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Full Length Research Paper

Effects of ethanol and aqueous leaf extracts of *Pterocarpus mildbraedii* on the liver function and CD4 cells of the immune system in albino rats

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ABSTRACT

Pterocarpus mildbraedii Harms (Leguminosae) leaf is among the commonly consumed leafy vegetables in Nigeria. Ethanol and aqueous extracts of the leaves had been found to exert anti-diabetic effect in rats. This study was designed to investigate the possible toxicological effects of the ethanol and aqueous leaf extracts of this plant on liver functions and the immune system in Wistar albino rats. Intraperitoneal administration of ethanol and aqueous extracts of *P. mildbraedii* leaves at the doses of 200 and 400 mg kg^{-1}, to two groups of rats for 28 days showed no adverse effects on serum levels of liver function indices estimated. No significant differences ($p > 0.05$) in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin (TBIL) were observed at the end of the 28 days-treatment when compared with control group that received saline. Furthermore, histopathological examination of the liver samples revealed normal architecture in both control and treated groups. However, significant increases in immune system of the extracts- treated rats were observed. Aqueous and ethanol extracts, at 200 mg kg^{-1} body weight, increased the CD_4 cell counts from 42.50±7.50 to 127.67±1.45 cells μl^{-1} and from 52.50±2.50 to 189.33± 5.2 cells μl^{-1} respectively. These effects of the extracts increased with increased doses. These results suggest non-toxic effects of leaf extracts of *P. mildbraedii* on the liver, as well as an ability to boost the immune system in rats. Hence, the plant can be considered safe for use as leafy vegetable in foods or pharmaceutical formulations.

Keywords: *Pterocarpus mildbraedii*, Immune system, amino transferases, Liver, histopathology, and toxicology.

INTRODUCTION

A wide variety of leafy vegetables are consumed in Nigeria.Plant-derived foods, particular vegetables and fruits, are beneficial components of the human diet. They contribute to life by providing wide range of nutrients, vitamins and other substances (Newman *et al.*, 2003). The selection of a particular vegetable for inclusion in the diet depends on a number

of factors such as availability, indigenous knowledge and cultural practice (Eyo *et al*., 2003). Different authors have reported on chemical studies carried out on the commonly used Nigeria leafy vegetable. Nevertheless, a careful examination of literature revealed that, there are some less commonly used and inexpensive leafy vegetables whose nutritional potentials as well as their toxicological effects on living organism have not been adequately studied. One of such is *Pterocarpus mildbraedii* Harms (Leguminosae) leaf which is locally known as Ora in Ibo (Akpayung *et al*., 1995).

P. mildbraedii belongs to the family Leguminosae. It grows mostly in the eastern part of Nigeria. The exudations produce gums and resins which have been used for various purposes. In some part of Eastern Nigeria, the young and tender leaves of this plant are used traditionally as vegetable for the preparation of soups and there has been claim that it possesses anti-diabetic properties.

Toxicity is an expression of being poisonous, indicating the state of adverse effects led by the interaction between toxicants and cells. This interaction may vary depending on the chemical properties of the toxicants and the cell membrane. The toxic effects may take place prior to the binding of the toxicants to the vital organs such as liver and kidneys. Hence, evaluation of toxic properties of a substance is crucial when considering a substance for public health consumption. In practice, the evaluation typically includes acute, sub-chronic, chronic, carcinogenic and reproductive effects (Asante-Duah *et al*., 2002).

This study was designed to scientifically investigate the possible toxicological effects of ethanol and aqueous extracts of *P. mildbraedii* on the liver and CD_4 cells of the immune system in albino rats.

MATERIALS AND METHODS

Plants materials

The fresh leaves of *P. mildbraedii* were collected from Abagana, Njikoka local Government area, of Anambra State. The leaves were then identified and authenticated by a taxonomist in the Department of Botany, Nnamdi Azikiwe University; Awka with the voucher specimen prepared and deposited in the herbarium. The leaves were separated from the stalk and air-dried under shade at room temperature after which they were grounded and sieved to obtain a fine powdered form of the leaf.

