Journal of Medicinal Plant Research

Volume 10 Number 37, 3 October, 2016

ISSN 1996-0875

Academic Journals

ABOUT JMPR

The Journal of Medicinal Plant Research is published weekly (one volume per year) by Academic Journals.

The Journal of Medicinal Plants Research (JMPR) is an open access journal that provides rapid publication (weekly) of articles in all areas of Medicinal Plants research, Ethnopharmacology, Fitoterapia, Phytomedicine etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JMPR are peer reviewed. Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

Contact Us

Editorial Office: jmpr@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/JMPR

Submit manuscript online http://ms.academicjournals.me/

Editorial Board

Journal of Medicinal Plants Research

Table of Contents: Volume 10 Number 37 3 October, 2016

ARTICLES

academicJournals

Vol. 10(37), pp. 640-648, 3 October, 2016
DOI: 10.5897/JMPR2016.6222
Article Number: BD4B32160804
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Caryocar brasiliense fruit intake ameliorates hepatic fat deposition and improves intestinal structure of rats

Lauane Gomes Moreno, Lidiane Guedes Oliveira, Dirceu Sousa Melo, Liliane Vanessa Costa Pereira, Karine Beatriz Costa, João Luiz Miranda, Etel Rocha Vieira, Flvio Castro Magalhes, Marco Fabricio Dias-Peixoto, Elizabethe Adriana Esteves*

Programa Multicêntrico de Pós-graduação em Ciências Fisiológicas Universidade Federal dos Vales do Jequitinhonha e Mucuri, Brazil.

Received 9 August, 2016; Accepted 19 September, 2016

***Caryocar brasiliense* (*pequi*) is an exotic fruit, high in monounsaturated fat acids (MUFA) and bioactive compounds, which have beneficial effects on cardiometabolic risk factors. However, this fruit is poorly studied in this context. In this study, the effects of *pequi* pulp intake on cardiometabolic risk factors of rats were evaluated. Therefore, 16 male weaned rats were divided into two groups: Control group and *Pequi* group. Control group was feed a standard diet and *pequi* group, the same diet added *pequi* pulp (3.26 g.100^{-1}) for 15 weeks. At the end, plasma lipids, glucose, insulin, Homeostasis Model Assessment of Insulin Resistance index (HOMA-IR), blood pressure, heart rate, hepatic and fecal lipids and intestinal histomorphometric parameters were accessed. Liver and heart samples were harvested for redox status assays. There were no differences between experimental groups for blood pressure, heart rate, glucose, insulin, HOMA-IR, triglycerides, cholesterol, HDL-cholesterol, and liver and heart redox status (p<0.05). *Pequi* group had lowered lipid hepatic deposition and increased fecal output (p<0.05), increased intestinal villus height and crypt depth. Thus, *pequi* pulp intake minimized liver fat deposition by increasing its intestinal output and improved intestinal structure of rats, which can contribute for reducing cardiometabolic risk factors. MUFA, carotenoids and fibres can be associated, at last in part, with these effects.**

Key words: *Caryocar brasiliense*, *pequi*, cardiometabolic risk, lipid metabolism, redox status.

INTRODUCTION

Non communicable diseases (NCD), such as type 2 diabetes and cardiovascular diseases, are major causes of mortality worldwide (up to 38 million by year), and are responsible for 80% of deaths occurring in developing countries (WHO, 2014). Increasing the intake of fruits and vegetables are one of the main recommendations for reducing NCD risk. From this perspective, consumer interest in foods with functional properties is increasing

*Corresponding author. E-mail: eaesteves@yahoo.com.br.

(Bumgarner et al., 2012; Jackix et al., 2013; Schreckinger et al., 2010).

In this context, *pequi* (*Caryocar brasiliense*) is an exotic fruit from a Brazilian savannah-like biome. Its pulp is sweet, yellowish and has a high energy density and lipid content (~33%), being monounsaturated fatty acids (MUFAs) (especially oleic, ~54%) its major constituent (Lima et al., 2007). It also has substantial amounts of fibres (18%) (Teixeira et al., 2013) and carotenoids (~42 $mg.100^{-1}g$), especially violaxanthin, lutein, zeaxanthin, β-cryptoxanthin, neoxanthin and β-carotene (Azevedo-Meleiro and Rodriguez-Amaya, 2004).

From *pequi* pulp chemical constituents, MUFAs have been associated with improvements in lipid profile, reduced platelet aggregation, favourable modulation of blood pressure, insulin sensitivity and glycemic control (Hammad et al., 2016). Fibres are related to the regulation of intestinal function, control of body weight and lipid metabolism by decreasing the absorption and increasing excretion of cholesterol and triglycerides, and they have indirect effects on the blood pressure and serum glucose control (Lattimer and Haub, 2010). Carotenoids are powerful antioxidants, and some, such as β-carotene, have vitamin A activity. They are associated with reduced risk for cancer and cardio-vascular diseases, and protection from cell oxidative damage (Gülçin, 2012; Ried and Fakler, 2011).

Thus, the composition of nutrients and bioactive compounds of *pequi* pulp suggests that it could be a food supplement and it can exert effects on metabolism, cardiovascular function and cell redox status as a functional food. However, this fruit has been poorly studied. To the author's knowledge, there are only some research showing healing (Quirino et al., 2009; Bezerra et al., 2015), chemopreventive (Palmeira et al., 2016; Colombo et al, 2015) and anti-inflammatory (Miranda-Vilela et al., 2009) properties of *pequi* oil. Studies regarding functional properties from *pequi* pulp intake are scarce (Teixeira et al., 2013).

Therefore, the aim of this study was to evaluate the effects of *pequi* pulp intake on cardiometabolic risk markers of rats. The *in vitro* antioxidant activity and the chemical composition of *pequi* pulp, were determined previously because some compounds could be related to its potential health benefits.

MATERIALS AND METHODS

Pequi pulp samples

Ripe *pequi* fruits were acquired from the local market of Diamantina city, Minas Gerais State, Brazil. They were washed with tap water and subsequently with distilled water. After drying at room temperature, each fruit was cut in half and the pulp was separated from the almond manually. Afterwards, the pulps were placed on trays and dried at 65°C for 48 h (Teixeira et al., 2013). After drying, the material was grounded, wrapped in a plastic bag, labeled and stored at -18±2°C until the analysis.

Chemical composition and *in vitro* antioxidant activity of *pequi* pulp

Protein, total lipids, dietary fibres (enzymatic–gravimetric method) and total carotenoids were determined as described by The Association of Official Analytical Chemists - AOAC methods (AOAC, 1995). Carbohydrates were calculated by difference, and the total energy value (TEV) was estimated using the Atwater factors (Buchholz & Schoeller 2004). Fatty acids were analyzed by gas chromatography (CGC Agilent 6850 Series GC System) according to The American Oil Chemist´s Society – AOCS (AOCS, 2009).

The *in vitro pequi* pulp antioxidant activity was performed in both 6:4 ethanol: water and 1:1 methanol: acetone extracts. Briefly, dehydrated *pequi* pulp samples were extracted with 40 mL of 1:1 methanol/water solution for 1 h, at room temperature. Afterwards, the mixture was centrifuged (Biosystems, Modelo 80-2B, Curitiba-PR) at 3.000 rpm for 15 min. The supernatant was harvested and the step was repeated, using a 7:3 acetone/water solution (Larrauri et al., 1997). After the solvents evaporation, the mixtures were diluted in 6:4 ethanol : water and 1:1 methanol : acetone solutions. The 2,2-diphenyl-1-picrylhydrazyl free radical scavenging (DPPH) and ferric reducing antioxidant power (FRAP) methods were used according to Rufino et al. (2010).

Rat study

Experimental protocols were performed in accordance with the EU Directive 2010/63/EU for animal experiments. They were approved by the Ethics Committee on Animal Use/Federal University of Vales do Jequitinhonha e Mucuri, Diamantina, MG, Brazil (Protocol 010/2012).

Sixteen male Wistar rats, four weeks aged, were housed in individual stainless steel cages and maintained in a room with controlled temperature (22±2°C) and a 12 h light/dark cycle, with free access to food and water during the experimental period.

A commercial chow (RhosterLab®) was used as a standard diet, and its energy density was 3.28 $kcal.g^{-1}$ (13.77 $kJ\ g^{-1}$). Based on the lipid composition of the *pequi* pulp, the standard diet was added, *pequi* pulp at 3.26.100 g^{-1}, which resulted in a 50% increase in total lipid content, so its energy density turned into 3.39 $kcal\ g^{-1}$ (or 14.2 $kJ.g^{-1}$, a 3.35% increase). The *pequi* pulp supplementation was also added, 1.19 g.100 g^{-1} of oleic acid; 0.63 g.100 g^{-1} of fibres and 0.14 $mg.100\ g^{-1}$ of carotenoids to the standard diet.

