HISTOLOGICAL CHANGES IN THE LIVER OF WISTAR RATS TREATED WITH CRUDE AQUEOUS EXTRACTS OF MANGIFERA INDICA STEM BACK

1Oaikhena G.A.; 1Izunya, A.M.; 1Olugbenga, M.A.; 1Ujaddughe, M.O., 1Blackies, H.O.T and 2Okhiai, O.
Department of 1Anatomy and 2Nursing Sciences, Faculty of Basic Medical Sciences, College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

Correspondence: oaikhenaganiyu@gmail.com

Received: 27th July, 2014 Accepted: 18th September, 2014 Published: 30th September, 2014

ABSTRACT

This study was designed to determine the effect of aqueous extract of Mangifera indica stem bark on the histology of the liver in animal models. Twenty Wistar rats weighing between 170-185g were used for this study. They were sub-divided into four groups: A, B, C and D (n=5 each). Group A served as control, while B, C, and D served as test groups. For 14 days, group A received normal feed (grower’s mash and water only, while groups B, C and D received oral doses of 0.25ml (25mg), 0.5ml (50mg) and 1ml (100mg) of aqueous extract of Mangifera indica stem bark respectively. At the end of the experiment, the animals were weighed and sacrificed to harvest the liver for tissue processing and microscopy using standard laboratory procedures. The results showed that liver sections in group A (control) and test group B, presented no remarkable histological changes, while mild to pronounced cytoarchitectural distortions were observed in the liver sections of groups C and D respectively; suggesting that at higher doses, Mangifera indica can be hepatotoxic.

Key Words: Aqueous extract, Liver, Mangifera indica, Weight changes,

INTRODUCTION

Mangifera indica (Mango) is a plant grown widely in different parts of Africa, especially in the southern part of Nigeria, where it is valued for use as edible fruit (Nwinuka, et al., 2008). It is one of the several plants with potent therapeutic active ingredients as available literature indicates that Mangifera indica is used medicinally to treat ailments such as asthma, cough, diarrhea, dysentery, leucorrhoea, jaundice, pains, malaria (Madunagu et al., 1990; Gilles, 1992) and diabetes (Ojewole et al., 2005; Muruganandan et al., 2005; Perpetuo et al., 2003; Mahabir and Gulliford, 1997).

Other therapeutic properties of Mangifera indica include analgesic, anti-inflammatory (Garrido et al., 2001), immune-stimulant (Makare, 2001; Garcia et al., 2002; Garcia et al., 2003a), antioxidant (Martinez et al., 2000; Sanchez et al., 2000; Sanchez et al., 2003), spasmyloytic, antidiarrhea (Sairam et al., 2003), dyslipidemic (Anila and Vijayalakshmi, 2002), antiabetic (Aderibigbe et al., 1999; Aderibigbe et al., 2001), antiamebic (Tona et al., 2000), antihelminthic, antiallergic (Garcia et al., 2003b) and antibacterial (Bairy et al., 2002). However, many have cautioned that beyond herbal drug-efficacy verification, there is an urgent need to adequately subject herbal preparations to sound toxicity studies in order to understand the collateral systemic consequences of such herbal
preparations; as there are existing scientific commentaries and assertions about drug mediated injuries (Huang et al., 2002) and herbal cytotoxicity potentials (Russel et al., 1997; Prohp and Alaiya, 2003; Prohp and Maduemezia, 2004; Prohp et al., 2004; Prohp et al., 2006a; Prohp et al., 2006b).

Moreover, previous studies have shown that aqueous extracts of M.indica stem bark contains active compounds like phenolic acids, phenolic ester, flavonoids and the xanthone mangiferin (Núñez-Sellés et al., 2002). This study therefore, examines the effect of Mangifera indica stem bark extract on the histology of the liver in Wistar rats.

MATERIALS AND METHOD

Location and Duration of Study: This study was conducted at the histology laboratory of the Faculty of Basic Medical Sciences, Delta State University (DELSU), Abraka. The preliminary studies, animal acclimatization, drug procurement, actual animal experiment and evaluation of results, lasted for a period of one month, while actual administration of the substance of study lasted for two weeks.

Animal Groupings: A total of 20 Wistar rats weighing between 170-185gm were used for this study. The animals were procured and maintained in the Animal house of the Faculty of Basic Medical Sciences, Delta State University, Abraka. The experimental animals were divided into four groups A, B, C and D (n = 5each) and housed in standard plastic cages. Group A served as the control, while groups B, C, and D served as test groups. All the rats in the groups were allowed to acclimatize for a period of 2 weeks during which they were fed with grower’s mash produced by Bendel Feeds and Flour Mills Limited, Ewu, (standard diet,), and water given ad libitum. They were allowed to acclimatize for one week before the commencement of study.

Ethical Consideration: Ethical approval was sought and obtained from the Department of Anatomy, Faculty of Basic Medical Sciences, DELSU, while the rules guiding the use of rats for scientific studies were strictly adhered to.

Preparation of Aqueous Extract: Fresh Mangifera indica stem bark was obtained from a farm in Ekpoma in Esan West Local Government Area (L.G.A), Edo State, Nigeria. The plant was identified and authenticated at the Botany department of Ambrose Alli University, Ekpoma. The stem bark of Mangifera indica was cut into smaller pieces and sun-dried for two weeks. The dried samples were then pulverized using mortar and pestle. The resultant powdery material was used for the extraction process. Extraction was carried out using the method described by Harboone (1972), Ekpe et al., (1990), Uhegbu et al. (2005) and Nwinuka et al. (2008). Using distilled water as solvent, 20 g of powdered sample of the herb was extracted by soaking in 200 ml of distilled water in a beaker, stirred for about 6 minutes and left overnight. Thereafter, the solution was filtered using filter paper (Whatman No. A-1) to remove cellulose fibers, while the extract was stored in a refrigerator at 4°C.

