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Evaluation of lipid profile and antioxidant activities of an herbal combination therapy on formalin-induced inflammation in albino rats

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Abstract

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. Combination of *Mangifera indica* Stem-bark (MSB), *Carica papaya* (PL) and *Eucalyptus* (EU) leaf ethanol 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 200 mg/kg, 400mg/kg 400 mg/kg, and 600mg/kg 600 mg/kg combined ethanol extracts MSB+PL respectively. Groups 4-6 received 200 mg/kg, 400mg/kg 400 mg/kg, and 600mg/kg 600 mg/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. There was significant reduction in the malondialdehyde (MDA), the levels of SOD and CAT increased significantly. There were significant changes/elevation in lipid profile. The study therefore justifies that the extracts could played a role in ameliorating the inflammatory process by mopping up the free radicals produced by the inflammation.

Keywords: Antioxidants; Lipid Profile; Anti-Inflammation

1. Introduction

Herbal medicine is gaining popularity both in developing and developed countries because of its natural constituents 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).

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, which 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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This study is aimed at evaluating lipid profile and antioxidant activities of an herbal combination therapy on formalin-induced inflammation in Albino rats**.**

2. Material 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 N5^{0}23'33.6876" longitude and E6^{0}59'10.5504 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 (300 g) powdered sample 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 ℃). The solvent evaporated and dripped into the extraction chamber where it was collected. The active component left behind in the flask was 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℃) (25 ℃) 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 were 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 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 their 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 received their treatments accordingly, while groups 7 and 9 received 5mls normal saline and group 8 received 5mg/kg 5 mg/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 then sent to the laboratory for analysis. Blood samples were centrifuged for 10mins at 3000rpm and the serum was use to examine malondialdehyde (MDA) according to the method of Ohkawa & Ohishi (1979), Catalase was determined using Clairborne method (1985), and Superoxide Dismutase (SOD) was assessed according to misra and fridovich method (1972).

Furthermore, for lipid profile parameters, Cholesterol and triglycerides were assessed using Enzymatic and-point method (Unit mmol/L) (Y Kayamori, *et al.,* 1979), and high density lipo-protein (HDL) – Cholesterol was assessed using Precipitant Method (Unit mmol/L) (L Kerscher, *et al.,* 1985).

2.6. 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≤0.05 were considered statistically significant.

3. Results

3.1. Lipid Profile Results

The Total Cholesterol (TC) (Mmol/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 no significant difference in the TC concentration of all the test groups compared to the normal control group except for the group treated with 600mg/kg 600 mg/kg MSB+PL which was significantly higher than the normal group but similar to the negative control group as shown in Figure 1.

Total Glyceride (TG) (Mmol/L) of inflamed Rats Treated With Different Concentrations of Combined Ethanol Extracts

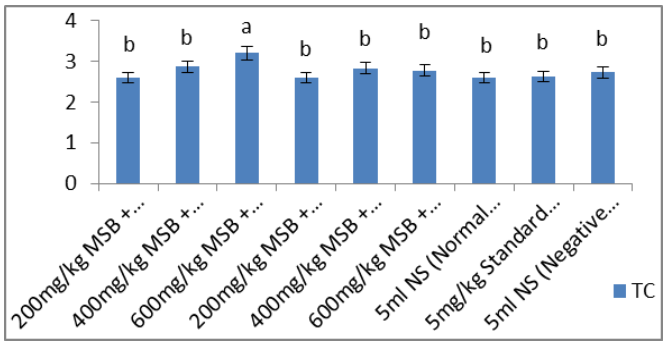

Figure 1 TC (Mmol/L) of inflamed rats treated with MSB+EU and MSB+PL respectively. Bars are mean ± standard deviation. Bars bearing different alphabet letters are statisticall significant (p<0.05)

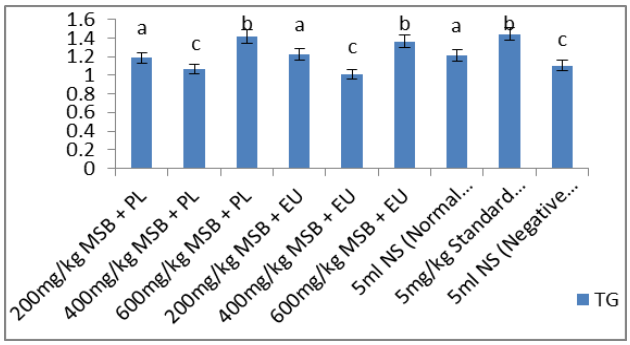

Figure 2 TG (Mmol/L) of inflamed rats treated with MSB+EU and MSB+PL respectively. Bars are mean ± standard deviation. Bars bearing different alphabet letters are statisticall significant (p<0.05**)**

