

The effects of Honey and Aloe Vera extract on Ibuprofen Induced Liver Damage in rats.

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Abstract: This study was designed to determine the effect of Aloe Vera extract and honey against ibuprofen induced liver damage in albino rats. A total of twenty five (25) adult male albino rats weighing 100-150g were divided into five groups (I, II, III, IV and V) of five rats each. Rats in group I served as control and were administered normal saline in a volume equivalent to the highest dosed group, rats in group II were administered 100mgkg⁻¹ of ibuprofen, rats in group III were administered 100mgkg⁻¹ of ibuprofen and 10 gkg⁻¹ of Aloe Vera extract, rats in group IV were administered 100mgkg⁻¹ of ibuprofen and 10 gkg⁻¹ of honey while rats in group V were administered with 100mgkg⁻¹ of ibuprofen, 10 gkg⁻¹ of Aloe Vera extract and 10 gkg⁻¹ of honey orally. The result showed a slight increase in liver weight, a significant increase ($P < 0.05$) in the levels of Aspartate aminotransferases (ASAT), Alanine aminotransferases (ALAT) and Alkaline phosphatase (ALP) in the ibuprofen treated rats when compared to the control rats. Administration of Aloe vera extract and honey did not reverse the damage caused. Histological findings indicate severe dilation of blood vessel, necrosis of vascular connective tissues, wide spread vacuolar degeneration, edema and bile duct hyperplasia. The result indicates that Aloe vera extract and honey has little or no effect in preventing ibuprofen induced toxicity.

Key words: Honey, Aloe vera, Ibuprofen, Hepatotoxic, hyperplasia

I. Introduction

The liver being the largest internal organ in the body is always in close contact with many harmful substances thus generating interest in natural remedies that are particularly effective in either protecting the liver or curing it from many ailments caused by these toxins. Ibuprofen, belongs to a class of drugs called non-steroidal anti-inflammatory drugs (NSAIDs) which are mostly and are used for the management of pain, fever and inflammation (Chen *et al.*, 2005). They are believed to work through the inhibition of at least 2 variations of cyclo-oxygenase (COX-1 and COX-2) thus inhibiting prostaglandins synthesis (Mahalakshmi *et al.*, 2010).

Aloe vera is one of the most popular plants used in alternative medicine and is chemically composed of anthraquinone, polysaccharide and carbohydrate (Vinson *et al.*, 2005). It is used for the treatment of ulcer, hepatitis, neoplasms and wound. It has also been reported that the plant possess anti-inflammatory properties and antigenotoxic, chemopreventive, antioxidative and antiviral effects (Kim and Lee, 1997, Zhang and Tizard, 1996, Kim *et al.*, 1999, Lee *et al.*, 2000; Hu *et al.*, 2003). Aloe vera is used for liver problems and as a hepatic stimulant (Alqasoumi *et al.*, 2008; Rajasekaran and Sathishsekar, 2007).

Honey a product of honeybees is used as a medicine in many cultures times and is known to exhibit a broad spectrum of activities including antiviral, antibacterial, antioxidant activity and immune-stimulant properties (Molan, 2002; Mato *et al.*, 2003; Mabrouk *et al.*, 2004; Aljadi and Kamaruddin, 2004; Khadr, *et al.*, 2007). Ibuprofen is widely abused by students, labourers and artisans in Nigeria because of its analgesic affect this study was designed to determine the effects of Aloe vera extract and honey against ibuprofen induced liver toxicity in rats.

II. Materials and Methods

Collection, identification and preparation of Aloe vera plant extract

Aloe vera plant were obtained from a commercial botanical garden in Maiduguri and the fresh leaves were identified and authenticated by Dr. S.S. Sanusi (Plant Taxonomist) of the Department of Biological Sciences, University of Maiduguri, Borno state. A total of 500g of the fresh leaves were harvested and squeezed to obtain a jelly sap that was then preserved in a refrigerator at 20 °C.

Preparation of Honey

Fresh, pure and undiluted honey was obtained from a bee hive directly. It was then sieved with a clear mesh to remove unwanted particles and stored at room temperature until used.

