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Der Pharmacia Sinica, 2011, 2 (2): 39-45

Der Pharmacia Sinica

ISSN: 0976-8688
CODEN (USA): PSHIBD

An evaluation of the effect of ethanolic fruit extracts of *Xylopia aethiopica* on haematological and biochemical parameters in male rats

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ABSTRACT

Xylopia aethiopica (African guinea pepper) is used mainly as spices, as postpartum tonic and to induce placental discharge postpartum by traditional birth attendants (TBA).In spite of its frequent and regular use, no attempt has been made to scientifically examine the effects of the spice on haematological and biochemical parameters. The present study was thus undertaken to fill this gap in knowledge in our effort to establish the effect of local spice and medications on these parameters. The extract of Xylopia aethiopica was administered (30, 100 and 300 mg/kg p.o) to different groups of male rats for sixty days. Blood samples were collected 24 hours after the last treatment by cardiac puncture into EDTA tubes for haematology parameters and into plain tubes for the enzyme and other biochemical assays. Oral administration of the extract produces significant ($p<0.001$) augmentation of Hb, total white blood cells and neutrophil in a dose dependent fashion. It however did not affect RBC, and HCT. The extract also cause a significant increase in serum total protein, Albumin, Globulin, HDL and total Cholesterol levels as well as indirect and total bilirubin dose dependently while decreasing serum ALT. It did not however have a significant effect on Renal function test (urea and creatinine). The present findings may be responsible for the usefulness of X. aethiopica fruit in the our local setting as an immune booster and postpartum tonic

Key words: *xylopia, haemoglobin, biochemical, evaluation*

INTRODUCTION

Fruits of *Xylopia aethiopica* (Dunal) A. Rich, an evergreen aromatic tree belonging to the family Annonacea growing mainly in the tropical forest of Ghana, Nigeria and Cameroon, have been used traditionally as spices, condiment, as postpartum tonic and as a medicinal herb for the

treatment and management of several diseases. The plant is therefore considered to be a useful herbin preventing and/ or ameliorating certain diseases such as asthma, bronchitis, neuralgia, rheumatism, amenorrhea in females[1] and has also been reported to have antimicrobial effect [2, 3]. Its anti-hypertensive and diuretic effects have also been reported [4]. The used of *Xylopia aethiopica* as a herb is very common in Ghana, especially in the rural areas where access to conventional medicine is still very low due to non-availability and/or affordability. Despites it's used there has been no comprehensive study on the effect of *Xylopia aethiopica* on haematological and biochemical parameters. It is therefore of interest to examine whether *Xylopia aethiopica* influences haematological and biochemical indices when taken. The present study was thus aim at finding the effect of *Xylopia aethiopica* on the biochemical and haematological profiles of rat treated with ethanolic fruit extract of *Xylopia aethiopica.*

MATERIALS AND METHODS

Plant material

Dried fruits of *Xylopia aethiopica* [Dun.] A. Rich, were bought from the Central Market, Kumasi/Ghana and identified by Dr. Abraham Mensah, Department of Pharmacognosy, KNUST, Kumasi, Ghana and a voucher sample was deposited at the department with a voucher number FP/08/76.

Preparation of extract

The powdered fruit (3.5 kg) was extracted with 70% v/v ethanol in a Soxhlet apparatus for 48 h. Using a vacuum rotary evaporator, the ethanol filtrate was concentrated at a low temperature, under reduced pressure. This yielded 325 g (9.29%) chocolate brown extract which was then stored in a refrigerator from which fresh solution was prepared using distilled water when required.

Animals

All experiment were performed with male albino rats weighing 150-200 g bought from Noguchi Memorial Institute for Medical Research, University of Ghana, Accra and kept at the Animal House Facility of the Department of Pharmacology, KNUST. The animals were allowed to acclimatize to the laboratory condition (temperature 24-28 ^{o}C and 12 hour light-dark cycle) for two week before commencement of the experiment with free access to solid pellet diet and water ad libitum throughout the study. All the animals were treated according to the National Institute of health Guidelines for the care and use of laboratory animals (NIH, department of Health and Human services publication no. 85-23, revised 1985).

Experimental design

Twenty five male rats were divided into five equal groups. Group I served as the control and the rats were given distilled water (vehicle for the extract). Groups II, III and IV rats were given 30, 100 and 300 mg kg^{-1} respectively, of *Xylopia aethiopica* fruit extract (X.A). The vehicle and extract were given orally for 60 days. Blood samples were collected 24 hours after the last treatment by cardiac puncture into EDTAtubes for haematology parameters and into plain tubes for the enzyme and other biochemical assays.