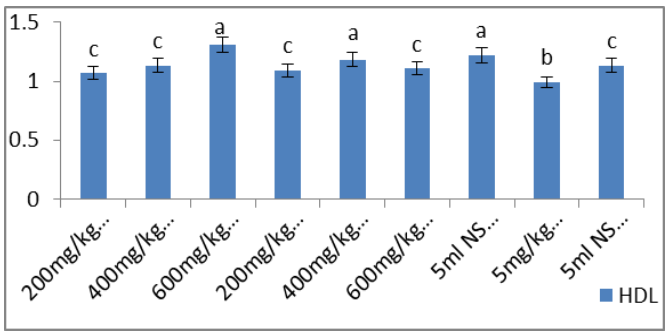

Figure 3 HDL of inflamed rats treated with MSB+EU and MSB+PL respectively. Bars are mean ± standard deviation. Bars bearing different alphabet letters are statisticall significant (p<0.05)

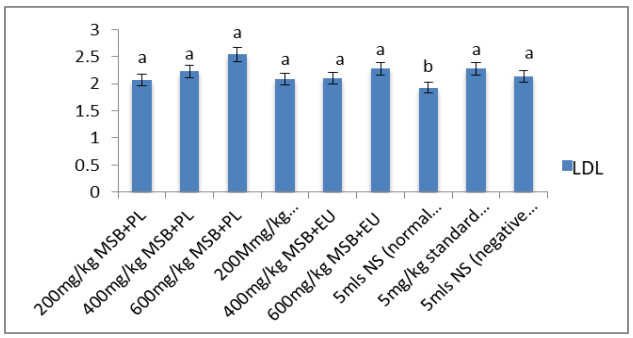

Figure 4 LDL of inflamed rats treated with MSB+EU and MSB+PL respectively. Bars are mean ± standard deviation. Bars bearing different alphabet letters are statisticall significant (p<0.05**)**

Legend: MSB= mango stem bark; PL= pawpaw leaves; EU= *Eucalyptus*; NS= normal saline

of Mango Stem Bark With Pawpaw Leaves And Mango Stem Bark With Eucalyptus Leaves showed a marked increase in the level of TG concentration of the groups treated with higher doses of the extract and negative control compared to the normal control. The groups fed with 200mg/kg 200 mg/kg MSB+PL and MSB+EU were similar to the normal control group while the groups fed with 400mg/kg 400 mg/kg were comparable to the negative control as shown in Figure 2.

There was a marked significant decrease in the high density lipoprotein (HDL) level of the standard control compared to all other groups and normal control group. Apart from the group treated with 600mg/kg 600 mg/kg MSB+PL which is similar to the normal control, all other groups decreased significantly and are comparable to the negative controls as shown in figure 3

The low density lipoprotein (LDL) of all the test groups revealed a significant increase compared to the normal control group as shown in figure 4.

3.2. Antioxidant Results

The Catalase Activity (CAT) (U/G) 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 a significant decrease in the catalase activity of the negative control compared to the normal control and other treated groups as shown in Figure 5

The superoxide dismutase (SOD) (μ/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 showed that there were clear significant decrease in the SOD level of the negative control, standard control and all the rats fed with low doses of the extract compared to the normal control. However, the groups fed with higher doses of the extract were comparable to the normal control as shown in Figure 6.

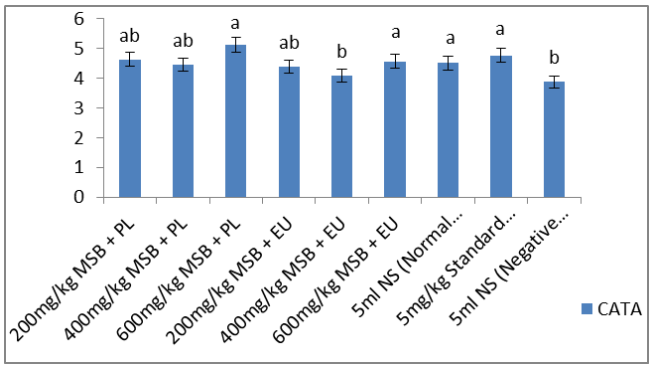

Figure 5 CATA of inflamed rats treated with MSB+EU and MSB+PL respectively. Bars are mean ± standard deviation. Bars bearing different alphabet letters are statisticall significant (p<0.05)

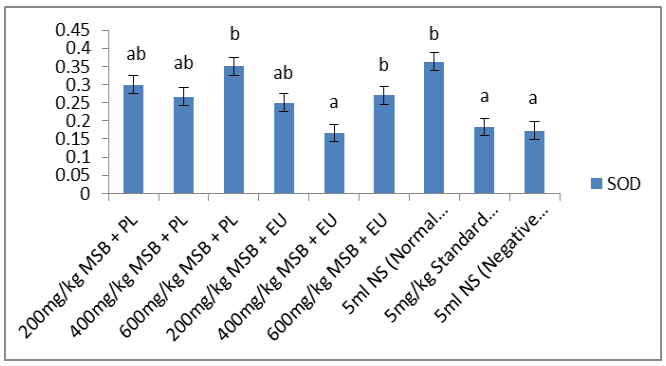

Figure 6 SOD of inflamed rats treated with MSB+EU and MSB+PL respectively. Bars are mean ± standard deviation. Bars bearing different alphabet letters are statisticall significant (p<0.05)

