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Adamu Adamu Ahmed
Department of Biological Science, Federal University of Kashere, Nigeria

Ismail Muhammad
Department of Zoology, Gombe State University, Gombe, Nigeria

Abdullahi Muhammad
Department of Chemical Pathology, Federal Teaching Hospital, Gomb, Nigeria

Abubakar Sulaiman Bapeto
Department of Biological Science, Federal University of Kashere, Nigeria

Corresponding Author:
Ismail Muhammad
Department of Zoology, Gombe State University, Gombe, Nigeria

Histopathological studies of liver of albino rat treated with *Mangifera indica* aqueous leaf extract

Adamu Adamu Ahmed, Ismail Muhammad, Abdullahi Muhammad and Abubakar Sulaiman Bapeto

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Abstract
Today, several plant extracts are employed as chemoprophylactics for a variety of illnesses. This is mainly because they are less restrictive, easily accessible, and extremely economical, and they are also known to contain bioactive components with antimalarial activities. The dosage of these extracts is typically not carefully controlled, which causes individuals to suffer serious harm to several important organs damage including the liver. Therefore, the present study aimed to determine the histopathological impact of *Mangifera indica* aqueous extract on the liver of the experimental rats. The mango leaves were collected and identified at the herbarium of the Botany Department, Gombe State University with voucher number GSUH46. The identified leaves were washed with distilled water and dried under shade at optimal ventilation for one week, after which it was crushed into labelled powder using electric blender. One hundred and fifty grams (150 g) of the sample was socked in 2 litres of distilled water for the extraction of the phytochemical components of the leaves using standard technique and the doses of the extract to be administered were determined according to the body weight of the experimental animals. For the oral acute toxicity test, five (5) albino rats were treated with 2000 mg/kg of the extract and monitored for any physical signs of toxic manifestations. The liver of two rats from each group was dissected and examined for morphological and histological changes. The morphological examination of the livers shows no morphological or histological difference. *Mangifera indica* aqueous extract is safe to be used as it has no effect on the liver of treated rat.

Keywords: Malaria, *Magnifera indica*, acute toxicity

Introduction
Even today, malaria is seen as a severe public health issue, particularly in the sub-Saharan African nations where the disease is still endemic. This is primarily because the region has favourable climatic and weather conditions that support the survival of the parasite and the disease's vector (Siwal *et al*., 2018) [28], as well as inadequate health facilities that can offer effective services like early diagnosis and effective treatment as recommended by World Health Organisation (WHO), thereby preventing the spread of the disease. The development of new pesticides to address the issue of vector resistance, radical treatment for the liver stage of the parasite, especially hypnozoites, clear gametocytes, thus preventing reinfection, and other commendable efforts are being made at various levels to prevent, control, and eradicate the disease (Omagha *et al*., 2022) [22]. This resulted in a decrease in malaria cases and mortality from 585, 000 to 405, 000 between 2010 and 2018, as well as a decrease in the number of malaria-endemic countries from 108 to 91 and also Ten (10) countries were certified as being malaria-free during this time, and 29 other countries were successful in preventing the reintroduction of new infections (Aigbiremo *et al*., 2021) [2].
Four *Plasmodium* parasite species (P. *malariae*, P. *vivax*, P. *ovale*, and P. *falciparum*) are responsible for the disease, which is spread by female Anopheles mosquitoes (Okpe *et al*., 2023; Santhy *et al*., 2016) [20, 26]. The most severe form of the disease, is caused by *Plasmodium falciparum*, has been linked to fevers that occasionally occur at intervals of less than 48 hours (Olayode *et al*., 2015) [21] and it can even be fatal especially in Africa (White, 2018) [31]. Other species also cause acute, severe illness, but mortality rates are low (Obimakinde *et al*., 2018) [16].

The majority of the disease's symptoms appear during the parasite's asexual stage when it invades and destroys the host's cells, causing symptoms such as high fever, headache, chills, excessive perspiration, discomfort, shivering, drowsiness, and confusion (Airaodion *et al*., 2021; Edem *et al*.,2020) [3, 32].