Haematological parameters

Various haematological parameters including white blood cell count (WBC), lymphocyte count (LYM), mid cell count (MID), granulocyte count (GRAN), red blood cell count (RBC), haemoglobin concentration (HB), packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW), and platelet concentration (PLT) were determined using 18 parameter automated haematology analyzer, Sysmex KX 21N (Sysmex Cooperation, Kobe, Japan)

Biochemical assays

Serum biochemistry was performed on each sample using Selector Junior Automated analyzer (Vital Scientific Ltd, Dieren,The Netherlands). Parameters that were determined includes: liver function tests - aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyltranspeptidase (GGT), lactate dehydrogenase (LDH), total bilirubin (T-BIL), direct bilirubin (D-BIL), indirect bilirubin (I-BIL), total-protein (T-PROT), albumin, globulin. Serum urea, serum creatinine, serum uric acid, was also analyzed. Also lipid profile which include total cholesterol (T-CHO), triglycerides (TG), high density lipoprotein (HDL), very low density lipoprotein (VLDL), low density lipoprotein (LDL) and coronary risk were determined.

Statistical analysis

Results were expressed as mean ± SEM. Significance difference between means was determined by one-way analysis of variance (ANOVA) with Newman-Keuls's aspost-hoc test. Graphpad prism for windows version 5.0 (Graphpad software, San Diago, CA. USA) was used for all statistical analysis. P value <0.05 was considered as significant.

RESULTS

Haematological parameters

From the results shown in table 1, there was a significant increase in total white blood cell count ($F_{3,12}$= 4.755, *P* = 0.0208)and differential Neutrophil count ($F_{3,11,}$= 6.880, *P* = 0.0071)in the extract treated groupcompared tothe control group.There was also a significant increasedin the haemoglobin concentration in the treated rats compare to the controls ($F_{3, 13}$ = 4.559, *P* = 0.0216). There were however no significant change in red blood cell count, Mean cell volume and Haematocrit between the treated animals and the control. Similar observations were seen in red cell distribution width, MCH and MCHC when the extract treated rats were compared to the respective controls (Table 1).

Serum biochemical indices

Themean serum total protein ($F_{3, 16}$= 9.800, *P* = 0.0007), albumin ($F_{3, 16}$ = 7.060, *P* = 0.0031) and globulin ($F_{3, 16}$ = 10.87, *P* = 0.0004) were significantly increasedin the *Xylopia aethiopica* extract treated rats compared with the control group (Table 2). There was also a dose dependent significant increase in the mean concentration of indirect bilirubin ($F_{3, 15}$ = 13.03, *P* = 0.0002)and total bilirubin ($F_{3, 15}$= 11.76, *P* = 0.0003) in the treatment groups compare to the control (Table 2). However, there was no significant change in the mean concentrations of serum direct bilirubin, creatinine, urea and uric acid in rats treated with X.A. extract and the control group (Table 2).

Table 1 Haematological parameters of animals treated for 60 days with X.A extract

PARAMETER	CONTROL	30 mg	100 mg	300 mg
WBC ($x10^9$L)	4.45 ± 0.54	5.35 ± 0.44	7 ± 0.46*	6.34 ± 0.63
RBC ($x10^{12}$/L)	7.30 ± 0.211	7.57 ± 0.523	7.41 ± 0.18	7.79 ± 0.11
Hb (g/dL)	13.20± 0.39	13.07± 0.65	14.40± 0.27	14.68 ± 0.31*
HCT (%)	45.54 ± 1.45	46.7 ± 3.47	45.04 ± 1.56	47.96 ± 0.98
MCV (fl)	62.4 ± 0.65	61.62 ± 0.50	60.72 ± 0.85	61.58 ± 0.59
MCH (pg)	18.78 ± 0.50	19.2 ± 0.16	18.9 ± 0.23	18.86 ± 0.38
MCHC (g/dL)	30.08 ± 0.91	31.22 ± 0.39	31.1 ± 0.30	30.6 ± 0.48
PLT ($x10^3$/µL)	985.3 ± 108	666.3 ± 80.99	664.7 ± 131.4	605 ± 63.04
LYM (%)	80.62 ± 3.13	80.8 ± 5.68	75.98 ± 2.48	78.48 ±2.84
NEUT (%)	14.77 ± 1.59	13.53 ± 0.39	18.68 ± 2.6	24 ± 1.80**
RDW-CV (%)	12.44 ±0.31	12.4 ± 0.15	12.48 ± 0.33	11.92 ± 0.16

*Data are express as mean ±SEM, *P ≤ 0.05, ***P ≤ 0.001 compared to respective controls (one-way ANOVA followed by Newman-Keulspost hoc)*