Preparation of Ibuprofen solution

Capsules containing 400 mg of ibuprofen were obtained commercially from a pharmaceutical store located in the University of Maiduguri student centre. The drug was dissolved in 10 ml of normal saline to form the stock solution

Animals: This study was carried out in the Department of Human Anatomy, University of Maiduguri, Nigeria. A total of twenty five (25) adult male Albino rats weighing 100- 150g were used for the study. The rats were obtained from the Department of Pharmacology, University of Jos, Nigeria and were maintained in the Animal holdings of the Department of Human Anatomy, University of Maiduguri, Nigeria. After an acclimatization period of two weeks the rats were individually indentified by color tattoo and weighed. The rats were then kept in plastic cages at room temperature and provided with rat pellets (Sanders Seepc, Nig. Ltd) and water *ad libitum*. The rats were cared for according to the Guiding Principles for the Care and Use of Animals based on the Helsinki Declaration as amended by World Medical Assembly, Venice, Italy (Declaration of Helsinki, 1996).

Experimental Protocol: The twenty five adult male albino rats were divided using simple random sampling into five groups (I, II, III, IV and V) of five rats each. Rats in group I served as control and were administered normal saline equivalent to the highest dosed group, rats in group II were administered 100 mgkg⁻¹ of ibuprofen, rats in group III were administered 100 mgkg⁻¹ of ibuprofen and 10 gkg⁻¹ of Aloe Vera extract , rats in group IV were administered 100 gkg⁻¹ of ibuprofen and 10 gkg⁻¹ of honey while rats in group V were administered with 100 mgkg⁻¹ of ibuprofen , 10 gkg⁻¹ of Aloe vera extract and 10 gkg⁻¹ of honey. The administration was by the oral method and it was for a period of 14 days.

At the end of the experimental period the rats were then sacrificed and the blood obtained was subjected to biochemical investigations. The liver was then harvested, trimmed of any adherent tissue, the wet weight taken and preserved in Bouin's fluid for subsequent histopathological examination.

Biochemical analysis: Blood collected from the animals by transaction of the jugular vein were put into sterile bottles and centrifuged at a rate of 12,000 revolutions per minute (rpm) for 10 minutes. The clear serum obtained was separated and labeled for the analyses of the serum levels of Aspartate aminotransferases (ASAT), Alanine aminotransferases (ALAT) and Alkaline phosphatase (ALP). The analyses were carried out at the Department of Chemical Pathology, University of Maiduguri, Maiduguri.

Tissue Processing: The liver tissues obtained were fixed in Bouin's fluid for 8 hrs, dehydrated, embedded in paraffin wax. From each specimen, 5µm serial sections were cut, with two sections separated by 20µm on each glass slide. All sections were stained with Haematoxylin and Eosin.

Statistical Analysis: The data obtained were recorded and analyzed for Duncan multiple comparison tests using statistical package (GraphPad Instat version 4.0) .The results were expressed as mean ± standard error of mean (SEM) and p value of less than 0.05 was considered significant.

III. Results

Effects of the administration of Ibuprofen, Aloe vera extract and Honey on mean liver weight.

The result showed a slight increase in the liver weight of the rats that were administered with 100 mgkg⁻¹ of ibuprofen when compared to the control group that was administered with normal saline only. The same results were also observed in the rats that were administered with 10 gkg⁻¹ of Aloe Vera extract / Honey and the combination of both when compared with the ibuprofen treated group (Table 1).

Table 1: The effect of the administration of Ibuprofen, Aloe Vera extract and honey on mean liver weight and biochemical parameters.

Groups	Doses administered			Parameters measured			
	Ibuprofen (mgkg ⁻¹)	Aloe Vera (gkg ⁻¹)	Honey (gkg ⁻¹)	Liver weight (mg)	ASAT (IUL ⁻¹)	ALAT (IUL ⁻¹)	ALP (IUL ⁻¹)
I	0	0	0	4.35±0.22	55.00±1.04	20.50±3.08	173.50±2.04
II	100	0	0	4.40±0.18	70.00±1.41***	29.50±2.12*	184.50±3.54**
III	100	10	0	4.49±0.67	81.00±2.82 ^a	30.00±1.41	190.50±7.78
IV	100	0	10	4.67±1.04	70.00±1.41	23.00±2.82	173.00±8.49 ^b
V	100	10	10	4.45±0.01	63.00±5.65	29.50±3.54	132.00±5.66 ^{a,a}

Results are presented as mean ± SEM; *significance relative to control and at P<0.05: ^asignificance relative to Ibuprofen control dosed group P<0.05. **ASAT** = Aspartate Aminotransaminases, **ALAT** = Alanine Aminotransaminases and **ALP** = Alkaline Phosphatase. N=5

Effects of the administration of Ibuprofen, Aloe vera extract and Honey on Biochemical parameters.

