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Effects of *Mangifera Indica* Leaf Extracts on the Biochemical Indices of the Liver Function and Some Haematological Parameters in Rabbits

I.A.A. Ibrahim [a, *], M. Chindo [a]

[a] School of Science and Technology, Federal Polytechnic, Nigeria

Abstract

Research was conducted to study the effect of Magnifera indica dried leaves on two important biochemical indices of the liver, (AST and ALT). Portion of the fractions each was subjected to phytochemical screening for the presence of secondary metabolites including, flavonoids, saponins, tannins and steroids/triterpenes and alkaloids using standard procedures (Silver *et al.*, 1998). A total of 8 rabbits containing four young and four adult rabbits of either sex (2kg) were used for the study. The test animals were divided into four groups of two rabbits each (containing a mixture of one young and one adult rabbit each). T_1 serves as the control, T_2, T_3 and T_4 serving as the test groups. The extract was administered orally to the test groups (T_2, T_3 and T_4) orally and distilled water (placebo) was administered orally to the control group (T_1). The dosage of administration sustained was 200ml/kg daily in divided doses for a month. The ALT & AST test procedure was conducted using RANDOX reagent according to the manufacturer's instruction. Results of preliminary phytochemical of the ethanol (EE) leaf extract of *M. indica* revealed the presence of all the constituent tested including alkaloids, flavonoids, anthraquinones, saponins, steroids, triterpenes, tannins except carbohydrates and glycosides. The aqueous extract (AE) revealed the presence of all the constituents except steroids, triterpenes, anthraquinones, tannins, carbohydrates and glycosides. The results obtained for alanine amino transferases (ALT) and aspartate amino transferases (AST) were found to be within the normal range of 10-45V/L for ALT and 10-120V/L for AST. The packed cell volume (PCV) and hemoglobin concentration were the only heamatological parameters tested. According to the result obtained in the analysis, there was a slight variation between the PCV and Hb concentration in the test animal and the control . Hence, the extract has no adverse effect on the circulating red blood cell as well as the Hb concentration but rather brings about the slight increase in the production of red blood cell as well as the Hb concentration. This may be attributed to the presence of active constituent that promote red cell production in the plant extracts.

Keywords: packed cell volume, heamatological, alamine amino transferase, aspartate amino transferase, phyrochemical.

1. Introduction

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances or constituents that produce a definite physiological action on the human body (Bhardwaj, Yadav, 2016; Kumar, Rana, 2017).

* Corresponding author
E-mail addresses: eliyah2001@yahoo.com (M. Chindo), iliyasuibrahim@gmail.com (I.A.A. Ibrahim)

Mangiferaindica commonly known as mango belongs to Anacardiaceae family. It is widely distributed in the tropical and subtropical region countries like India (Reddy, Sreedevi, 2016; Ogata et al., 2016). Mangifera indica grows up to 35-40m (115-130ft) tall. It is used for many medicinal purposes as well as dietary functions. The leaves are used as herbal remedy and when soak overnight and drink is reported to have antihyperglycemic activity (Deb et al., 2016; Akhter et al., 2016; Munni et al., 2016). The leaves are astringent, refrigerant styptic, vulnerary and constipating. They are useful in vitiated conditions of cough and pitta, hiccup, hyperdipsia, burning sensation, hemorrhages, haemoptysis, haemorrhoids, wounds, ulcers, diarrhoea, dysentery, pharyngopathy, scorpion string and stomachopathy. The ashes of burnt leaves are useful in burns and scalds. A powder of tender leaves is given in diarrhoea and diabetes. The smoke from burning leaves is inhaled for relief in hiccup and throat diseases (Rajasekaran, Mahimaidoss, 2016; Parvez, 2016). The roots and bark are astringent, acrid, refrigerant, styptic, antisyphilitic, vulnerary, anti-emetic, anti-inflammatory and constipating. They are useful in vitiated conditions of pitta, metrorrhagia, calonorrhagia, pneumorrhagia, lecorrhoca, syphilis, uteritis, wounds, ulcers and vomiting. The juice of fresh bark has a marked action on mucous membranes, in menorrhoea, leucorrhoca, bleeding piles and diarrhoea. An ointment of resinous gum of tree bark is dressing for scabies and other parasitic skin diseases (Macfoy, 2013; Eluwa et al., 2014).