Over a billion people are at high risk of contracting malaria globally (Obeagu *et al*., 2017) [15] According to, Ojurongbe *et al*., (2013) [17] there were roughly 229 million cases of malaria worldwide in 2019 and 409,000 deaths, with more than 90% and 93% of these cases respectively occurring in sub-Saharan Africa (Obimakinde *et al*., 2018) [16] where Nigeria account for 27% global malaria case and 23% global death (Muhammad *et al*., 2022; Okpe *et al*., 2023) [14, 20]. According to Obimakinde *et al*., (2018) [16], the disease is responsible for 30% of hospital admissions and 60% of outpatient visits to healthcare facilities in Nigeria. Due to their low immunity, young children and pregnant women are especially susceptible to the illness.

The use of synthetic medications from various antimalarial drug classes, such as Quinolines, Artemesin, and Antifolate, which specifically target the parasites' asexual stage (*Plasmodium species*), as well as synthetic insecticides, which specifically target the disease's vector (female Anopheles mosquitoes), is essential for both treating and preventing the disease. One of the biggest drawbacks of all of these is that the vector and parasites have, respectively, gained resistance to medications and insecticides, including the recently advised Artemisinin-based Combination Therapy (ACT) (Didier & Alfredo, 2020; Wang *et al*., 2015) [6, 30]. Alternative medications are required for the disease to be effectively treated.

However, quite several people, particularly in rural and remote areas where access to healthcare is limited, have developed the habit of self-medicating for their ailments using a variety of plants and extracts, which can vary from one region to another (Maharaj *et al*., 2022) [12]. Many plant components, including leaves, bark, stems, roots, and others, have been utilised for ages to treat a variety of illnesses, including malaria (Alaiya & Odeniyi, 2023) [4]. Traditional herbalists have suggested a variety of herbs and plant extracts, such as *Azadirachta indica*, *Moringa oleifera*, and *Mangifera indica*, for the treatment of malaria because they have been shown to have antiplasmodial effects (Oseni & Akwetey, 2012) [24]. The chemical components in plant parts like seeds, leaves, roots, and the peels/skin of some fruits are what give plants their medical value. According to According to Okolie & Kristhien, (2019) [19] and Joseph *et al*., (2021) [10]. These chemical compounds have distinct physiological effects on humans. Hence there is a need to investigate the impact of these plant extracts on some vital organs. Therefore this paper aimed to investigate some histopathological changes associated with the liver of albino rats after being treated with aqueous *Mangifera indica* extract.

Methodology

Study Area

This research was conducted in Gombe Local Government Area, Gombe State, Nigeria. The local Government is located between latitudes 10^0 08^1 N and 11^0 24^1 E and longitudes 11^0 02^1 N and 11^0 18^1E with a total land mass area of 52km^2. The climate of Gombe is characterized by a dry season and rainy season with a mean annual rainfall of 835 mm and a mean annual temperature of about 26 °C whereas relative humidity is higher (94%) in August and drastically dropping to less than 10% in the months of January-April (Mbaya *et al*., 2012) [13].