The effects of the administration of Ibuprofen, Aloe vera extract and honey on biochemical parameters are presented in Table 1. Biochemical analysis of blood showed a significant increase (P< 0.05) in the levels of ASAT, ALAT and ALP in the ibuprofen treated rats when compared to the control rats. Administration of 10 gkg⁻¹ of Aloe vera extract against ibuprofen induced liver damaged mostly caused significant (P< 0.05) increases in the serum levels of ASAT, ALAT and ALP when compared to the ibuprofen treated rats. Administration of 10 gkg⁻¹ of honey showed a slight and significant decrease in the serum levels of ASAT, ALAT and ALP when compared to the ibuprofen treated rats while the administration of a combine dose of 10 gkg⁻¹ of Aloe vera extract and honey also showed slight decreases in the serum levels of ASAT, ALAT and ALP when compared to the ibuprofen treated rats.

Histological findings

Liver sections from the control group showed normal liver parenchyma with the central vein clearly seen along with bile ducts and hepatic arteries. The hepatocytes were neatly arranged in anastomosing plates with the sinusoids radiating from the central vein (Fig. 1). Severe dilatation of blood vessels, necrosis of vascular connective tissues and wide spread areas of vacuolar degeneration were the histopathological changes observed in the liver of rats administered 100 mgkg⁻¹ Ibuprofen (Fig.2). Severe congestion and dilatation of blood vessels were the changes observed in rats administered with 10 gkg⁻¹ Aloe vera and 100 mgkg⁻¹ Ibuprofen (Fig.3) while rats treated with 10 gkg⁻¹ honey and 100 mgkg⁻¹ Ibuprofen presented with marked edema and bile duct hyperplasia (Fig.4), multi-focal blood channels and hepatic necrosis were the histopathological changes associated with the administration of 10 gkg⁻¹ honey, 10 gkg⁻¹ Aloe vera and 100 mgkg⁻¹ Ibuprofen (Fig.5).

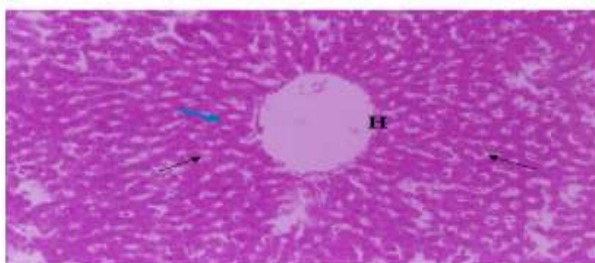


Fig. 1 Photomicrograph of control rat liver showing normal hepatocytes (arrow blue), sinusoids (arrow) and central vein (H) H & E x 200.

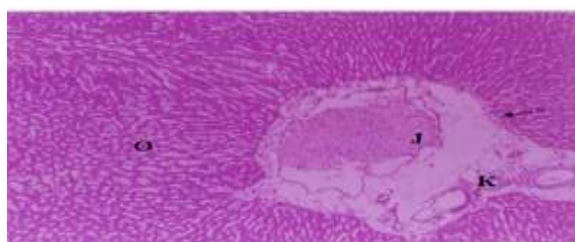


Fig. 2: Photomicrograph of rat liver treated with 100mgkg⁻¹ Ibuprofen showing severe dilation of blood vessel (arrow) necrosis of vascular connective tissues (K) and wide spread areas of vacuolar degeneration (O), H & E x200.

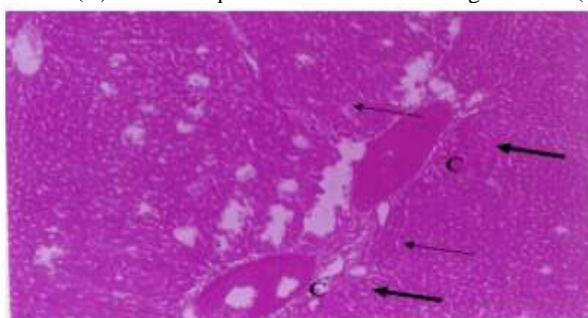


Fig. 3: Photomicrograph of rat liver treated with 10gkg⁻¹ Aloe Vera+ 100mgkg⁻¹ Ibuprofen showing severe congestion (C), fibrous connective tissues (arrow) and dilation of blood vessels (thick arrow) H & E x200.

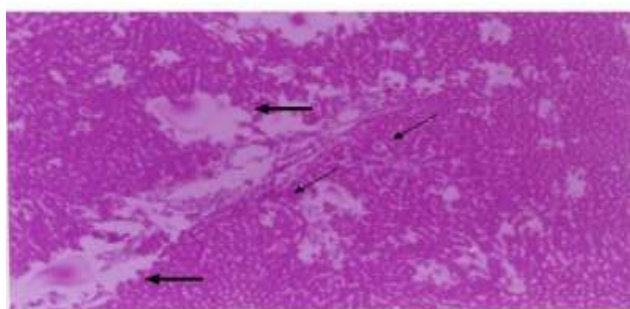


Fig. 4: Photomicrograph of rat liver treated with 10 gkg⁻¹ honey + 100mgkg⁻¹ Ibuprofen showing edema (thick arrow), bile duct hyperplasia (thin arrow) H & E x200.