The unripe fruits are acidic, acrid, antiscorbutic, refrigerant, digestive and carminative. They are useful in dysentery ophthalmia, eruptions, urethrorrhoea and vaginopathy. Sun dried slices of unripe fruits are excellent remedy for scurry while fried skin of unripe fruit is given in menorrhages. The ripe fruits are refrigerant, sweet, emollient, laxative, cardiotonic, haemostatic, aphrodisiac, and tonic. They are used in vitiated conditions vata and pitta, anorexia, dyspepsia, cardiopathy, haemoptysis, haemorrhages from uterus, lungs and intestine, emaciation and anemia. Rind of fruit is astringent, stimulative, tonic and useful in general debility of stomach (Macfoy, 2013; Eluwa et al., 2014). The flowers are astringent, refrigerant, styptic, vulnerary, constipating and haematinic. The dried flowers are useful in vitiated conditions of pitta, haemorrhages, haemoptysis, wounds, ulcers, anorexia, dyspepsia, uro-edema gleet, catarrh of bladder, diarrhoea, chronic dysentery, and anaemia, The seed kernel in rich source of protein (8.5 %) and gallic acid, it is sweet, acrid, astringent, refrigerant, anthelmintic, constipating, haemostatic, vulnerary and uterine tonic. The medicinal purposes of *Mangiferaindica* have been widely studied, *Mangiferaindica* leaf extract inhibited lipid peroxidation (Núñez Selles et al., 2016; Lauricella et al., 2017 ; Lauricella et al., 2017), exerted antifungal activity (Ahmed et al., 2016), exhibited antiulcerogenic action (Lauricella et al., 2017 ; Lauricella et al., 2017). It was also reported as an anti-gouty arthritis agent, antidiabetic agent and management of gastro-intestinal disorders (Pal et al., 2016).

Blood is an important index of physiological and pathological changes in an organism (Raina, 2016; Pal et al., 2016). The primary function of the blood is to transport oxygen from respiratory organs to body cells (López-Barneo et al., 2016; Lumb, 2016), distributing nutrients and enzymes to cells and carrying away waste products , thereby maintaining homeostasis of the internal environment (Olivier, 2017). The various functions of the blood are carried out by the individual and collective actions of its constituents – the haemotological and biochemical components (Pitsikas, 2016; Singh et al., 2017). Haematological tests have been widely used for the diagnosis of various diseases and nutritional status of animal. The information gained from the blood parameters would substantiate the physical examination and together with medical history provide excellent basis for medical judgment (Kurtz et al., 2016 ; Silverman et al., 2016 ; Goodman et al., 2017).

Therefore, the aim of the present study was to determine the effect of *Mangiferaindica* leaf extract on the biochemical indices of liver function and some haematological parameters in rabbits.

2. Materials and methods

Experimental Site

The research work was carried out at Chemistry/Biology Laboratories of the School of Science and Technology, AbubakarTatari Ali Polytechnic and Pathology Laboratory section of Darusssalam Health Clinic Center, DutsenTanshi Bauchi, Bauchi State, Nigeria.

Collection and Identification of Plant Material

Fresh leaves of *M. indica* were collected from the schools garden. The identification and authentication of the plant was done by a Botanist, Ibrahim Shuaibu, College Agriculture, Bauchi. The leaves were sorted, shade dried, pulverized to powder and stored in a clean container for onward analysis.

Extraction

Extraction with ethanol

The Powdered leaves (200 g) were extracted with 400ml ethanol using maceration method for 2days with occasional shaking. The extract was filtered using Whatmann No. 1 filter paper and the filtrate was freed from solvent with the aid of a water bath (30-40^{o}C) to obtain a gummy greenish product (28 g) subsequently referred to as the crude ethanol extract (EE).

Extraction with distilled water

The Powdered leaves (100 g) were extracted with 200ml distilled water using maceration method for 2days with occasional shaking. The extract was filtered using Whatmann No. 1 filter paper and the filtrate was freed from solvent with the aid of a water bath (30-40 ^{o}C) to obtain a gummy greenish product (28 g) subsequently referred to as the aqueous extract (AE).

Preliminary Phytochemical Investigation

Portion of the fractions each was subjected to phytochemical screening for the presence of secondary metabolites including, flavonoids, saponins, tannins and steroids/triterpenes and alkaloids using standard procedures (Silver et al., 1998).

Test for Alkaloids

0.5g of the extract was stirred with 5ml of 1 % aqueous hydrochloric acid on a water bath and filtered. 3ml of the filtrate was divided into two. To the first 1ml few drops of freshly prepared Dragendoff's reagent was added. To the second, 1 drop of Meyer's reagent was added and observed.

Test for Flavonoids and Phenols

Ferric chloride test: To a small portion of the extract, distilled water was added. A drop of ferric chloride was added to a solution of the extract and observed.

Test for Anthraquinones 0.5 g of the extract was shaken with 5ml carbon tetrachloride, this was filtered and 10 % dilute ammonia solution was added. The mixture was shaken and observed.

Test for Saponins 0.5g of the extract was shaken with distilled water in a test tube. It was allowed to stand for 10 minutes and observed.

Test for Steroids and Triterpenes *Liebermann-Buchard test*: A small portion of the extract was dissolved in chloroform. Equal volume of acetic anhydride and concentrated H_2SO_4 were added down the test tube and observed.

Test for Tannins *Lead Sub-acetate Test*: To a small portion of the extract, distilled water was added. 3-5 drops of lead acetate solution was added and observed.

Test for Carbohydrates *Fehling's Test*: To a small portion of the extract, distilled water was added. 2ml Fehling's

Test for Glycosides *Legal's test*:To a small portion of the extracts, sodium nitropruside in pyridine and sodium hydroxide was added and observed.