All 16 animals were randomly assigned to two treatment groups (n=8): Control group - animals fed the standard diet and *Pequi* pulp group - animals fed the standard diet added *pequi* pulp. The study lasted for 15 weeks. During this period, body weight and food intake were monitored for energy efficiency ratio (EER = weight gain/kcal) and feed efficiency ratio (FER = weight gain/g of diet) calculations. Faeces were collected in the last 72 h of the experiment, dried and kept at -80°C until analysis. The body weight and length (nose–anus length) were measured in all anaesthetized rats (quetamin + xilazin/50 mg/kg + 10 mg/kg) in the previous day to the euthanasia for the Lee index calculation (weight body.$g^{0.33}$ /nose–anus length).

On the last day, overnight fasted animals were anesthetized (quetamin + xilazin/50 mg/kg+10 mg/kg), euthanized by decapitation for blood and tissues (adipose tissues, liver, heart, duodenum) harvesting. Retroperitoneal and epididymal fat pads were used for adiposity index ([retroperitoneal + epididymal pads/body weight – (retroperitoneal + epididymal pads)]*100)) calculation. Blood was centrifuged in heparinized tubes to obtain plasma, and aliquots were transferred to Eppendorf tubes and kept at -80°C until analysis. Liver and heart tissues were processed for redox status analysis. Duodenum fragments of 5 cm were harvested and stored in a 10% formaldehyde solution for complementary histological analyses.

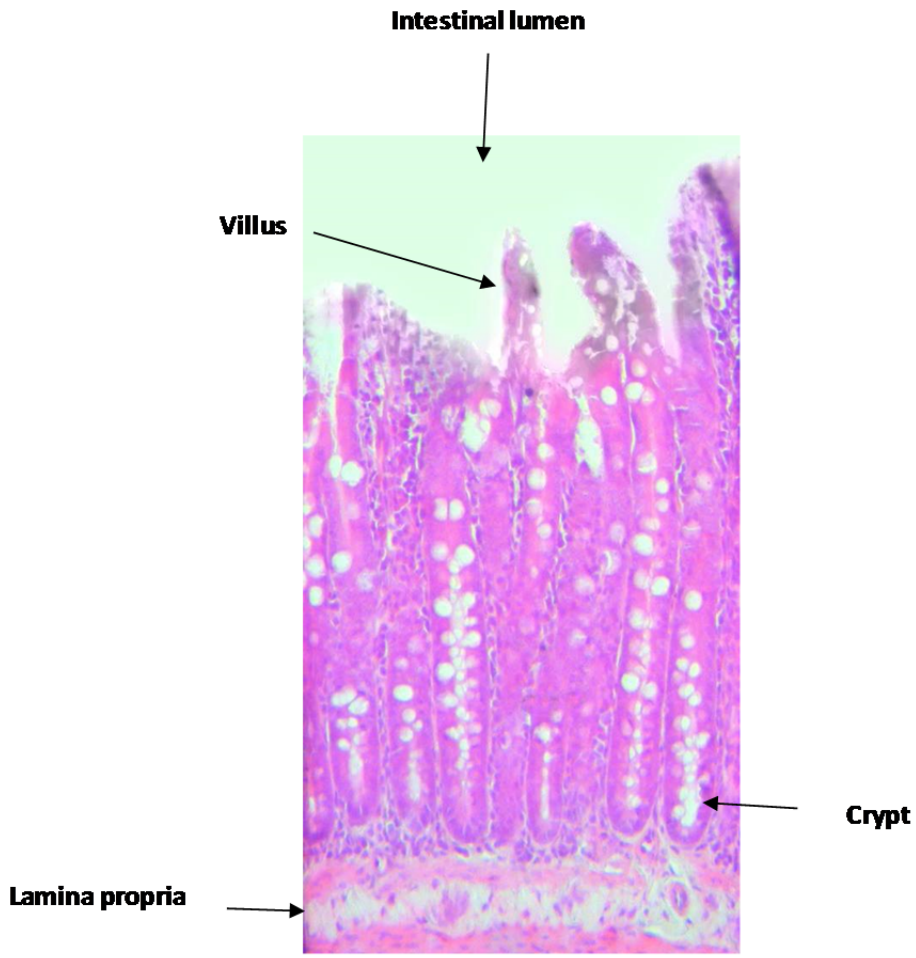

Figure 1. Morphometric analysis of duodenum: villus height and crypt depth. 5 µm cross-section, 100x magnification.

Cardiometabolic risk factors

Tail blood pressure (BP) and heart rate (HR) were measured in the last week of the experimental protocol by the non-invasive tail plethysmography method. The animals were heated to cause vasodilation of the caudal artery. The pulses were recorded by system (AD Instruments Ltd, UK). The BP and HR values were used for the double product calculation (BP x HR). The heart weight and body weight were used for cardiac hypertrophy evaluation, by heart weight/body weight calculation.

Fasted plasma glucose levels (GLU) were measured by means a commercial kit according the procedures recommended by the manufacturer and using a semi-automatic biochemical analyzer (PIOWAY-3000). Fasted plasma insulin (INS) was determined using a commercially available Enzyme-Linked Immunosorbent Assay kit (Linco Research Inc., St. Louis, MO, USA) and a micro-plate reader (Spectra MAX 190, Molecular Devices, USA). Insulin resistance was accessed by the homeostasis model assessment of insulin resistance (HOMA-IR index), from fasted glucose and insulin levels according to Matthews et al. (1985).

Total plasma cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels were determined using a semi-automatic biochemical analyzer (PIOWAY-3000) and commercial kits according the procedures recommended by the manufacturer. Liver and faeces samples were oven-dried (60°C ± 2°C for 72 h) after harvesting, grounded, and their lipids were extracted according to Folch et al. (1957). CHOL and TG levels were determined using commercial kits according the procedures recommended by the manufacturer and using a semi-automatic biochemical analyzer (PIOWAY-3000).

Considering the possible influences of some chemicals from *pequi* pulp in the intestinal morphology, which could affect nutrient digestion and absorption, we also preceded histomorphometric assays. For that, fragments of duodenum were removed and fixed in a 4% buffered formaldehyde solution. After dehydration and fixation in paraffin, two 5 µm cross-sections which were stained with haematoxylin/eosin was performed. Results were obtained by means of a digital camera coupled to a microscope. All images were analysed using the Axion Vision software. The villus height (VH) and the crypt depth (CD) were expressed as the arithmetic mean determined from 20 measurements of each sample. The villus height/crypt depth ratio (VH/CD) was also calculated. The villi density per optical field (920764.14 µm²) was taken from five photos from each animal. All measurements were made in µm, at 100x magnification (Figure 1).

For the redox status analysis, liver and heart samples were homogenized in phosphate-buffered saline (PBS) (T 20 basic ULTRA-TURRAX; IKA Labortechnik, China), pH 7.2, and

Table 1. Nutritional composition (g.100 g^{-1}), energy density (kcal.g^{-1}) and total carotenoids (mg.100 g^{-1}) of boiled and dehydrated *pequi* pulp (*Caryocar brasiliense*).

Component	Amount*
Lipids	66.76±1.16
Proteins	4.76±0.38
Fibres	19.19±0.09
Carbohydrates	1.41±3.27
Energy density**	6.25±0.10
Carotenoids	43.3±0.03

*Values expressed in mean ±standard deviation. **26.27± 0.42 kj.g^{-1}.

Table 2. Fatty acid profile from boiled and dehydrated *pequi* pulp oil (*Caryocar brasiliense*).

Nomenclature	g.100g^{-1}
Lauric (C12:0)	0.04±0.01
Myristic (C14:0)	0.11±0.01
Palmitic (C16:0)	40.14± 0.01
Stearic (C18:0)	1.50±0.00
Arachidonic (C20:0)	0.16±0.00
Behenic (C22:0)	0.05±0,01
Lignoceric (C24:0)	0.08±0,00
Total of saturated	42.13±0.01
Palmitoleic (C16:1)	0.89±0.00
Oleic (C18:1)	54.76±0.01
Linoleic (C18:2)	1.53±0.01
α-Linolenic (C18:3)	0.34±0.00
Eicosenoic (C20:1)	0.26±0.00
Total of unsaturated	57.87±0.01

*Values expressed in mean ±standard deviation.

centrifuged for 10 min at 10,000x and 4°C (Jouan BR4i, Thermo Fischer Scientific, USA). The supernatant was harvested and used for the protein determination (Bradford, 1976) and the biochemical assays described below. The total antioxidant capacity was measured using the ferric reducing antioxidant power (FRAP) assay, according to the method of Benzie and Strain (1996). The formation of thiobarbituric acid-reactive substances (TBARS) during a hot acid reaction was used as an index of lipid peroxidation, according to Ohkawa et al. (1979).