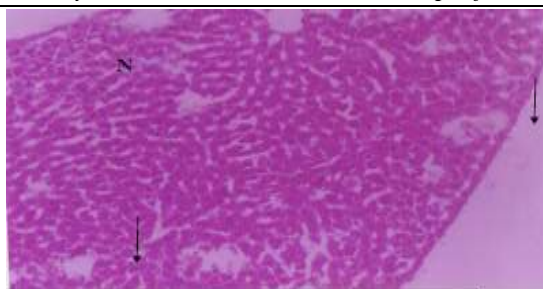


Fig.5: Photomicrograph of rat liver treated with 10gkg^{-1} honey + 10gkg^{-1} aloe Vera+ 100mgkg^{-1} Ibuprofen showing multi-focal blood channels (arrows) and hepatic necrosis (N) H & E x200.

IV. Discussion

In the assessment of liver damage certain biomarkers of hepatotoxicity are measured and one of such biomarkers are enzyme levels such as ASAT and ALAT because liver damage arising from necrosis or membrane damage normally releases the enzymes into circulation; therefore, measurement of these enzymes in serum gives an indication of the health status of the liver. High levels of ASAT indicate liver damage, as that due to viral hepatitis as well as cardiac infarction and muscle injury. ALAT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. It is known that an increase in the enzymatic activity of ALAT and ASAT in the serum directly reflects a major permeability or cell rupture, and thus ALAT is more specific to the liver, and is thus a better parameter for detecting liver injury (Benjamin, 1978 ; Wittwer and Bouhmwald, 1986). An increase in ASAT and ALAT, a hepatospecific enzyme that is principally found in the cytoplasm of rats following administration of a hepatotoxin is attributed to the increased release of enzymes from the damaged liver parenchymal cells (Benjamin, 1978; Ringler and Dabich, 1979; Singh, 1980 ; Garba, *et al.*,2009). The elevated level of alkaline phosphatase an enzyme produced in the liver, bone and placenta indicates liver injury or bile duct obstruction as a result of ibuprofen administration. Liver injuries are mostly caused by interference with the metabolic pathways essential for parenchymal cell integrity. They lead to diversion, competitive inhibition or structural distortion of molecules essential for metabolism or to selectively blockade of key metabolic pathways required to maintain the intact hepatocyte. The biochemical and physiological lesions induced by these agents lead to degenerative changes such as steatosis, necrosis or both (Garba *et al.*,2006).

Oedema in this result denotes excess plasma fluid in the interstitium which normally causes an expansion in the intracellular matrix and a major local manifestation of acute inflammation in dividing resident of lymphocytes or macrophages (Kumar, *et al.*, 2004). Serum ASAT, ALAT is the most sensitive biomarkers used in the diagnosis of liver diseases (Pari and Kumar, 2002). During hepatocellular damage, varieties of enzymes normally located in the cytosol are released into the blood flow. Their quantification in plasma is useful biomarkers of the extent and type of hepatocellular damage (Pari and Murugan, 2004). Serum ALAT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner.

Therefore, serum ALAT is more specific to the liver, and is thus a better parameter for detecting liver injury (Williamson *et al.*, 1996). In conjunction with the reports of (Cho *et al.*, 2009; Hegde and Joshi, 2009; Kim *et al.*, 2010), data from the present study showed that CCl_4 caused hepatic damage with a significant increase in serum levels of ASAT and ALAT. Serum ALP level is also related to the status and function of hepatic cells. CCl_4 administration in the present study also caused significant increase in the serum ALP which may be due to increased synthesis in presence of increasing biliary pressure (Moss and Butterworth, 1974).

The distortion of the radial arrangement of the sinusoids from the central vein, the distortion of the hexagonal shape of the hepatocytes with evidence of hepatic necrosis may be due to the cleaving of sialic acid by the enzyme sialidase from the haemoglobin-free erythrocytes, plasma and the liver, thus exposing the liver to the damage noticed (Wilson *et al.*, 2011). The reduction in some of the damage caused by ibuprofen administration may be attributed to the beneficial effect of honey in the prevention of hepatic damage induced by obstruction of the common bile duct (Erguder *et al.*, 2008) who suggested that honey supplementation might give beneficial results.

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