Animals

Wistar albino rats (40) adult male were purchased from the animal house of the Veterinary Medicine Department, University of Nigeria, Nsuka. They were housed in standard rat cages well ventilated in the animal house of Applied Biochemistry Department, Nnamdi Azikiwe University, Awka.

2.3 Chemicals

All the chemicals used were of analytical grade and were products of BDH Ltd, Poole, England.

Preparation of Crude Extracts

The extraction was carried out at room temperature with 500 g of the powdered leaves macerated in 2 litre of water (distilled) for 72 hr to obtain aqueous extract while another 500 g of the powdered leaves in 2 litre of 80 % ethanol was used to prepare ethanol extract. The ethanol extraction was carried out with the use of soxhlet extraction. The extracts were filtered through clean muslin cloth and the extraction process was repeated by adding another 2 litre of distilled water and ethanol to the sample residue. The filtrate from each extraction was combined and concentrated to evaporate the excess ethanol to obtain thick slurry of both extracts.

Study Design

The animals were kept on commercial feed manufactured by Guinea Feeds Ltd. Asaba, Delta State for the period of the experiment (28 days). The animals were sorted and divided into five groups (A-E) with eight animals per group. Group A is control which was not given the extracts of *P. mildbraedii* but were given 1 ml kg^{-1} of normal saline, group B and C received 200 mg kg^{-1} and 400 mg kg^{-1} of ethanolic extract of *Pterocarpus mildbraedii* intraperitoneally respectively while group D and E were given 200 mg kg^{-1} and 400 mg kg^{-1} of aqueous extracts of *Pterocarpus mildbraedii*respectively**.**

Studies on Hepatic and Immune Systems

Effects of extracts on Liver Function Parameters

The rats were sacrificed on day 0, day 14 and day 28 of the administration, 2 animals were sacrificed from each group on day 0 while 3 animals were sacrificed from each group on day 14 and 28. The blood samples were collected into plain bottles to examine the possible effects of the extracts on certain biochemical parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin (TBIL) used for assessing liver function.

Effects of Extracts on CD4 cells

The control animals (group A) were given only the normal feed and 1ml kg^{-1} of normal saline. Two doses "200 mg kg^{-1} and 400 mg kg^{-1} body weight" of ethanol extracts of the plant leaves were administered intraperitoneally to group B and C for 28 days. Two doses of aqueous extracts of the plant leaves were administered intraperitoneally to group D and E for 28 days. The rats were sacrificed at day 0, day 14 and day 28 of the administration and the blood samples were collected into ethylenediaminetetra- acetic acid (EDTA) bottle for CD4 test. The effects of the extracts on immune cells precisely CD4 T-lymphocytes were examined. The CD4 analysis carried out at day 0 before extracts administration was used as the baseline analysis while that of days 14 and 28 were used as a follow up analysis after the exposure of the rats to the extracts.

Histopathology Studies: Kidneys, Liver and Spleen

All the vital organs isolated from each rat were fixed in 10% buffered formalin, routinely processed and embedded in paraffin wax. Paraffin sections considered (5μm) were cut on glass slides and stained with haematoxylin and eosin. The slides were examined under a light microscope and the magnified images of the tissue structures were captured for further analysis (Mcmanus *et al*., 1984).

Statistical analysis

Statistical analysis was conducted Statistical Package for Social Sciences (SPSS). Data is reported as mean ± SEM of 3 measurements and was analysed using't' test. *P* values less than 5% was considered significant ($p < 0.05$).

RESULTS

Effects of extracts on Liver function parameters

Figures 1 and 2 below show the effects of ethanol and aqueous extracts of *P. mildbraedii* on the liver of albino rats. The extracts of *P. mildbraedii* did not have any significant effects on serum level of alanine aminotransferase, aspartate aminotransferase, alkaline phosphasphate and total bilirubin ($p>0.05$) when comparing the results obtained from the extracts treated rats with the results obtained from baseline analysis carried out on all the groups before treatment with the extracts of *P. mildbraedii*. Also, there were no significant differences when the results

obtained from extracts treated rats was compared with the results obtained from follow up liver function analysis carried out on the rats in the control group.