Table 2.Serum liver and renal function test following 60 days of exposure to X.A.extract

PARAMETER	**CONTROL**	**30 mg**	**100 mg**	**300 mg**
Albumin (g/L)	38.54 ± 0.96	39.24 ± 1.24	39.98 ± 0.83	44.94 ± 1.27**
Globulin (g/L)	30.02 ± 1.04	34.04 ± 1.28**	26.84 ± 0.99	33.94 ± 0.84
T. Protein (g/L)	68.54 ± 1.84	73.26 ± 1.27	66.84 ± 1.73	78.88 ± 1.98**
ALK.Phos (U/L)	700.4 ± 52.77	964.8 ± 206.1	562.4 ± 33.87	709.2 ± 78.88
ALT (U/L)	148.3 ± 8.83	128 ± 7.11	118.4 ± 5.63	114.9 ± 4.11*
AST (U/L)	3.45 ± 0.85	3.2 ± 2.0	3.3 ± 1.40	0.95 ± 0.56
GGT (U/L)	0.02 ± 0.02	0.44 ± 0.25	0.74 ± 0.45	11.68 ± 7.45
DBIL (µmol/L)	1.08 ± 0.04	1.04 ± 0.05	0.98 ± 0.06	1.05 ± 0.05
INDBIL (µmol/L)	0.344 ± 0.10	0.58 ± 0.04	0.91 ± 0.08*	1.27 ± 0.20**
TBIL (µmol/L)	1.44 ± 0.09	1.66 ± 0.07	1.88 ± 0.10	2.33 ± 0.17**
Creatinine (µmol/L)	53.54 ± 4.20	56.6 ± 2.99	55.96 ± 1.50	62.76 ± 4.38
UREA (mmol/L)	7.48 ± 0.36	6.58 ± 0.90	6.65 ± 0.61	7.46 ± 0.65
URIC acid (mmol/L)	64.98 ± 3.53	58.8 ± 9.51	51.5 ± 4.66	52.7 ± 5.36

*Results are express as mean ±SEM, *P ≤ 0.05, ** P ≤ 0.01, ***P ≤ 0.001 compared to respective controls (one-way ANOVA followed by Newman-Keulspost hoc)*

Serum enzymes

From table 2 treatment of rats with the extract of *Xylopia aethiopica* induce a significant decrease ($F_{3, 13}$ = 4.617, P = 0.0207) in the activity of serum ALT compared to the control group. However, the extract did not cause any significant changes in levels of serum AST, Alkaline phosphatase (ALK.Phos) and γGlutamyltranferase.

Serum lipid profile

Rats administered X.A extract showed a significant increase ($F_{3, 14}$ = 7.063, *p* = 0.0040) in serum total cholesterol level compare to the control group. There was also a dose dependent significant increased ($F_{3, 16}$ = 8.422, *P* = 0.0014) in serum HDL levels in the treated group compare to the control group.Howeverthere was no significant difference in the mean concentrations of serum LDL, triglycerides and the coronary risk ratio between the treated group and the control (Table 3).

Table 3: Lipid profile (mean ± SEM) following 60 days of exposure to X.A. extract

Parameter	Control	30 mg	100 mg	300 mg
Cholesterol(mmol/L)	2.06 ± 0.14	2.47 ± 0.05*	2.1 ± 0.08	2.39 ± 0.01
Triglyceride(mmol/L)	0.95 ± 0.09	0.77 ± 0.09	0.83 ± 0.03	0.86 ± 0.11
HDL (mmol/L)	2.59 ± 0.13	3.11 ± 0.04**	3.14 ± 0.10**	3.15 ± 0.06*
LDL (mmol/L)	0.84 ± 0.07	0.99 ± 0.05	0.90 ± 0.04	0.99 ± 0.06
Coronary risk (ratio)	1.16 ± 0.02	1.10 ± 0.02	1.12 ± 0.01	1.08 ± 0.03

*Results are express as mean ±SEM, *P ≤ 0.05, ** P≤ 0.01, ***P ≤ 0.001 compared to respective controls (one-way ANOVA followed by Newman-Keulspost hoc)*

DISCUSSION

Treatment of rats with ethanolic fruit extract of *Xylopia aethiopica* for 60 days significantly increased the body weight of the animals in a dose dependent manner. This increase in body is an indication that the extract did not interfere with growth but rather promoted growth possible by increasing the synthesis of protein. We have shown in our laboratory that extract of *Xylopia aethiopica* has androgenic properties (unpublished data) and androgens are known to possess anabolic properties [5] and this could thus be responsible for the significant increase in body weight of the animals in the present study.

