such as alkaloids, phenols, glycosides, tannins, terpens, flavonoids, saponins, which have been proven to have anti-inflammatory effect (Ighodaro and Akinloye, 2017, Amraoui, et al., 2019).

Inflammation causes the release of chemicals from tissues and migrating cells such as prostaglandins (PGs), leukotrienes (LTs), histamine and bradykinin and more recently platelet activating factor (PAF) and interleukin-1. Inflammatory drugs inhibits cyclo-oxygenase and reduces the synthesis of prostanoids corticosteroids, thus preventing the formation of both PGs and LTs by causing the release of lipocortin which inhibits phospholipase A2, reduces arachidonic acid and thus suppresses the inflammation of rheumatoid arthritis. Non-steroidal anti-inflammatory drugs inhibits cyclo-oxygenase (COX1 and 2), the enzyme involved in prostaglandin biosynthesis. COX-2 induced by inflammatory stimuli such as cytokins, produces prostaglandins that contribute to the pain and swelling of inflammation (Omodamiro and Jimoh, 2014, Ezeani, et al., 2019).

The histological examination of the liver section revealed histologically normal liver, Normal Hepatocyte (Hep), Patent central vein (CV), Sinusoid (sin) containing Von Kupffer cells in all the test groups except the groups treated with 400mg/kg and 600mg/kg body weight MSB+EU which revealed Histologically distorted liver, sinusoids filled with inflammatory cells

Liver sinusoids are made of highly unique cells which functions to maintain liver function and hepatocyte. However, liver damage due to disease or exposure to toxic substances induces the deregulation of these cells, which switch from maintaining proper liver homeostasis to a pro-inflammatory phenotype which further compromises liver function (Gibert-Ramos, et al., 2021, Thomson and Knolle, 2010). These results in the recruitment of immune cells by the liver sinusoids endothelial cells (LSECs) such as natural killer T cells (NKT) and lymphocytes, leukocytes from the lumen of sinusoids into the liver tissues (Gibert-Ramos et al., 2021, Shetty, et al., 2018).

The distortion of the liver and inflammatory cells observed in the sinusoids of the animals treated with MSB+EU extract may suggest a mark of possible or potential toxicity.

5. Conclusion and recommendation

Early detection of inflammation and proper treatment to control/manage it, offers the highest likelihood of preserving function and preventing disability.

The result of the present study reveals the toxicity and anti-inflammatory efficacy of combined ethanol extracts of mango stem bark + pawpaw leaves and mango stem bark + *eucalyptus* leaves respectively.

The result on hematology revealed a significant change ($p<0.05$) in platelet and white blood cell; indicating the action of the immune system responding to the inflammatory/stress. There was no significant change in the red blood cells at high doses of the extracts, hence indicating the presence of nutritional content of the extracts and anti-nociceptive properties of the extracts.

The present study established that combined ethanol extracts of mango stem bark + pawpaw leaves did not show any toxic effect on the liver of the rats, hence may possibly be safe for consumption. However, mango stem bark + *eucalyptus* leaves at high doses may be toxic.

The antioxidant examination revealed increased level of Malondialdehyde (MDA) in the negative control. This indicates production of free radicals due to the presence of formaldehyde in the tissues and prevention of it in the treated groups.

The tumor necrosis alpha examination (TNF-a) revealed no significant change, hence the acute inflammation induced by formalin is non-immunologic.

The result also showed that combined ethanol extracts of mango stem bark + pawpaw leaves and mango stem bark + *eucalyptus* leaves, have significant anti-inflammatory effect due to the marked reduction in paw size volume of the rats. Hence these extracts may be used in the management of painful arthritic inflammatory condition.

Histological examination carried out on the liver of the rats revealed no alteration in the liver cell integrity of the rats treated with the mango stem bark + pawpaw leaves extracts. However, questions are raised on its effect on liver integrity with mango stem bark + *eucalyptus* leaves extract due to the distortion of the liver and inflammatory cells observed in the sinusoids.

Contribution to knowledge

The study justifies the anti-inflammatory use of both extract combinations and suggests MSB+EU maybe most effective, however, questions are raised on its effect on liver integrity.

Recommendation for further study

Based on the results, Further studies are suggested on effects on other body organs and to determine the anti-inflammatory effect of these extracts on immunological pro-inflammatory molecules (such as cytokines, IL-6, IL-1β, TNF-α, ESR and C-reactive proteins) on arthritic rats induced by complete Fraud's Adjuvant. Further studies are also required for isolation of active constituents and cellular characterization so as to exclusively establish these plant parts (mango stem bark, eucalyptus leaves and pawpaw leaves) as a potential safer disease modifying agent in the management/treatment of arthritic inflammation.

Compliance with ethical standards

Acknowledgments

The authors firstly thank the Almighty God (The Lord Jesus Christ) for His Grace throughout the duration of this work. And also the supervisors, Biochemistry Department, Federal University of Technology Owerri for their assistance

Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper

Statement of ethical approval

Ethical approval was obtained.

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