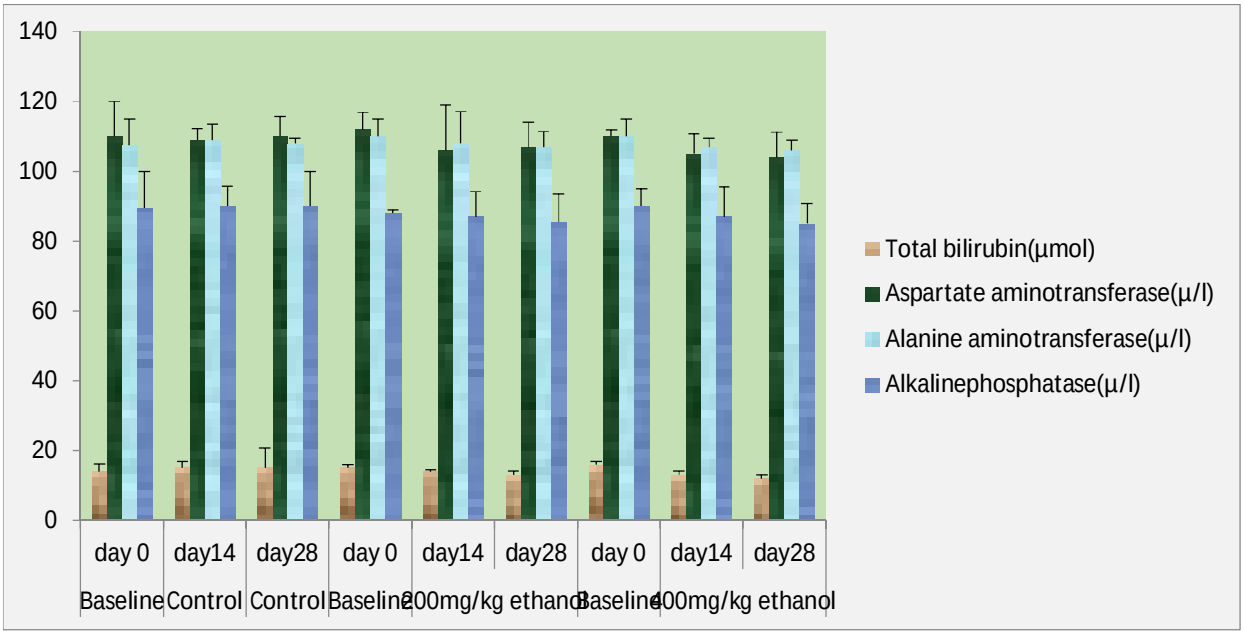


Fig. 1: The effects of ethanol extract of *P. mildbraedii* on Liver function parameters