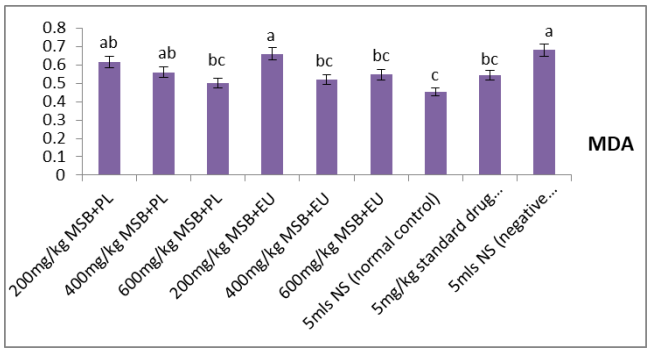

Figure 7 MDA of inflamed rats treated with MSB+EU and MSB+PL respectively. Bars are mean ± standard deviation. Bars bearing different alphabet letters are statisticall significant (p<0.05)

Legend: MSB= mango stem bark; PL= pawpaw leaves; EU= eucalyptus; NS= normal saline

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 the untreated inflamed group (group 9), this significant increase was also seen in the lowest dose of MSB+EU group (200mg/kg 200 mg/kg body weight) and all doses of the MSB+PL groups except the highest (600mg/kg 600 mg/kg body weight animals). There was no significant difference observed between the rest of the groups and the normal control as shown in Figure 7

4. Discussion

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).

Inflammation could affect serum lipid level and lipoprotein function. In the face of inflammation, LDL is more easily oxidized as the ability for HDL to prevent the oxidation of LDL is diminished. However, it is worthy to note that the greater the severity of the underlying inflammation, the more consistently these abnormalities in lipids and lipoproteins are observed (Anyasor, *et al.,* 2015). The changes in the serum concentrations of LDL, cholesterol, HDL and triglyceride, may suggest a possible disposition of the animals to dyslipidemia and this is comparable to the report by Abiola *et al.,* 2021) who reported changes in the concentration of lipids. The marked reduction in serum HDL level in the group treated with standard drug (piroxicam) in this study may indicate a significant metabolic disorder in the rats caused by piroxicam administration (Abiola, *et al.,* 2021). However, higher doses of the extract especially MSB+EU were observed to reverse this observation. Increase in serum triglyceride and cholesterol could suggest that the extract contains ingredients capable of increasing the hepatic VLDL production and decreasing the clearance of triglyceride rich lipoproteins; enhancing the activities of hepatic lipogenic, cholesterogenic enzymes like malic enzymes, fatty acid synthase, glucose 6-phosphatate dehydrogenase and HMG-CoA reductase which are required for cholesterol synthesis (Feingold and Grunfeld, 2022; Knowlton, *et al.,* 2012; Emejulu, *et al., 2014;*).

The enzymatic antioxidant systems such as catalase and superoxide dismutase play a corresponding role in the avoidance of oxidative damage by reactive oxygen species. SOD is one of the chief cellular defense enzymes that dismutate superoxide radicals to water and oxygen. Catalases (CAT) on the other hand are heme-containing proteins that defend the cells from toxic effects of reactive oxygen species by converting hydrogen peroxide to water and molecular oxygen. The increase in the activities of antioxidant enzymes is comparable to that reported by udegbunam, *et al., (*2014) who reported increase in the SOD levels, and Ruma *et al.,* 2015) who also reported increase in the antioxidant activity. This may suggest the fact that upon injection of inflammatory agent, excess reactive oxygen species are generated causing oxidative stress, the production of ROS could overwhelm the capacity of endogenous antioxidant enzymes system resulting to significant decrease. However, administration of the extracts increases the antioxidant enzyme activity, thereby neutralizing the free radicals generated. It may also be suggested that the standard drug had no significant effect on the SOD enzyme activity.

Malondialdehyde was highest in the negative control and low-dose groups (200mg/kg 200 mg/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 200 mg/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).

5. Conclusion

It is now widely acknowledged that early diagnosis of inflammation and aggressive treatment to control disease activity offer the highest likelihood of preserving function and preventing disability.

The result of the present study reveals the effects of combined ethanol extracts of mango stem bark + pawpaw leaves and mango stem bark + *eucalyptus* leaves respectively on lipid profile and antioxidant activities.

The result on antioxidant revealed a significantly increased level of Malondialdehyde (MDA), decreased SOD and CAT in the negative control. This indicates production of free radicals due to the presence of formaldehyde in the tissues.

The lipid profile assessment revealed significant changes in the LDL, HDL, cholesterol, and triglycerides; which indicate possible disposition of the animals to dyslipidemia.

Contribution to knowledge

The study revealed that the combined extracts of mango stem bark + pawpaw leaves and mango stem bark + *eucalyptus* leaves is observed to reverse the significant changes on the lipids caused by the inflammation and piroxicam (the standard drug) may cause significant metabolic disorder in prolong usage . The extracts reduced the level of MDA in the groups treated with the extracts, and increase the antioxidant activity of SOD and CAT. This justifies the anti-inflammatory use of both extract combinations in ameliorating the inflammatory process by mopping up the free radicals produced.

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

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Disclosure of conflict of interest

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

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors

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