The extract also caused a significant increased in the level of Hb, WBC and Neutrophil count in the treated animals.Increased in these parameters could bedue to a direct effect of the extract on haemopoitic activity in these animals. According to Eteng et al., [6]alkaloid, which is one of the phytochemicalcomponents of *Xylopia aethiopica*, is known to cause similar effect by inhibiting phosphodiesterase leading to the accumulation of cAMPwhich in turn stimulates protein synthesis.Locally in Ghana, decoction of dry fruits of *Xylopia aethiopica* is taken as a postpartum tonic as well as an immune booster in individuals with low immunity [1] Neutrophils are the main type of White blood cells that are responsible for fighting infectious agents by phagocyctosis. Antimicrobial activity of *Xylopia aethiopica* has since been reported [3, 7]. The findings of the present study is agreement with the work of Taiwo et al., [8], who found that aqueous extract of *Xylopia aethiopica* was able to significantly increased the levels of Hb, PCV,WBC and Neutrophil in rats. These findings thus validate the traditional use of the extract as a tonic and an immune booster.

The extract also induced a significant increase in total protein, albumin and globulinin the treated animals compared to the control group.Albumin binds and transports metal ions, bilirubin, and drugs. Its levels is use to assess the synthetic function of the liver. Significantincrease in the level of these parameters is an indication that the extract had stimulated its synthesis in the liver. Serum protein levels are regulated via synthesis in the liver and its levels thus reflect the synthetic ability of the liver[9]. The extract also causes a significant reduction in the level of serum ALT but did not affect the levels of AST, GGT and alkaline Phosphatase. It is known that an increase in the enzymatic activity of ALT, AST and ALP in the serum directly reflects hepatocellular damage [10].Results of the enzyme analysis therefore suggest that extract of *Xylopia aethiopica* has no hepatotoxicity with reference to the doses used in this study. This finding is similar to the resultof Taiwo et al., [8]. There was no significant change in the serum direct bilirubin levels. However there was a significant increased in the serum levels of total and indirect bilirubin in the treated rats compared to the control animals. An increased in indirect

serum bilirubin (unconjugated bilirubin) could be due to increased breakdown of red cell or decreased uptake by hepatocytes.Increase in the serum unconjugated bilirubin in this study may arise from haemolysis rather than liver injuries since the liver enzymes, total protein, albumin and total cholesterol levels were not affected. Serum urea and creatinine levels are an indication of kidney function both in man and in rodents [11]. The levels of urea and creatinine in the treated rats did not show any significant difference with respect to the control values, an indication that fruit extract of *Xylopia aethiopica* is not nephrotoxic at the dose levels used in this study.

Treatment of rats with extract of *Xylopia aethiopica*in the present study resulted in a significant increased in total cholesterol and HDL-cholesterol with a concomitant decreased in coronary risk ratio. Serum lipid levels have been shown to be strong risk factors for coronary heart diseases [12]. Other workers have also shown strong correlations between increased plasma total cholesterol, low density lipoprotein cholesterol and increased incidence of coronary heart disease [13, 14]. The significant increased in total cholesterol observed in this study is probably due to the significant increased in high density lipoprotein (HDL) since there was no corresponding significant increased in the low density lipoproteins (LDL). According to Eder and Gidez[6], an increased in HDL can solely be responsible for an observed increased in total cholesterol since the extract might be affecting the metabolism of HDL in the liver. Fruits of *Xylopia aethiopica* have been found to contain high level of monounsaturated fatty acids and may be responsible for the beneficial effect shown in this study as they have been found to inhibit the heart – damaging oxidation of low density lipoprotein (LDL) cholesterol [15]. This in an indication the extract do not posed any cardiovascular risk since it only increased HDL (good cholesterol) without affecting the “bad cholesterol” (LDL).

CONCLUSION

In conclusion, crude extract of *Xylopia aethiopica* showed haematopoitic activity by increasing formed element in blood (WBC, Hb and neutrophils) in rats. It also has the ability to boost immunity and growth by increasing the plasma protein and albumin levels whiles possessing antiatherogenic activity by increasing HDL (good cholesterol) and decreasing bad cholesterol (LDL), thus reducing the risk of coronary artery diseases. This may be associated with its usefulness in Ghanaian traditional medicine as an immune booster and postpartum tonic as well as treatment of inflammatory disorders and in healing of wounds.

Acknowledgement

The authors would like to thank Mr. Thomas Ansah, Chief Technician of the Pharmacology Laboratory, Kwame Nkrumah University of Science and Technology for his invaluable technical assistance.

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