Statistical analysis

Results from chemical composition and the *in vitro* antioxidant activity assays were expressed in mean ± standard deviation. Results from rat study were expressed in mean ± standard error. The experiment was performed in a completely randomized design with two treatments (experimental groups) and eight repetitions. Data were analyzed by one way ANOVA at $p<0.05$, using the Statistica 10.0 software. Figures were drawn by means of the SigmaPlot 11.0 software.

Table 3. Antioxidant activity of *pequi* pulp (*Caryocar brasiliense*) extracts by different methods (umol TE/g).

Extract	Method	
	DPPH	FRAP
Ethanol/Water 6:4	0.076±0.001	0.445±0.063
Methanol/Acetone 1:1	0.569*±0.086	1.256*±0.128

*$p<0.05$ by *t-test*. Ethanol/water 6:4 vs Methanol/Acetone 1:1. Values are expressed as mean ± standard deviation. DPPH=2,2-diphenyl-1-picrylhydrazyl free radical scavenging; FRAP = ferric reducing antioxidant power.

RESULTS

Chemical composition of pequi pulp and *in vitro* antioxidant assays

Pequi pulp had expressive amounts of total lipids (Table 1). The main fatty acids from *pequi* lipids were oleic followed by palmitic (Table 2). *Pequi* pulp also had high amounts of fibres, being majorly insoluble, and total carotenoids (Table 1). For the *in vitro* antioxidant activity assays, *pequi* pulp methanol/acetone extract had higher antioxidant capacity, by both FRAP and DPPH methods ($p<0.05$) (Table 3).

Rat study

Similar body weights were found at the beginning and end of the experiment. The food and caloric intake, FER, EER, Lee index and adiposity index did not differ between groups (Table 4). There were no differences for BP, HR, double product, hypertrophy index, plasma markers of glucose and lipids metabolism markers (glucose, insulin, HOMA-IR, triglycerides, cholesterol and HDL-C) (Table 5).

Regarding hepatic and faecal lipids, *pequi* pulp animals had lower hepatic levels of CHOL and TAG when compared with controls ($p<0.05$) (Figure 2A and B). Faecal CHOL did not differ between groups. *Pequi* group had higher faecal TAG levels when compared with C ($p<0.05$) (Figure 2D).

From the duodenum histomorphometric assays, an increase in the villus height (VH), Crypt depth (DC) and number of villous (NV) per optical field for *pequi* pulp group ($p<0.05$) was observed. However, differences were not observed between treatments for VH/DC ratio (Table 6). There were no differences between groups for both liver and heart lipid peroxidation levels and antioxidant capacity (Figure 3). However, for hearts, *pequi* group had a 23% increase in the antioxidant capacity and a 28% decrease in the peroxidation levels as compared to the control.

Table 4. General characteristics of experimental groups.

Variables	Control	*Pequi* pulp
Body weight (g)	286.37 ± 52.26	261.52 ± 25.69
Food Intake (g)	2209.54 ± 255.38	2148.23 ± 243.76
Caloric Intake (Kcal)	7247.29 ± 837.64	7475.84 ± 848.28
FER(g/g)	0.10 ± 0.02	0.10 ± 0.01
EER (g/kcal)	3.18 ± 0.57	2.82 ± 0.20
Lee Index	0.10 ± 0.02	0.10 ± 0.01
Adiposity Index	2.87 ± 0.63	3.01 ± 0.51

Values are expressed as mean ± standard error. FER: feed efficiency ratio; EER energy efficiency ratio.

Table 5. Cardiometabolic risk factors of the experimental groups.

Variables	Control	*Pequi* pulp
BP (mmHg)	152.38 ± 20.38	146.23 ± 26.22
HR (bpm)	392.37 ± 58.17	397.43 ± 33.72
Double product	60451.59 ± 16078.48	58502.86 ± 14663.45
Cardiac hypertrophy (g/g)	0.46 ± 0.06	0.51 ± 0.04
Glucose (mg/dL)	123.75 ± 20.68	116.98 ± 16.02
Insulin (ng/mL)	0.89 ± 0.28	1.17 ± 0.34
HOMA-IR	7.91 ±2.95	9.64 ± 3.59
Triglycerides (mg/dL)	32.56 ± 4.95	34.57 ± 10.46
Cholesterol (mg/dL)	58.11 ±7.91	62.19 ± 10.36
HDL-cholesterol (mg/dL)	22.90 ± 5.50	22.13 ± 4.08

Values are expressed as mean ± standard error. BP: blood pressure; HR: heart rate.

DISCUSSION

Pequi is an exotic fruit with a heavy potential to be a functional food, since it has a peculiar nutritional composition and is high in several bioactive compounds. In this study, the amount of lipids found in the *pequi* pulp samples was in accordance with previous data from the laboratory (Teixeira et al., 2013) and higher than that found by Cardoso et al. (2013) and Lima et al. (2007). Also, *pequi* pulp has a paradoxal composition in fatty acids, since oleic acid, a MUFA, is its major constituent and related to cardiometabolic risk reduction. Otherwise its second higher constituent is palmitic acid, a saturated fatty acid with several cytotoxic effects described (Akazawa et al., 2010; Eitel et al., 2002).

Its amount of fibres and carotenoids were comparable to other commonly consumed foods that are high in those compounds (Rodriguez-Amaya et al., 2008). Otherwise, carotenoids were lower than other samples analysed elsewhere (Cardoso et al., 2013; Lima et al., 2007; Teixeira et al., 2013). These differences may be due the different processing forms of *pequi* pulp samples in those studies. In these samples, boiling and dehydrating may have lowered carotenoid content and also, increased other nutrient concentrations, such as lipids and fibres. The higher antioxidant activity of methanol/acetone *pequi* pulp extract indicated that lipophilic compounds account significantly for the pulp antioxidant power.

Based on these findings, it is clear that this fruit has expressive amounts of certain nutrients and bioactive compounds that have been associated with protection in many biochemical processes that underly the development of cardiometabolic diseases. Therefore, study of some biological effects of these compounds together in a single food which is still poorly studied, is proposed.

Adding *pequi* pulp did not influence body weight gain, food intake, glucose and plasma lipids. In addition, this increase also did not affect BP and HR and it did not cause cardiac overload, since no changes in the double product and cardiac hypertrophy index was observed. There was 50% increase in total lipid content of standard diet by adding *pequi* pulp. Although, there was increase in calories and lipids, the diet did not turned into a high fat, which could cause metabolic disturbance. According to Buettner et al. (2006) and Hariri and Thibault (2010), to have high fat, a diet must have at least 30% of its energy from lipids. The *pequi* group diet had 18.51%.