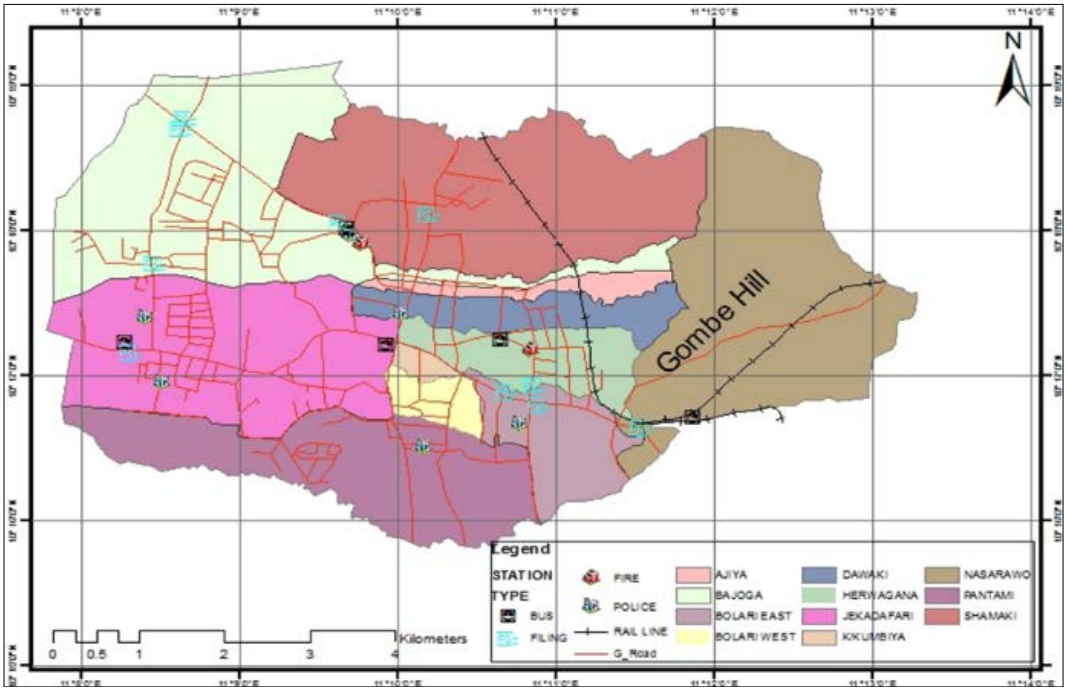

Fig 1: Map of Study Area

Plant Material Collection, Identification, and Preparation

Mango (*Mangifera indica*) leaves were collected from a location close to the Dukku Local Government Secretariat in Gombe State, and a botanist in the Herbarium section of the Department of Botany at Gombe State University identified leaves as *Mangifera indica* leaves with specimen voucher number (GSUH46). Fresh plant material leaves were carefully washed with distilled water to remove dirt and soil, then dried for a week in the shade with good ventilation.

Using an electric blender, the dried leaves were mashed into a powder that was labelled. In a tightly closed coloured amber vial, a sample of the processed powder was stored at room temperature. Two litres of distilled water were used to encapsulate 150 grams of the material, which was then left at room temperature for seven days. To make sure the solvent reached the sample, the mixture was thoroughly shaken every six hours with an orbital shaker (KJ-201BS). The mixture was then filtered using Whatman No. 1 filter paper, and the filtrate was then concentrated at a temperature of 40 °C in a rotatory evaporator while under vacuum, producing a sticky residue as leaf extracts. Until it was needed for anti-malarial screening, the extract was maintained in a tightly closed, labelled specimen vial in a refrigerator at 4 °C.

Phytochemical Screening of *Mangifera indica* Leaves Extract

Following the procedures outlined by Sofowora (1993) [33], an aqueous extract of *Mangifera indica* leaves was produced and examined for the presence of active phytochemicals such as Saponins, Reducing Sugars, Tannins, Cyanogenic Glycosides, Phenols, Terpenoids, Flavonoids, and Alkaloids.

Determining *Mangifera indica* leaf extract concentrations for the *in vivo* study

The dosages of the extract given were calculated based on the experimental animal's body weight, this is by the methodology used by (Ceravolo *et al*., 2021) [5], the precise dose was calculated using the formula below.

$$\text{Volume (dose) of extract (ml) administered} = \frac{\text{Weight of animal (kg) x dose (mg)}}{\text{Concentration of extract (mg/ml)}}$$

Acute Oral Toxicity Test

Female albino rats that were not diseased, nulliparous, or pregnant were employed. The oral toxicity research was carried out by the widely recognised methodology created by OECD Guidelines 425. For this investigation, 5 female rats were employed, and they were later watched to make sure they were eating and drinking regularly and moving about. All of the animals were then starved for 4 hours before and 2 hours following the administration of the extract. The first rat received a single oral gavage dose of the extract equal to 2,000 mg/kg of body weight, followed by continuous monitoring for the first 30 minutes after extract administration and intermittent monitoring for the following 4 hours and throughout 24 hours. When there was no sign of the first rat dying, the extract was given to four other rats at the same dose (2000 mg/kg). For 14 days following extract administration, all rats were watched to look for toxic manifestations such as lack of appetite, hair erection, lacrimation, diarrhoea, tremors, convulsions, death, and other toxic consequences. The control group were treated with distilled water.