Preparation of Animal Sample

A total of 8 rabbits containing four young and four adult rabbits of either sex (2kg) obtained in Bauchi metropolis were used for the study. They were kept in the School Laboratory Garden for 40 days so as to acclimatize with the environment. The test animals were divided into four groups of two rabbits each (containing a mixture of one young and one adult rabbit each). T_1 serves as the control, T_2, T_3 and T_4 serving as the test groups.

Mode of Administration and Dosage

The extract was administered orally to the test groups (T_2, T_3 and T_4) orally and distilled water (placebo) was administered orally to the control group (T_1). The dosage of administration sustained was 200ml/kg daily in divided doses for a month .

Blood Collection Procedure The blood was collected from the central auricular artery (ear) with a 20g needle. Vasolidation was achieved with the aid of heating lamps, 70 % alcohol swabs and warm compression.

Liver Function Test (ALT & AST) using Randox Reagent

The ALT & AST test procedure was conducted according to the manufacturer's instruction in the following three steps;

Step 1: Two test tubes were set as TEST and BLANK. 200 U/L of reagent 1 was added to both test tubes. To the TEST tube, 40 U/L of serum was added to it and 40 U/L of distilled water to the BLANK tube and incubated at 37^{o}C in water bath for 35 minutes.

Step 2: 200 U/L of reagent 2 was added to both the TEST and BLANK tubes, incubated for 20 minutes at room temperature

Step 3: To the TEST and BLANK tubes, 2000 U/L of sodium hydroxide (NaOH) solution was added and incubated for 5 minutes at room temperature. The result was displayed at 530 mm with the aid of photoelectric colorimeter. ALT and AST were calculated below;

ALT (U/L) = Optical density of sample mixture × concentration = OD of sample mixture x 160

AST (U/L) = Optical density of sample mixture × concentration = OD of sample mixture x 350

PCV

Capillary tube (75mm) was filled to approximately with EDTA and anticoagulated blood (3 quarter of its length). The excess blood was wiped from the outside of the tube and scatted with a sealer. The tubes were placed in a microhaematocrit centrifuge with the sealed end pointing outwards. The inner lid was firmly secured and the outer lid was also closed and centrifuge for five minutes at 11,000 revolutions per minute. When the centrifuge stopped, the tubes were removed and read as the fraction of red cells column to the total length of the sample.

3. Results and discussion

The result of phytochemical screening, effects of *Mangiferaindica* leaf extracts on rabbits serum enzymes and haematological indices are presented in Tables 1-3 respectively;

Table 1. Phytochemical Constituents of Ethanol and Aqueous Extracts of *M. indica*

Constituents	**Test**	**Observation**	**Inference**	
			EE	**AE**
Saponins	Frothing	Frothing persist for 15mins	+	+
Alkaloids	Mayer's Draggondorf's	White-cream ppt Orange ppt	+ +	- -
Flavonoids	$FeCl_2$	Green or violet ppt	+	+
Tannins	Lead subacetate	Cream ppt	+	+
Steroids & Terpenes	Lieberman-Buchard	Blue-green color at interphase	+	-
Anthraquinones	Borntragers	Pink or violet	-	-
Carbohydrates	Molisch's Fehling's	Reddish ring Red	-	-
Phenols	$FeCl_2$	Bluish black color	+	+
Glycosides	Fehling's	Red ppt	-	-

Table 2. Effect of *M. indica*leaf extracts on rabbit serum enzyme

Group	**ALT (u/L)**	**AST (u/L)**
T_1	12.80	21.00
T_2	12.80	31.50
T_3	16.00	17.50
C	17.60	17.50
Normal range	10-45	10-120

Key: T=Test; C=Control

Table 3. Effect of *M. indica*leaf extracts on rabbit haematological indices

Group	PCV (%)	HB (g/dl)
T_1	44	14.60
T_2	41	13.70
T_3	46	15.30
C	36	15.30
Normal range	33-50	94-174

The result of preliminary phytochemical of the ethanol (EE) leaf extract of *M. indica* revealed the presence of all the constituent tested including alkaloids, flavonoids, anthraquinones, saponins, steroids, triterpenes, tannins except carbohydrates and glycosides. The aqueous extract (AE) revealed the presence of all the constituents except steroids, triterpenes, anthraquinones, tannins, carbohydrates and glycosides. These constituents have been reported to be responsible for most biological activities of plants (Cowan, 1999).

The results obtained for alanine amino transferases(ALT) and aspartate amino transferases (AST) were found to be within the normal range of 10-45V/L for ALT and 10-120V/L for AST. However, there was no significant alteration with the level of the serum enzymes in the control rabbit which is an indication that the extract of *M. indica*did not alter the stoichiometry of the liver marker enymes and the liver.The packed cell volume (PCV) and hemoglobin concentration were the only heamatologicalparameters tested. According to the result obtained in the analysis, there was a slight variation between the PCV and Hb concentration in the test animal and the control . Hence, the extract has no adverse effect on the circulating red blood cell as well as the Hb concentration but rather brings about the slight increase in the production of red blood cell as well as the Hb concentration. This may be attributed to the presence of active constituent that promote red cell production in the plant extracts.

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