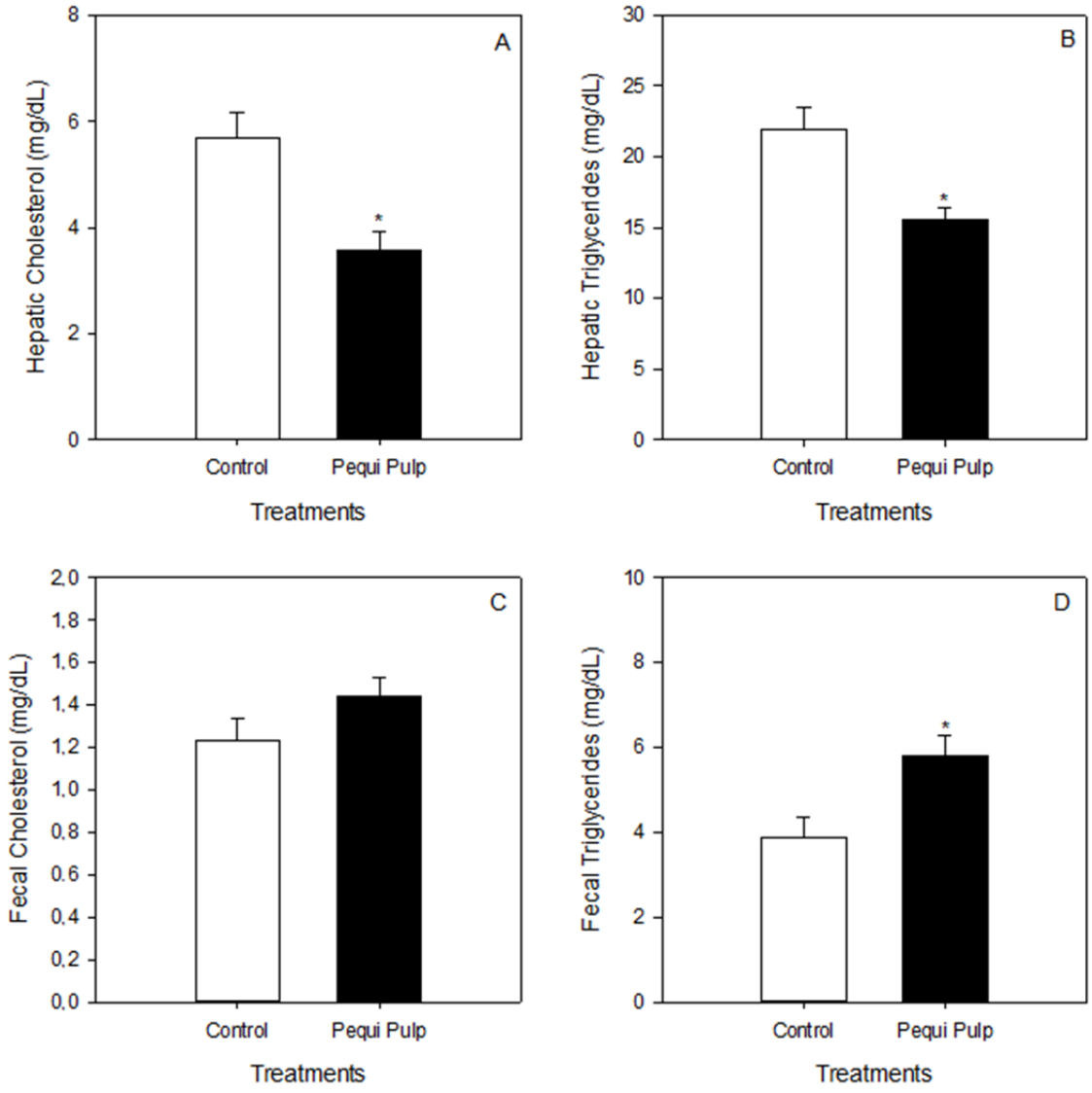

Figure 2. Hepatic and faecal cholesterol and triglycerides levels of experimental groups. Values are expressed as mean ± standard error. * represent statistically significant difference ($p<0.05$) by one way ANOVA.

Table 6. Morphometry of the duodenum: villus height (VH), crypt depth (CD), VH/CD ratio (µm) and villous number (VN) (units per optical field) of the experimental groups.

Variables	Control	*Pequi* pulp
HV (µm)	398.11 ± 42.02	480.14 ± 65.79*
DC (µm)	247.01 ± 9.93	296.63 ± 38.34*
HV/DC Ratio	1.61 ± 0.11	1.62 ± 0.05
VN	19.03 ± 1.57	21.99 ± 2.07*

Values are expressed as mean ± standard error. * represent statistically significant difference ($p<0.05$) by one way ANOVA.

Although, the main chemical constituents from *pequi* pulp (mainly MUFAs and fibre) are related to CHOL, TG and LDL-lowering effects (Ried and Fakler, 2011) as well as having antioxidant properties (carotenoids) (Gülçin,

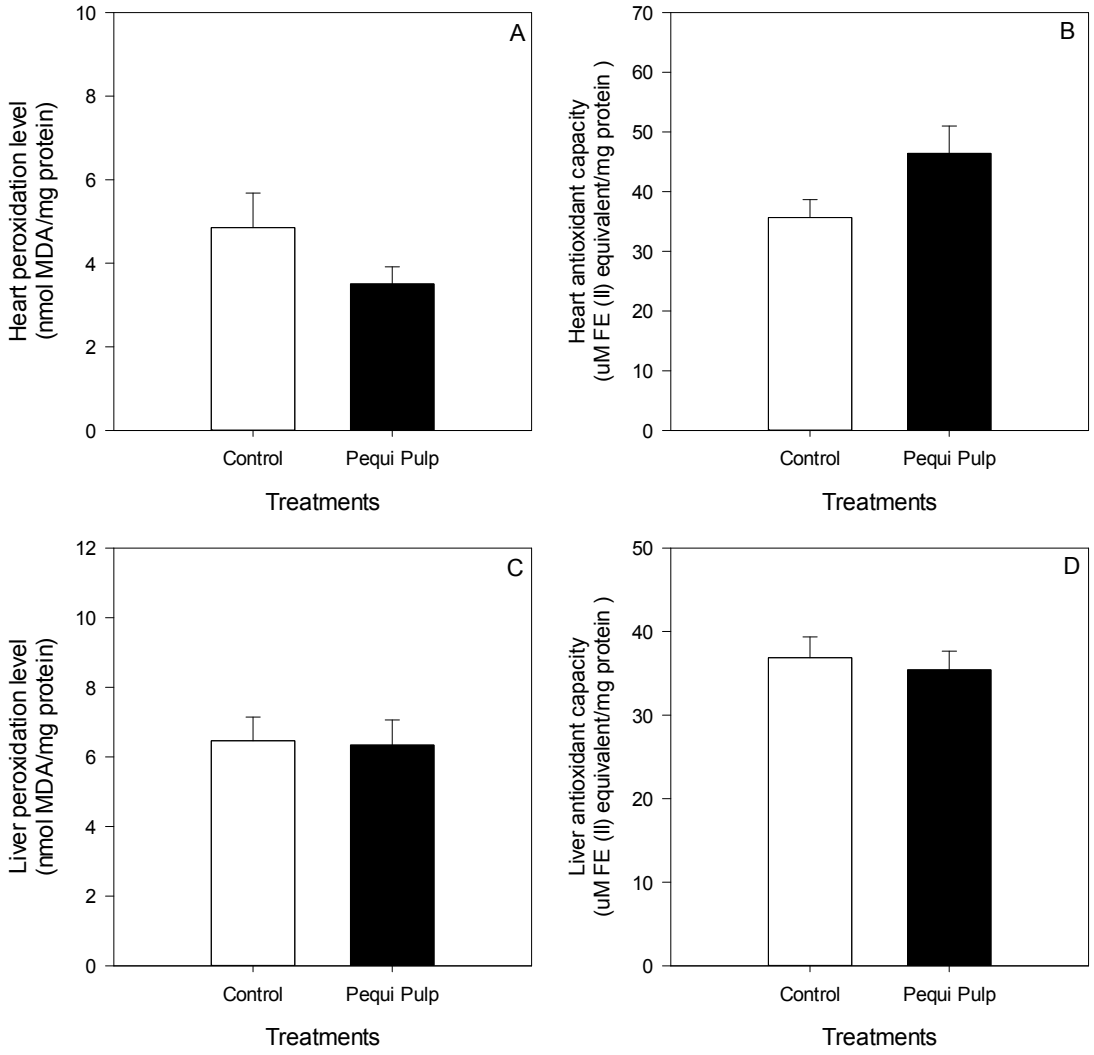

Figure 3. Lipid peroxidation levels and total antioxidant capacity of hearts and livers of experimental groups. Values are expressed as mean ± standard error.

2012), we did not observe these effects on plasma lipids. Otherwise, there were pronounced effects from *pequi* pulp supplementation in the hepatic and faecal lipids. The lower hepatic levels of TRI and CHOL and higher TRI faecal output in the *pequi* group may be associated with the higher *pequi* pulp fibre content.

Fibres, especially soluble, can increase the lumen viscosity, bind to cholesterol, triglycerides, bile acids and other lipids, impairing their digestion/absorption and increasing their faecal output (Lattimer and Haub, 2010). They can also be fermented by microflora and generate products, as acetate, propionate and butyrate, which affect the endogenous synthesis of theses lipids (Ngoc et al., 2012). Insoluble fibres, in turn, regulate intestinal transit-time, contributing to the lower absorption of those nutrients (Lattimer and Haub, 2010). Furthermore, insoluble fibres are related to a higher expression of hepatic genes that increase fatty acid oxidation (Isken et al., 2010).

Therefore, the *pequi* pulp could have modulated the function of the gastrointestinal tract to increase the lipids excretion. Histological data corroborated these findings. *Pequi* group showed higher villous height and crypt depth, implying this food exerted a positive effect in the mucosa integrity. Conversely, the DC increase in this group indicated a high rate of cell differentiation in crypts. In addition, the increase in VH indicated cell migration and renovation to the villus (Rosa et al., 2010).