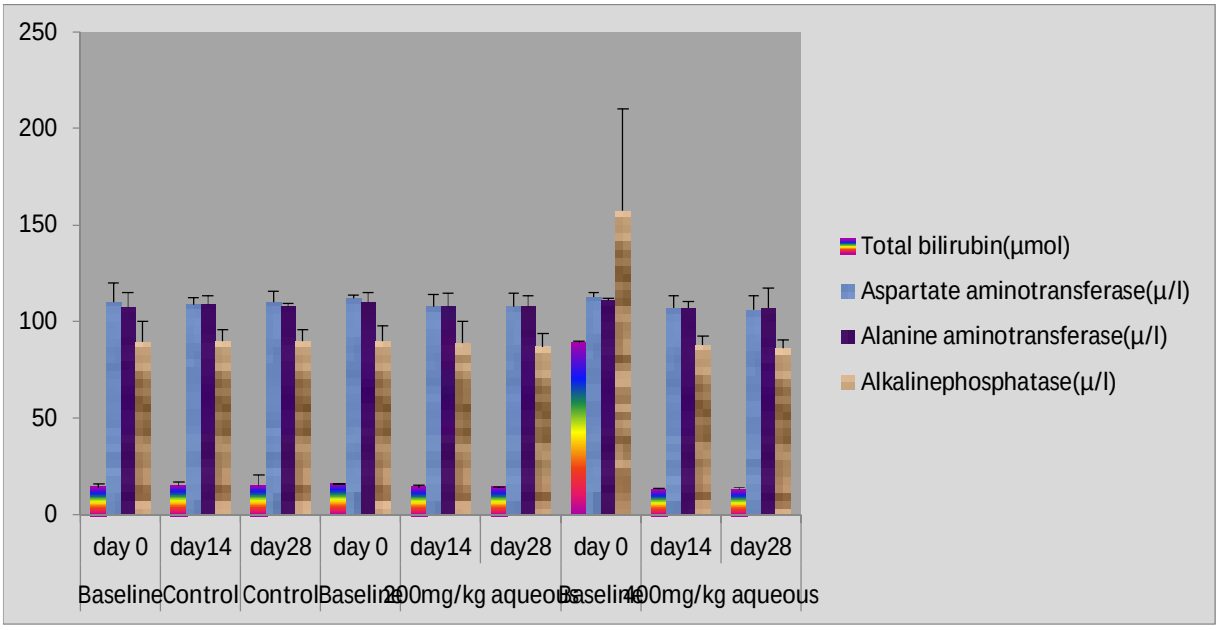


Fig. 2: The effects of aqueous extract of *P. mildbraedii* on Liver function parameters

Effects of extracts on CD4 cells

Figures 3 and 4 below show the effects of ethanol and aqueous extracts of *P. mildbraedii* on CD4 cells of albino rats. Both 200 mg kg^{-1} and 400 mg kg^{-1} B. W. of ethanol and aqueous extracts of *P. mildbraedii* significantly increased the CD4 cell counts in the blood of the *P. mildbraedii* extracts treated rats. The increase in the CD4 cells count of the extracts treated rats was also observed when compared to the results obtained from the entire baseline CD4 analysis carried out on all the groups. Also, there was a significant difference in the CD4 cell counts of the extract treated rats compared to the results obtained from the follow up CD4 analysis carried out on the control groups ($p < 0.05$) with the CD4 cell counts of extracts treated rats significantly increased compared to that of the rats in the control group.