Histopathological examination

Microtome was used to cut the liver of the treated and non-treated (control) rats into chunks 0.5 mm thick and placed in 10% formalin at room temperature and then transferred into 70% alcohol for dehydration after fixing. Before being put into two changes of molten paraffin wax for 20 minutes each in an oven at 57 °C, the tissues were each run through 90% alcohol and chloroform for 10 minutes each. Using a microtome, serial sections of 5 m thick of solid block tissue were cut. They were then each individually stained with hematoxylin and eosin stains before being run through a solution of equal parts xylene and alcohol. After being cleared in xylene, each tissue was dried in an oven. As illustrated in Plate 1, photomicrographs were taken using a coloured digital camera attached to an Olympus light microscope.

Results

Phytochemical Analysis of Leave Extract of *Mangifera indica*

The qualitative phytochemical study of the aqueous leave extract of *Mangifera indica* contained most of the active phytochemicals as presented in Table 1. Alkaloids, Tannins, Saponins, Cardiac Glycosides, Steroids Terpenoids, and Phenols were present. Flavonoids and Reducing sugars were absent.

Table 1: Result of qualitative phytochemicals analysis of *Mangifera indica* leave extract

Test	Inference
Alkaloids	Present
Saponins	Present
Flavonoids	Absent
Steroids	Present
Tannins	Present
Reducing sugars	Absent
Phenols	Present
Terpenoids	Present
Cardiac glycosides	Present

Acute Toxicity Study

The acute oral toxicity test of aqueous leave extract of *Mangifera indica* at the limit test dose of 2,000 mg/kg revealed that all female albino rats in the extract-treated group exhibited no gross physical and behavioural changes, such as vomiting, loss of appetite, excitement, sleep, diarrhoea, abnormal secretion, or hair erection from the first hour to 24 hours period. In addition, all rats survived during the 14-day observation period. It was also recorded that altered feeding was observed, and the food and water intake increased. The mean body weight of the extract-treated rats at the dose of 2000 mg/kg body weight at the end of the observation period was found to be 80.5±7.5g. There was a significant increase when compared with the mean body weight of the rats before the treatment (70.0±10g).

Histopathological Analysis of Liver section of Albino rats

The histological examinations of the liver sections from rats treated with 2000 mg/kg body weight of the extract showed normal morphology, where hepatocyte plates, sinusoids, portal triads, and congested central veins appeared normal as compared to histology of the negative control rat (Non-treated rats) (Plate I). The results from the oral toxicity evaluation indicated that there was virtually no toxicity caused by the extract at the maximum single oral dose of 2000 mg/kg body weight (acute toxicity). In addition, there were no differences in the histopathological examinations of the liver tissues between the treated and non-treated (Control) groups.

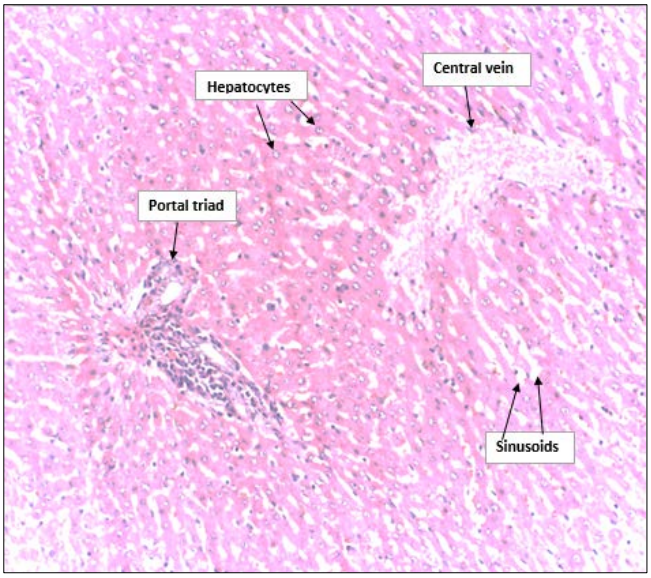

Plate 1: Histopathological examination showing normal liver of a rat without the administered leave extract of *Mangifera indica.*