Some compounds of *pequi* pulp can be related to that. Fibres were associated with VH increase (Ashraf et al., 2013) and oleic acid was associated with a better gut development and a DC increase (Rosa et al., 2010). In addition, carotenoids, as vitamin A precursors can act on intestinal cell growth and differentiation (Allen et al.,

2002). As antioxidants, they can decrease damage caused by oxidative agents and therefore, contribute to cell preservation (Turan et al., 2009). Then, it can be inferred that *pequi* pulp may have increased the duodenal absorption surface area and cell renovation, helping the maintenance of the mucosa integrity.

Regarding redox status, statistical differences were not observed between groups for livers and hearts. According to several authors (Feillet-Coudray et al., 2009; Sour et al., 2015), changes on redox status parameters are easily detectable when dietary lipid and caloric overload occurs, or when there are some physiological disturbance, such as inflammation, obesity and dyslipidaemia. Adding *pequi* pulp did not increase significantly lipid content of the diet. In addition, the lipid lower liver accumulation and increased faecal output upon *pequi* pulp intake may also be related to these results, since it can have contributed to a less generation of reactive oxygen and nitrogen species with subsequent lower peroxidation of membrane lipids.

However, in the heart, it is important to consider that *pequi* pulp led to a trend in increasing antioxidant capacity and decreasing lipid peroxidation levels. It seems that the heart was the more sensitive organ upon *pequi* pulp intake. Carotenoids are natural antioxidants, have lipophilic characteristic and may be incorporated into mitochondrial membranes, which are the main site for free radical production during the electron flow (Vega et al., 2009). Furthermore, this *in vitro* assay showed that methanol and ethanol extracts of *pequi* pulp had a high antioxidant capacity.

Conclusion

Taken together, the results indicate that *pequi* pulp intake minimized liver fat deposition by increasing its faecal output and improved intestinal structure, which could account for reduction of cardiometabolic risk in rats. Fibres, MUFA and carotenoids from this fruit may be responsible, at last in part, for these effects.

Conflicts of interests

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by the Brazilian agencies Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The authors gratefully acknowledge the Instituto de Pesquisas e Estudos de Lassance (IPEL) for the contribution of the biochemical kits.

REFERENCES

American Oil Chemists' Society (AOCS) (2009). Official methods and recommended practices of the AOCS Urbana: AOCS.

Akazawa Y, Cazanave S, Mott JL, Elmi N, Bronk SF, Kohno S, Charlton MR, Gores GJ (2010). Palmitoleate attenuates palmitate-induced Bim and PUMA up-regulation and hepatocyte lipoapoptosis. J. Hepatol. 52:586-593.

Allen S, Maden M, Price J (2002). A role for retinoic acid in regulating the regeneration of deer antlers. Dev. Biol. 251:409-423.

Association of Official Analytical Chemists (AOAC) (1995). Official Methods of Analysis of AOAC International Washington DC: AOAC International.

Ashraf S, Zaneb H, Yousaf M, Ijaz A, Sohail M, Muti S, Usman M, Ijaz S, Rehman H (2013). Effect of dietary supplementation of prebiotics and probiotics on intestinal microarchitecture in broilers reared under cyclic heat stress. J. Anim. Physiol. Anim. Nutr. (Berl). 97:68-73.

Azevedo-Meleiro CH, Rodriguez-Amaya DB (2004). Confirmation of the identity of the carotenoids of tropical fruits by HPLC-DAD and HPLC-MS. J. Food Compost. Anal. 17:385-396.

Benzie IF, Strain J (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239:70-76.

Bezerra NKMS; Barros TL, Coelho NPMF. A ação do óleo de pequi (Caryocar brasiliense) no processo cicatricial de lesões cutâneas em ratos (2015). Rev. Bras. Plant Med. 17:875-880.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Buchholz AC, Schoeller DA (2004). Is a calorie a calorie? Am. J. Clin. Nutr. 79(5):899S-906S.

Buettner R, Parhofer K, Woenckhaus M, Wrede C, Kunz-Schughart L, Schölmerich J, Bollheimer L (2006). Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. J. Mol. Endocrinol. 36:485-501.

Bumgarner NR, Scheerens JC, Kleinhenz MD (2012). Nutritional Yield: A proposed index for fresh food improvement illustrated with leafy vegetable data. Plant Foods Hum. Nutr. 67:215-222.

Cardoso LDM, Reis BDL, Hamacek FR, Sant'ana HMP (2013). Chemical characteristics and bioactive compounds of cooked pequi fruits (*Caryocar brasiliense* Camb.) from the Brazilian savannah. Fruits 68:3-14.

Colombo NBR, Rangel MP, Martins V, Hage M, Gelain DP, Barbeiro DF, Capelozzi VL (2015). Caryocar brasiliense camb protects against genomic and oxidative damage in urethane-induced lung carcinogenesis. Braz. J. Med. Biol. Res. 48:852-862.

Eitel K, Staiger H, Brendel MD, Brandhorst D, Bretzel RG, Häring H-U, Kellerer M (2002). Different role of saturated and unsaturated fatty acids in β-cell apoptosis. Biochem. Biophys. Res. Commun. 299:853-856.

Feillet-Coudray C, Sutra T, Fouret G, Ramos J, Wrutniak-Cabello C, Cabello G, Cristol J, Coudray C (2009). Oxidative stress in rats fed a high-fat high-sucrose diet and preventive effect of polyphenols: Involvement of mitochondrial and NAD (P) H oxidase systems. Free Radic. Biol. Med. 46:624-632.

Folch J, Lees M, Sloane-Stanley G (1957). A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Gülçin I (2012). Antioxidant activity of food constituents: an overview. Arch. Toxicol. 86:345-391.

Hammad S, Shuaihua P, Jones PJ (2016). Current evidence supporting the link between dietary fatty acids and cardiovascular disease. Lipids 51(5):507-17.

Hariri N, Thibault L (2010). High-fat diet-induced obesity in animal models. Nutr. Res. Rev. 23:270-299.

Isken F, Klaus S, Osterhoff M, Pfeiffer AFH, Weickert MO (2010). Effects of long-term soluble vs. insoluble dietary fiber intake on high-fat diet-induced obesity in C57BL/6J mice. J. Nutr. Biochem. 21:278-284.

Jackix AE, Monteiro EB, Raposo HF, Amaya-Farfán J (2013). Cholesterol reducing and bile-acid binding properties of taioba (Xanthosoma sagittifolium) leaf in rats fed a high-fat diet. Food Res.

Int. 51:886-891.
Lattimer JM, Haub MD (2010). Effects of dietary fiber and its components on metabolic health. Nutrients 2:1266-1289.
Larrauri JA, Rupérez P, Sauracalixto F (1997). Effect of drying temperature on the stabilitity of polyphenols and antioxidant activity of red grape pomace peels. J. Agric. Food Chem. 45:1390-1393.
Lima AD, Silva AMdOe, Trindade RA, Torres RP, Mancini-Filho J (2007). Composição química e compostos bioativos presentes na polpa e na amêndoa do pequi (*Caryocar brasiliense* Camb.). Rev. Bras. Frutic. 29:695-698.
Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R (1985). Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412-419.
Miranda-Vilela AL, Pereira LCS, Gonçalves CA, Grisolia CK (2009). Pequi fruit (Caryocar brasiliense Camb.) pulp oil reduces exercise-induced inflammatory markers and blood pressure of male and female runners. Nutr. Res. 29:850-858.
Ngoc T, Hong T, Len N, Lindberg J (2012). Effect of fibre level and fibre source on gut morphology and micro-environment in local (Mong Cai) and exotic (Landrace Yorkshire) pigs. Asian-Australas J. Anim. Sci. 25:1726-1733.
Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95:351-358.
Palmeira SM, Silva PR, Ferrão JS, Ladd AA, Dagli ML, Grisolia CK, Hernandez-Blazquez FJ (2016). Chemopreventive effects of pequi oil (Caryocar brasiliense Camb.) on preneoplastic lesions in a mouse model of hepatocarcinogenesis. Eur. J. Cancer Prev. 25(4):299-305.
Quirino GS, Leite GO, Rebelo LM, Tomé AR, Costa JGM, Cardoso AH, Campos AR (2009). Healing potential of Pequi (Caryocar coriaceum Wittm.) fruit pulp oil. Phytochem. Lett. 2:179-183.
Ried K, Fakler P (2011). Protective effect of lycopene on serum cholesterol and blood pressure: Meta-analyses of intervention trials. Maturitas 68:299-310.
Rodriguez-Amaya DB, Kimura M, Amaya-Farfan J (2008). Fontes brasileiras de carotenoides: Tabela brasileira de composição de carotenoides em alimentos Brasília: Ministério do Meio Ambiente.
Rosa DD, Sales RLd, Moraes LFdS, Lourenço FC, Neves CA, Sabarense CM, Ribeiro SMR, Peluzio MdCG (2010). Flaxseed, olive and fish oil influence plasmatic lipids, lymphocyte migration and morphometry of the intestinal of Wistar rats. Acta Cir Bras. 25:275-280.
Rufino MSM, Alves RE, de Brito ES, Pérez-Jiménez J, Saura-Calixto F, Mancini-Filho J (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. Food Chem. 121:996-1002.
Schreckinger ME, Lotton J, Lila MA, de Mejia EG (2010). Berries from South America: a comprehensive review on chemistry, health potential, and commercialization. J. Med. Food 13:233-246.
Sour S, Belarbi M, Sari N, Benammar C, Baghdad C, Visioli F (2015). Argan oil reduces, in rats, the high fat diet-induced metabolic effects of obesity. Nutr. Metab. Cardiovasc. Dis. 25:382-387.
Teixeira TN, Esteves EA, Oliveira LG, Oliveira MLP, Santana RC, Rodrigues AP (2013). *Caryocar brasiliense* pulp increases serum HDL and reduces hepatic lipid accumulation in rats fed a high fat diet. J. Med. Plant Res. 7:963-969.
Turan A, Gill R, Dudeja PK, Mohan H, Mahmood A (2009). Effect of fat feeding on pro-oxidant and anti-oxidant enzyme systems in rat intestine: possible role in the turnover of enterocytes. Dig. Dis. Sci. 54:1229-1236.
Vega VA, Anzulovich AC, Varas SM, Bonomi MR, Giménez MS, Oliveros LB (2009). Effect of nutritional vitamin A deficiency on lipid metabolism in the rat heart: Its relation to PPAR gene expression. Nutrition 25:828-838.
World Health Organization (WHO) (2014). Global status report on noncommunicable diseases 2014. Available at: http://www.who.int/nmh/publications/ncd-status-report-2014/en/ Accessed in March 6, 2016.