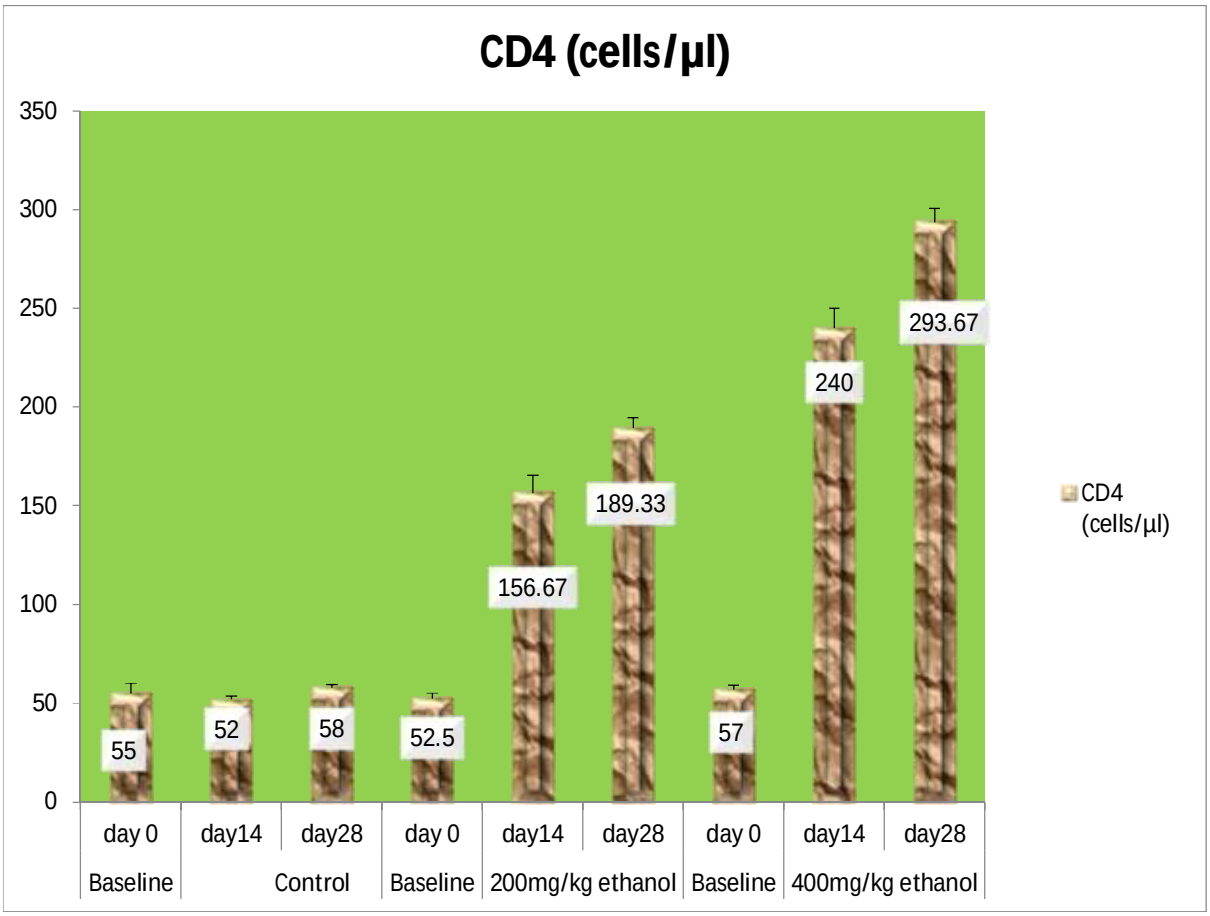


Fig. 3: The effects of ethanol extract of *P. mildbraedii* on CD4 cells

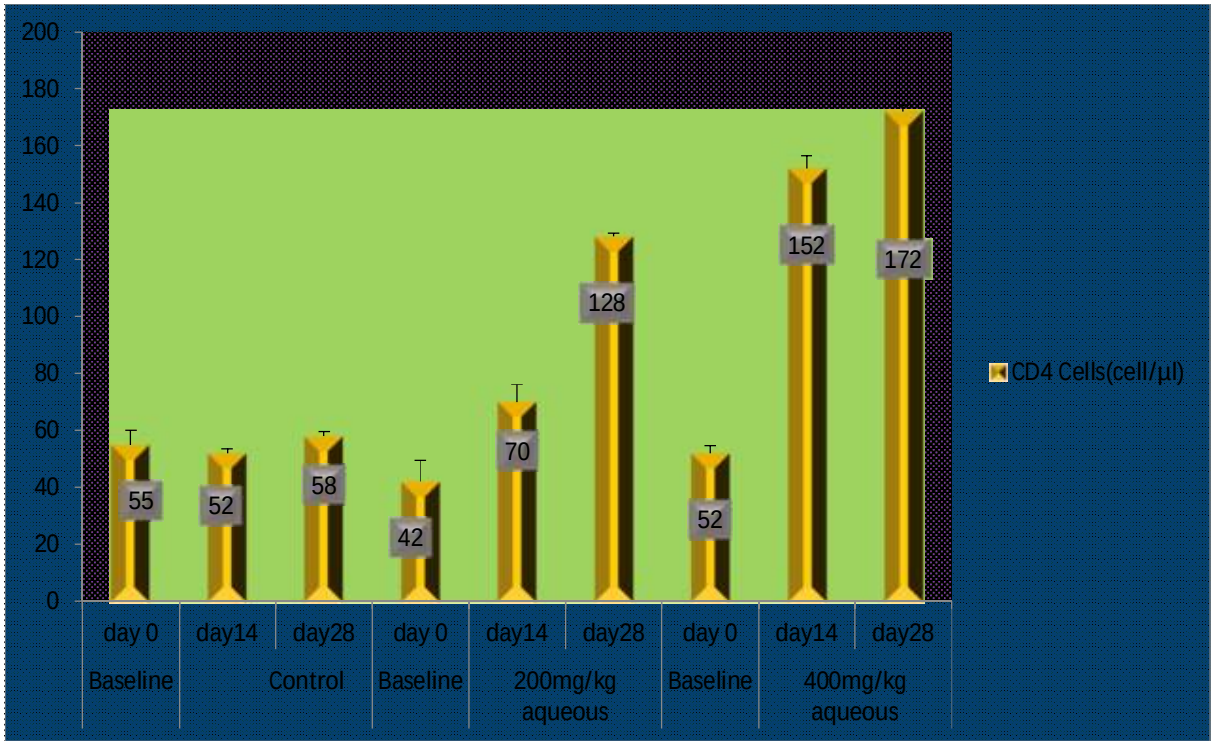
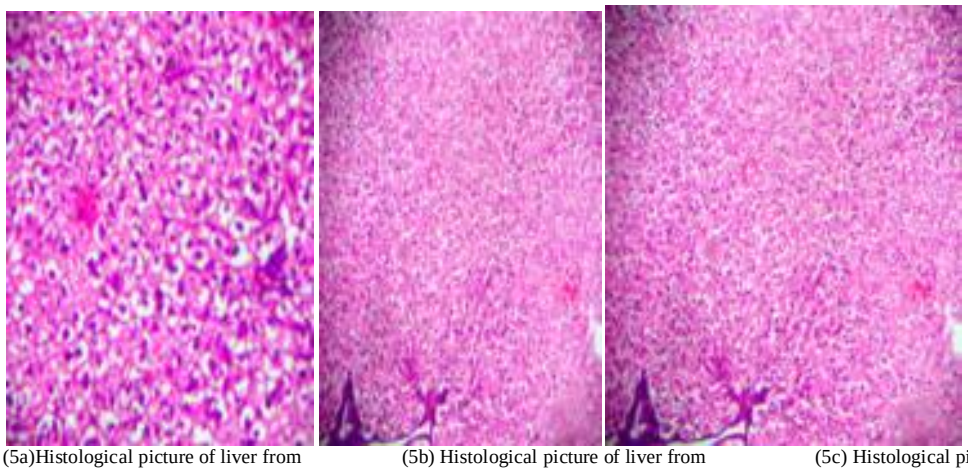


Fig. 4: The effects of aqueous extract of *P. mildbraedii* on CD4 cells

Histopathology analysis

Macroscopic examination of the organs of the animals treated with extract showed no changes in color compared to control. Autopsy at the end of the experiment period revealed no apparent changes in the liver from both control and extracts treated rats in the histopathology analysis. The microscopic structures of the organs depicted through Figure 5a, 5b and 5c below shows unnoticeable differences between the control and test groups. The microscopic examination revealed that, the liver from the extract treated rat did not show any alteration in cell structure or any unfavorable effects when viewed under a Carl Zeiss research microscope using multiple magnification power (Axioskope 40, Germany) with a digital camera attached with the representations area of the slide digitally photomicrographed. The structure or coordination of cells in extract treated organs is more or less similar compared with the control organs. Hence, the results of the histopathological analysis correlate with the results obtained from biochemical analysis.



(5a)Histological picture of liver from control rats

(5b) Histological picture of liver from ethanol extract treated rats

(5c) Histological picture of liver from aqueous extract treated rats

Figure 5:Showing Histological examination of liver (5a, 5b, 5c)

DISCUSSION

Phytotherapeutic products from medicinal plants have become universally popular in primary healthcare, particularly in developing countries, and some have been mistakenly regarded as safe just because they are a natural source. Nevertheless, these bioactive products from medicinal plants are presumed to be safe without any compromising health effect, and thus widely used as self-medication (Vaghasiya *et al*., 2011).