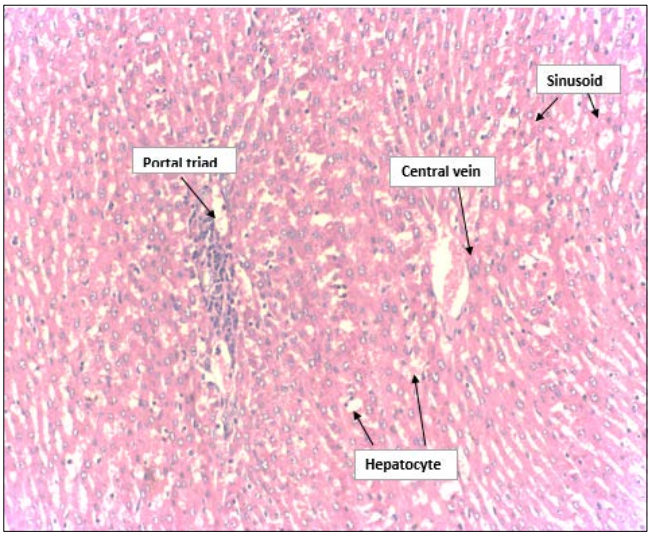

Plate 2: Histopathological examination of the liver of a rat administered with 2000mg/kg body weight of Leave Extracts of *Mangifera indica.*

Discussion

Many individuals prefer utilising extracts and other preparations prepared from the leaves and bark of medicinal plants instead of synthetic or conventional medicine since they are more widely available, less constrained, and, most importantly, less expensive(Koffi *et al*., 2020) [11]. In addition, different parts (bark, root, stem, leaves, etc.) of several plants are known to have antimalarial properties (Okokon *et al*., 2022) [18]. *Mangifera indica* is one of the plants that could have a significant group of pharmaceutically significant antimalarial, antiparasitic, and antibacterial chemicals (Okolie & Kristhien, 2019; Parvez, 2016) [19, 25]. The present study evaluated the phytochemical constituents and safety profile of the aqueous leaf extract of *Mangifera indica* as an antimalarial drug in the liver of the tested rats. An extensive array of bioactive substances, including Alkaloids, Saponins, Terpenoids, Cardiac Glycosides, Tannins, And Phenolics, have been identified. This finding is comparable to that made by Uniyal & Rahal, (2022) [29], who identified the same bioactive substances in *Mangifera indica* leaf tissue. Flavonoids were not listed as one of the bioactive chemicals in the current study; this is consistent with findings by Omotayo *et al*., (2022) [23] and Agrawal, (2021) [1], who also noted the absence of flavonoids from mango leaf extracts. Flavonoids and reducing sugars, for example, may not be present in a plant extract due to differing geographic sources of the plant being used or the method of extraction, notably the solvent employed. These secondary metabolites might be the cause of the plant's medicinal effects. This was consistent with Wen-Hui *et al*., (2018) [34] reported that several phytochemical substances have significant antimalarial properties. The antiplasmodial action may have been elicited by alkaloids alone or in combination with other metabolites in the extract, according to Ettebong *et al*., (2015) [8], who claim that alkaloids have been known to exhibit antimalarial activities by preventing protein synthesis in the Plasmodium parasite.

The aqueous leaf extract of *Mangifera indica*'s acute toxicity was determined to be larger than 2000mg/kg, indicating that the extract may have low toxicity. This is consistent with earlier research that found that any chemical with an oral LD_{50} value of more than 2000 mg/kg body weight may be regarded as having low toxicity and being safe for humans (Hagazy *et al*., 2020) [9]. Additionally, according to this study, no deaths or signs of toxicity were seen in the tested rats from the first four hours onwards, and no toxic effects were noted in the subsequent rats tested. Sidi *et al*., (2008) [27] reported that no toxicity or signs of toxicity were seen in rats treated with 2000mg/kg over a 90-day observation period also supports this conclusion. The safety of the traditional herb (*Mangifera indica*) in its antimalarial use on several crucial organs, such as the liver, is therefore indicated by such data. Additionally, based on a photomicrograph of a normal liver with no effects, the histological analysis of the liver organs of treated and untreated rats revealed no hepatotoxicity in the 2000 mg/kg-administered animals.

Conclusion

The extract of *Mangifera indica* possesses some basic bioactive component of medicinal importance, and these compounds are not harmful to the organs of the experimental animals. Therefore *Mangifera indica* can be effectively used for the treatment of recommended diseases.

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