academicJournals

Vol. 10(37), pp. 649-655, 3 October, 2016
DOI: 10.5897/JMPR2016.6204
Article Number: 1BA8B1B60806
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Screenings of *In-vitro* antimicrobial, cytotoxic and anti-inflammatory activity of crude methanolic extracts of *Crinum latifolium (Leaves)*

Md. Abdur Rahman[1]*, Md. Saddam Hussain[1], Md. Shalahuddin Millat[1], Mithun Chandra Ray[1], Mohammad Tohidul Amin[1] and Md. Mizanur Rahman Moghal[2]

[1]Department of Pharmacy, Noakhali Science and Technology University, Sonapur-3814, Noakhali, Bangladesh.
[2]Department of Pharmacy, Mawlana Bhashani Science and Technology University, Santosh, Tangail-1902, Bangladesh.

Received 21 July, 2016; Accepted 9 September, 2016

Crude methanolic extracts of *Crinum latifolium* plant (Leaves) was assayed to identify various pharmacological properties. Antimicrobial potential of crude methanolic extracts of *C. latifolium* was accomplished by most commonly used disc diffusion method against a wide range of Gram positive (+*ve*) and Gram negative(-*ve*) bacteria. Extracts showed slight antimicrobial activity against Gram positive (+*ve*) bacteria while surprisingly showed significant antimicrobial activity against Gram negative (-*ve*) bacteria *Escherichia coli.* In contrast to vincristine sulphate, the crude methanolic, n-hexane soluble, petroleum ether soluble and chloroform soluble extracts showed slight to moderate cytotoxic properties with LC_{50} value of 7.06, 48.978, 242.83 and 153.93 µg/ml respectively. Plant extract showed significant (*P*<0.05) anti-inflammatory properties, that is, 16.21 and 20.55%10 mg/ml for hypotonic solution and heat induced condition respectively. So, this plant extract demands further research for revealing all its potency to have new safe drug for the entire respective field of medical science.

Key words: *Crinum latifolium*, zone of inhibition, brine shrimp lethality bioassay, anti-inflammatory.

INTRODUCTION

Cancer or tumor is the most common cause of death in both developed and developing countries. There are many methods are available to describe how cancer spread throughout the body. One method showed cancer is preliminary effect on specific part of our body and then invade to the other parts of our body very quickly and ultimately causes death of the patient (Evan, 2002; Ueda et al., 2002). So it is very necessary to identify or diagnosis of cancer at early stage otherwise if it is spread other part of the body then difficult to treat. However, there are several approaches of cancer treatments are available including surgery, radiation therapy and chemotherapy. All of these approaches are aimed to destroy cancerous cell from the body. Each approach

*Corresponding author. E-mail: arahmanphar5.nstu@gmail.com Tel: +8801816299081.

possesses several side effect (Kintzios et al., 2004). That is why it is now demand of the present era to discover drug with fewer side effect. There are many chemotherapeutic agents is being invented to treat various cancer. They utilize sometimes in individual form or in conjunction with other drugs in the form of chemotherapy. But most of these drugs are synthetic and shown numerous side effects. We know that plant is always the safer source for treating any kind of disease. By considering this universal truth our present study was undertaken to discover drug from natural source with fewer side effect for treating different types of cancer.

Again, Antibiotic resistance has become a great concern of treating infectious disease globally which offers great challenges for clinicians and pharmaceutical industry (Bauer et al., 2003). Many of our currently used antibiotics have become less active against a wide range of pathogen due to emergences of drug resistance. On the other hand, newly discovered drug possess many unwanted side effect. So the analysis of medicinal plants to explore antimicrobial agents will be a fruitful task in generating new way of treatment (Shahidi, 2004; Runyoro et al., 2006). That is why our present study was undertaken.

Another outcome of our present research is to determine anti-inflammatory potentials of our plant part. In the perspective of inflammatory disease it is established that stabilization of lysosomal membrane limiting the inflammatory response through inhibiting the release of lysosomal constituents such as bactericidal enzymes and proteases which cause further tissue inflammation and damage upon extracellular release (Rajendran et al., 2008). It is evidence that RBC membrane represents the lysosomal membrane. So, if the drugs effect on the stabilization of erythrocyte membrane could be resembled to the stabilization of lysosomal membranes (Omale et al., 2008). Anti-inflammatory agent causes the red blood cells membrane stabilization, subjected to hypotonic stress, through the release of hemoglobin (Hb) from RBCs (Naibi et al., 1985). Therefore, the stabilization of red blood cells hypotonic solution induced condition represent useful technique for the assessing the anti-inflammatory activity of various plant extractives (Oyedapo et al.,1999).

Our present research was conducting on *Crinum latifolium*, which is an herb belonging to the family Amaryllidaceae that arises from an underground bulb. It is locally known as sukhdarsan. Phytochemical screening of leaves reveals the presence of a wide variety of compounds such as alkaloids, phenolic compounds, tannins, flavonoids, terpenoids, amino acids, steroid saponins, and antioxidants. Traditionally Bulbs are used as a rubefacient for rheumatism. Juices of the leaves are used for earaches. Crushed and toasted bulbs are used for piles and abscesses to hasten suppuration (Dewan et al., 2013). The purpose of our current study is to analyze antimicrobial, cytotoxic and membrane stabilizing potentials of the plant methanolic extract.

MATERIALS AND METHODS

Collection and identification of plant material

The fresh leaves of *C. latifolium* were collected from Noakhali, a coastal region of Bangladesh on 26th July, 2012 and were taxonomically identified by taxonomist and botanist of Bangladesh National Herbarium, Mirpur, and Dhaka. Their given Accession number was -37751.

Plant extracts preparation and isolation

The leaves were collected by hand plucking from plant and cleaned of debris. The leaves were then air-dried by using mechanical graded e aluminum foil and finally kept at room temperature for 14 days (Atata et al., 2003). From which 400 gm of pounded material was taken into a suitable clean, flat-bottomed glass container and extracted with 1600 ml of 80% methanol. Then the container with plant part in powder form was made air tight by using mechanical graded e aluminum foil and finally kept at room temperature for 14 days. During this time the sample mixture were shacked and stirred at regular interval of time. The mixture was then passed through Markin cloth in order to obtain maximum quantity of extract. It was then filtered through Whatman filter paper and allowed to evaporate at a convenient rotary evaporator. The filtrate (Methanol extract) was then placed in a water bath. After a certain period of time the extract converted into a brownish black color residue, properly preserved at 4º C temperature, which was then used as a sample for further study.

Antimicrobial activity

To determine the antimicrobial potential of this plant, antimicrobial screening was performed by using disk diffusion method with slight modification for convenience. Many of the recent work was done by this method we used here (Bauer et al., 1966; Prabhu et al., 2011; Pratibha et al., 2012).