Therefore, toxicity study is vitally needed not only to identify the range of doses that could be harmful to human health but also to reveal the possible clinical signs elicited by the substances under investigation (Rang *et al*., 2001). Toxicity results from animals study will be crucial in definitively judging the safety of plants (medicinal and foods) if they are found to have sufficient nutritional potential as well as therapeutic potentials for development into pharmacological products (Moshi, 2007). In this study, rats in control group were only exposed to commercial feed and 1ml kg^{-1} saline whereas the rats in test group were exposed to 200 and 400 mg kg^{-1}.

Finding from our study revealed that, all the used doses of ethanol and aqueous extracts of *P. mildbraedii* caused a significant increase in the CD4 cell counts of the extracts treated rats ($P < 0.05$) compared to the result obtained from the rats in the control group. The results of the CD4 cell counts analysis also showed that extracts treated rats has higher CD4 cell counts compared to the results obtained from baseline CD4 analysis conducted for all the groups at day 0 of exposure. The effects of the extracts are dose dependent as the 400 mg kg^{-1} was discovered to increase the CD4 cell counts at a higher rate than 200 mg kg^{-1} body weight of the same extracts administered into the rats for the same number of days. The CD4 cells count was increased by 200 mg kg^{-1} ethanol extract from 52.50±2.5 to189.33±5.21 while CD4 cells count was increased from 57.00±2 to 293.67±6.84 by 400 mg kg^{-1} ethanol extract within the same period. The ethanol extract was also discovered to significantly increased CD4 cells count level in rats than aqueous extracts at all the doses used in this study.

Furthermore, ethanol leaf extract of *P. mildbraedii* was also significantly more potent as an immune response booster compare to aqueous leaf extract of *P. mildbraedii.* The CD4 cell counts was increased by 200 mg kg^{-1} ethanol leaf extract from 52.50±2.5 to189.33±5.21

while 200mg/kg of aqueous leaf extract increased CD4 cell counts from 42.50±7.5 to 127.67±1.45.The CD4 cell counts was increased from 57.00±2to293.67±6.84 by 400 mg kg^{-1} of ethanol leaf extract while 400mg/kg of aqueous leaf extract increased CD4 cell counts from 52. 50±2.5 to171.67±2.19 as shown in figures 1 and 2. After 28 days of treatment, no significant changes was observed in serum level of TBIL, ALT, AST and ALKP in extracts treated rats at all the used doses compared to rats in control group which indicate the non-toxic nature of the leaf extracts.

Apart from biochemical analysis, histological analysis was done to further confirm the absence of alteration in cell structure of the organs. The histological examination is the golden standard for evaluating treatment related pathological changes in tissues and organs (OECD. 1995). The liver is the main target organ of acute and chronic toxicity where exposed to the foreign substances being absorbed in intestines and metabolized to other compounds which may or may not be hepatotoxic to the rat (Rhiouania *et al*., 2008). In the present study, histopathological evaluation of intrperitoneally ingestion of *P. mildbraedii* leaves indicated that the extracts did not adversely affect the morphology of the rat liver. This agrees with the results of biochemical analysis. However, the liver is capable of regenerating damaged tissue; hence the liver function may not be impaired early following an insult from a toxicant (Salawu *et al*., 2009).

CONCLUSIONS

The present results show that ethanol extract of *P. mildbraedii*does not cause any apparent *in vivo* toxicity in an animal model. No death or signs of toxicity were observed in rats treated with extracts (ethanol and aqueous) at doses 200 and 400 mg kg^{-1} thus establishing its safety in use. *P. mildbraedii* leaf extracts however significantly increased CD4 cells level in extracts treated rats and can serve as an immune response booster. Hence, the use of *P. mildbraedii*can be encouraged both as a leafy vegetable and as a medicinal agent in known dosages, especially in rural communities where conventional drugs are unaffordable because of their high cost. A detailed experimental analysis of its chronic toxicity is essential.

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