Experimental Design: After acclimatization period, the rats were weighed and divided into groups A, B, C and D (n=5 each). Group A rats (control) received normal food and water, while B, C and D (test groups) were treated with daily oral doses of 0.25ml (25mg), 0.5ml (50mg) and 1ml (100mg) of the aqueous extract daily for 14 days respectively. The 1ml of crude aqueous extract used in this study was based on the previous work done by Nwinuka et al. (2008). At the end of the experiment, the rats were weighed and anaesthetized with chloroform in order to dissect them and harvest the liver. The harvested tissues were immediately fixed in 10% formal-saline to avoid autolysis and putrefaction pending tissue processing which were performed using standard tissue processing techniques. Microscopy was performed using a light binocular microscope with a digital camera for photomicrography and at x400 magnification.

RESULT

The histological findings showed that the liver sections of groups A (control) and B presented normal cytoarchitectural features with relatively no remarkable histological changes (see plates 1, 2 and 3). However, the liver section of group C and D presented cytoarchitectural distortions. Specifically, group C presented mild cell pyknosis and eosinophilic cells, while group D presented hepatic cell necrosis with loss of cytoplasm and shadowy cell membranes surrounding centrally placed nucleus (vacuolar cytoplasm) (see plate 4).
DISCUSSION

The histological alterations observed in group D simply suggest that at higher doses, *M. indica* is hepatotoxic. This is supported by the fact that polyphenols at certain concentrations have the capacity to cause oxidative stress and liver toxicity in vivo (Lambert *et al*., 2007). In fact, reports from several other studies have it that phenolic constituents - triterpenes, flavonoids, phytosterols, and polyphenols in *M. indica* (Saleh and El-Ansari, 1975; Anjaneyulu *et al*., 1994; Kharn *et al*., 1994; Selles and Castro, 2002; Singh *et al*., 2004) in *M. Indica*, accounts for its antioxidant properties (Martinez *et al*., 2000; Sanchez *et al*., 2003). Some other studies have revealed that polyphenols exhibit clear cytoprotective effect on rat or tumour hepatocytes injury system (Sugikara *et al*., 1999; Lima *et al*., 2006; Yao *et al*., 2007), and human hepatocytes against oxidative injury induced by hydrogen peroxide (H$_2$O$_2$) or Carbon tetrachloride (CCl$_4$) in vitro (Zhao and Zhang, 2009). Judging by these beneficial effects of *M. Indica* on the liver, it is obvious that the balloon degenerative changes (vacuolar cytoplasm) observed in group D, could be due to overdose. The signs of such alterations seen in group D, probably reflects what was observed in the earlier group C with mild cell pyknosis and thus, signifying a dosage dependent effect.

According to Loy *et al*., (2002), the concentration at which aqueous extract of *M. indica* exhibits its antioxidant effect in terms of lipid peroxidation inhibition, protection from the oxidation damage, and scavenger capacity of reactive oxygen species, are extremely low. Nwinuka *et al*., (2008) in their study on the effect of *M. indica* on the haematopoietic system reported an improvement on the haematological parameters with a similar dose (1ml of crude AE) and duration. This however, might not be applicable to the hepatobiliary system due to the frontal metabolic role of the liver in the concept of bioavailability. Most importantly, it has been asserted that plant products are more hepatoprotective at low doses (Ojiako and Nwanjo, 2006; Ojekale *et al*., 2007). Even vitamin E, an antioxidant, has been reported to have perooxidative effects at higher doses (Mukai, 1993; Thomas *et al*., 1996; Eder, 2002), while green tea polyphenols can cause oxidative stress and liver toxicity in vivo at certain concentrations (Lambert *et al*., 2007).

Plate 1: Photomicrograph of control Liver sections (H&E ×400) showing normal cytoarchitectural features with intact hepatocytes (see arrows) and clear cytoplasmic boundaries.
Plate 2: Photomicrograph of group B Liver section (H&E ×400) showing comparably normal cytoarchitecture with intact hepatocytes (see arrows) and clear cytoplasmic boundaries (see arrows).

Plate 3: Photomicrograph of group C Liver section (H&E ×400) showing mild cell pyknosis (in squares) and eosinophilic cells (see arrows).
Over all, it is unfortunate that more often than not, emphasis on herbal products have been on efficacy and not on therapeutic safety. This fact prompted Nwaopara (2013) to opine that the advancement of herbal medicine is inextricably tied to the conscientious effort to constantly evaluate the therapeutic potentials of all medicinal plants around us and as such, cannot fathom why trained researchers abandon herbal medicine in the hands of diviners and herbalist. It is our recommendation therefore, that indiscriminate ingestion of *M. indica* plant products be avoided.

**ACKNOWLEDGEMENT**

We acknowledge all those who in one way or the other, contributed towards the success of this study. Special thanks to the staff of Anthonio Research Center, Ekpoma, Edo State, Nigeria, for their technical assistance towards making this publication a reality.

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AUTHOR’S CONTRIBUTIONS

Oaikhena, G.A. and Izunya, A.M., conceptualized the research. Data analysis was done by Oaikhena, G.A, while Oaikhena, Izunya, Olugbenga and Ujaddughe, joined in writing and reviewing this paper. All authors funded it. No conflict of interest declared.