Test organism

Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa*) bacteria were used as a test organisms for antimicrobial activity. The strains of these organisms were collected from the Department of Microbiology, Noakhali Science and Technology University, Sonapur-3814, Noakhali, Bangladesh and are sub-cultered in nutrient broth and nutrient agar culture media.

Media preparation

To prepare fresh cultures and to test the sensitivity of the materials against micro-organism we used Nutrient agar medium (DIFCO). For preparing the media specified amount of nutrient agar was taken in a conical flask and distilled water was added to it to make the required volume of 1000 ml. For perfect dissolution the contents were heated in a water bath with continuous steering and the pH (at 25°C) was maintained at 7.2-7.6 using NaOH or HCl. The tip of the flask was mounted with a flag of cotton and aluminum foil and subjected to sterilization by autoclaving machine at a pressure of 15 lbs/sq inch, for 25 min at 125ºC temperature. About 10 ml and 5 ml

of the medium was then transferred into screw cap test tubes to prepare plates and slants respectively and lower the temperature to 45-50°C. The slants were used for making fresh culture of microorganisms that were in turn used for sensitivity study.

Application of discs, diffusion and incubation

Freshly prepared sample discs and commercially available standard antibiotic disc were transferred to each petri dish. The plates were then inverted and kept in a refrigerator for about 24 h at 4°C to allow sufficient diffusion of the materials from the discs to the surrounding area of the medium. The dishes were then incubated at 37°C for 24 h to allow optimal growth of microorganism.

Measurement of zone of inhibition

Antibacterial activity of test sample was measured by calculating zone of inhibition (Scalbert, 1991), which can be expressed in millimeter or centimeter unit by using suitable antibiotic zone scale. Different antibiotics discs (Ampicillin, Imipenem, Penicillin and Cefixitime) and sterile filter paper disc with respective solvent (methanol) of 25 µl were used as positive and negative control respectively. If the test sample possesses any antimicrobial activity, it will reduce the growth of the microorganisms and a clear, distinct zone of inhibition will be appeared surrounding the medium.

Brine shrimp lethality bioassay

The measurement of toxicity plays a vital role in drug discovery and is a useful tool in biological, especially ecological investigations (Opler et al., 2002). It also serves as a tool for screening plant extracts of possible medicinal value. In this study, we used simple brine shrimp bioassay test of Meyer with slight modification by using *Artimia salina* as test organism, which was collected from a pet shop (Meyer et al., 1982).

Brine shrimp hatching

Sea water was prepared by dissolving 38 g sea salt (pure NaCl) in one liter of distilled water, which is then filtered to get clear solution of 3.8% concentration (Krishnaraju et al., 2006). In a suitable plastic or glass vessel sea water was taken and shrimp eggs were added to one side of the vessel and allowed to hatch for 24 h till the mature nauplii were found. Continuous oxygen and light supply were provided to support the hatching process.

Sample preparation

All the test samples were taken in vials and dissolved in 100 µl of pure dimethyl sulfoxide (DMSO) to get stock solutions. Then 50 µl of solution was taken in the first test tube containing 5 ml of simulated seawater and 10 shrimp nauplii. Thus, final concentration of the prepared solution in the first test tube was 400 µg/ml. Then a series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. In every case, 50 µl samples were added to test tube and fresh 50 µl DMSO was added to vial.

Negative control group test

100 µl of DMSO was added to each of three pre-marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii to use as negative control groups.

Positive control group test

Here we used vincristine sulphate (VINCRIRST ®, Techno Drugs Ltd., Bangladesh) as a positive control. Measured amount of vincristine sulphate was dissolved in DMSO to get an initial concentration of 40 µg/ml from which serial dilutions were made using DMSO to get 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625 and 0.078125 µg/ml respectively. Then the positive control solutions were added to the pre-marked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water to get the positive control groups (Islam et al., 2009).

Counting of nouplii

After 24 h, the number of survived nauplii in each vial was counted by using magniflying glass. From this data the percent (%) of mortality of brine shrimp nauplii was calculated for each concentration.

Membrane stabilizing activity

The membrane stabilizing activity of the extractives was assessed by evaluating their ability to inhibit hypotonic solution hemolysis of human erythrocytes following the method developed by Omale et al. (2008).

Statistical analysis

All the above assays were conducted in triplicate and repeated threes for consistency of results and statistical purpose. The data were expressed as Mean±SD and analyzed by one way analysis of variance (ANOVA) followed by Dunnett 't' test using SPSS software of 10 version. $P<0.05$ was considered statistically significant.

RESULTS

Antimicrobial activity

From the experiment, we observed that, crude methanolic extracts of *C. latifolium* showed slight activity against Gram positive *(+ve) S. aureus* bacteria. On the other hand, it showed good antibacterial properties against Gram negative *(-ve) E. coli* bacteria. The overview of the results is shown in Table 1.

Findings of brine shrimp lethality bioassay

By using brine shrimp bioassay, developed by Meyer we could understand the cytotoxic potential and anti-tumor properties. In our current study we used various solvent soluble extracts of *C. latifolium.* Different solvent soluble extracts showed various rate of mortality at different concentration. By plotting the log of concentration against percent of mortality for all test sample, we found a linear correlation. On the basis of this correlation the LC_{50} (the concentration at which 50% of mortality of brine shrimp

Table 1. Antimicrobial effect of crude methanolic extract of *Crinum latiflium.*

Test microorganism(Bacteria)		Zone of inhibition of extract in various concentration				Response of the standard against microorganism
Gram Positive		25 µl	50 µl	75 µl	100 µl	10/30 µl/Disc
(+ve)	*Staphylococcus aureus*	-	-	-	1.0±0.039* cm (+)	Ampicillin (10 µl) +++
Gram Negative (-ve)	*Salmonella typhi*	-	-	-	-	Penicillin (10 µl) +++
	E. coli	1.0±0.025* cm (+)	1.2±0.18 cm (+)	1.4±0.37 cm (++)	1.6±0.004** cm (++)	Cefoxitin (30 µl) +++
	Pseudomonas aeruginosa	-	-	-	-	Imipenem (10 µl) +++

Here, (+++) = highly active; (++) = moderately active; (+) = slightly active; (-) = No activity against microorganism. *** = P < 0.001, ** = P < 0.01, * = P < 0.05.

Table 2. Results of brine shrimp lethality bioassay of crude methanolic extract of *Crinum latiflium.*

Sample	LC_{50} (µg/ml)	Regression Equation	R^2
Vincristine Sulphate (positive Control)	0.79	y = 2.65x+2.60	1.71
Crude methanol extract	7.06	y = 2.74x + 2.57	3.40
Chloroform fraction	48.978	y = 42.88x - 22.502	0.671
Carbon tetrachloride	242.83	y = 66.137x -102.82	0.9125
Petroleum ether fraction	153.93	y = 29.79x - 15.16	0.93

nauplii occurred) was determined for each solvent soluble extracts. We also found that, there was no rate of mortality obtained, in case of control study. The overview of the results is shown in Table 2.

Anti-inflammatory activity

The anti-inflammatory activities of the Crude methanolic extracts of *C. latifolium* are showed in Tables 3 and 4. The crude methanolic extracts dose dependently increased in anti-inflammatory study, whereas 10 mg/ml concentration most significantly showed 16.21 and 20.23% inhibition of hemolysis respectively by hypotonic solution and heat induced hemolysis. Acetyl salicylic acid was used as standard in membrane stabilization. ASA (0.10 mg/mL) revealed 70.01 and 56.32% inhibition of hemolysis, respectively induced by hypotonic solution and heat induced hemolysis correspondingly.

DISCUSSION

Antimicrobial activity

The medicinal properties of the plants lie in a several chemical group such as tannins, flavonoids, alkaloids and phenolic compound. Many parts of the plant especially leaves possess antimicrobial properties due to presence of tannins and flavonoids (Scalbert, 1991; Chung et al., 1998). Plants also synthesize huge amount of aromatic compound among which phenols or their oxygen-substituted derivatives are predominant (Geissman, 1963). These compounds provide protection against microbes for the plant (Cowan, 1999). This is great to see our plant extract showed to have phytochemicals responsible for anti-microbial effect (Dewan et al., 2013).

May be that is why Extracts showed slight antimicrobial activity against Gram positive (*+ve*) bacteria while Surprisingly showed significant antimicrobial activity against Gram negative (*-ve*) bacteria *E. coli.*

Cytotoxic activity

Cancer-related research is conducted all over the

Table 3. Effect of different conc. of methanolic extract of C. latifolium on hypotonic solution-induced haemolysis of erythrocyte membrane.

Treatment	Concentration (mg/ml)	Optical density of samples in hypotonic solution (Mean ± SD)	% inhibition of haemolysis
Control	_ _ _	3.701±0.058	
ME	2	3.423±0.075	7.51±0.0077
ME	4	3.265±0.108	11.78±0.0080
ME	6	3.234±0.082	12.61±0.0088
ME	8	3.123±0.1012*	15.62±0.0092
ME	10	3.101±0.098**	16.21±0.0101
Acetyl salicylic acid	0.10	1.712±0.043***	53.74±0.0265

Table 4. Effects of different concentration of methanolic extract of *C. latifolium* on heat induced hemolysis of erythrocyte membrane.

Treatment	Concentration	OD of sample ±SD		% inhibition of hemolysis
		Heated solution	Unheated solution	
Control	- - -	1.093±0.035	-	-
ME	2	0.879±0.107	0.856±0.011	9.7±0.069
ME	4	0.780±0.004	0.743±0.017	10.57±0.043
ME	6	0.715±0.014	0.650±0.022	14.67±0.062
ME	8	0.587±0.082	0.461±0.342*	19.93±0.036
ME	10	0.405±0.008	0.227±0.021**	20.55±0.087
Acetyl Salicylic Acid	0.10	0.672±0.025	0.129±0.029***	56.32±0.228

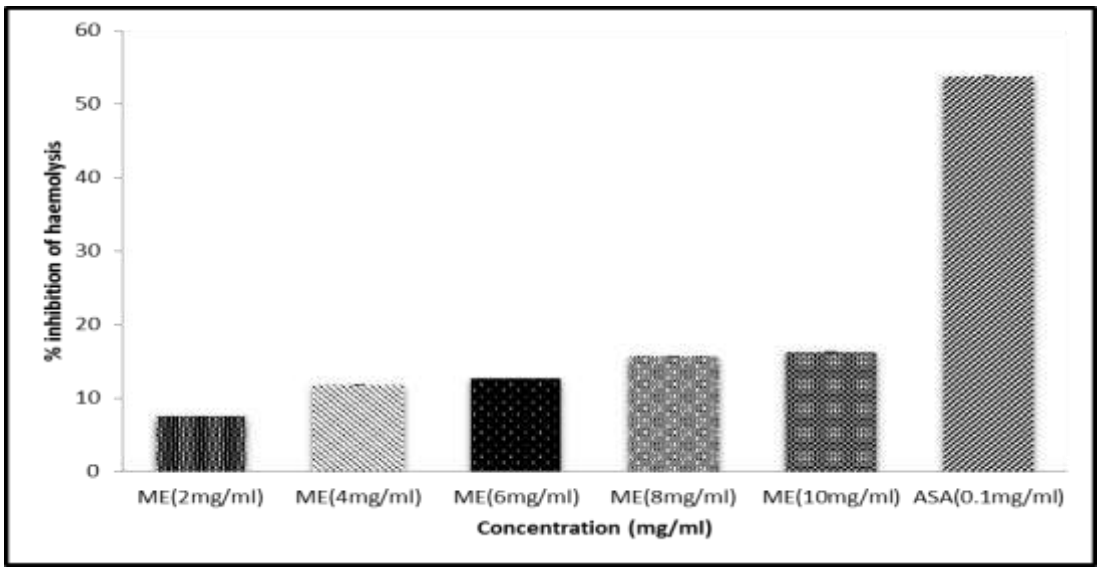

Figure 1. Effect of different conc. of C. latifolium on hypotonic solution induced haemolysis of erythrocyte membrane.

world for discovering new hopes for patient suffering with cancer. These studies frequently able to originate biologically active agents from plants used and will be used for treating different carcinoma (Mukherjee et al., 2001). In addition, it is important to understand the mechanisms of anticancer agents for future application in cancer therapy (Half et al., 2009). Our present study investigated the cytotoxic activity of the methanolic

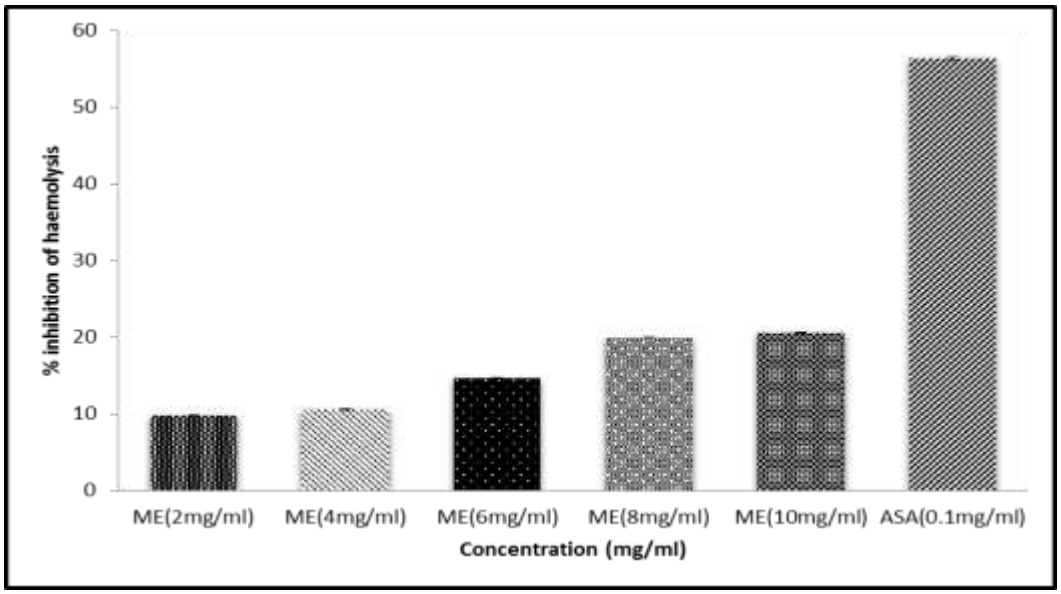

Figure 2. Effect of different conc. of C. latifolium on heat induced hemolysis of erythrocyte membrane.

extract of *C. latifolium*. It was found that many of the phytochemicals provide protection against cancer due to poly-phenyl antioxidant and anti-inflammatory effect. Several studies also suggest that these phytochemical provide protection against colorectal plus other types of cancer (Michaud et al., 2000; Greenberg et al., 1994; Birt et al., 2001). Our plant part also contain polyphenol so this plant was one will be one of the most trusted source for discovering anticancer drug, that was so far established through our present study as our plant methanolic extract showed remarkable cytotoxic activity.

Membrane stabilizing activity

C. latifolium methanolic extract inhibited hypotonic solution and heat induced hemolysis of erythrocyte at varying percentage that was comparable with membrane stabilizing activity shown by standard acetyl salicylic acid. As through the standard anti-inflammatory drug showed higher stabilization activity than the experimental plant methanolic extract, but our plant extract will be the existing source of anti-inflammatory activity with fewer or no side effects. The moderate membrane stabilizing activity shown by our plant methanolic extract may be due to the presence of flavonoid contents. It has been established by many experimental study that plants with flavonoids shown profound stabilizing effects on lysosomes both *in vitro* and *in vivo* laboratory condition (Middleton, 1996).

Conclusion

From the above experiments we could terminated that the crude methanolic and various solvent soluble extracts of *C. latifolium* (leaves) showed slight to moderate cytotoxic activities. We also confreres that, it also revealed excellent antibacterial and membrane stabilizing activities (Figures 1 and 2).

Conflict of interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENT

The authors would like to express their heartfelt gratitude, indebtedness, profound appreciation to all honorable teacher, staff and supervisor Md. Mizanur Rahman Moghal, Assistant Professor, Department of Pharmacy, Mawlana Bhashani Science and Technology University, for their continuous support, untiring inspiration, scholastic supervision, constructive criticism, affectionate feeling and optimistic counseling throughout the project work.

REFERENCES

Atata RF, Sani A, Ajewole SM (2003). Effect of Stem Bark Extracts of *Enantia chloranta* on some clinical isolates. Niger. Soc. Exp. Biol. 15(2):84-92.

Bauer AW, Kirby WMM, Sheriss JC, Turck M (1966). Antibiotic susceptibility testing by standarised single method. Am. J. Clin. Pathol. 45:493-496.

Bauer J, Rojas R, Bustamante B (2003). Antimicrobial activity of selected Peruvian medicinal plants. J. Ethnopharmacol. 88:199-204.

Birt DF, Hendrich S, Wang WQ (2001). Dietary agents in cancer prevention: flavanoids and isoflavonoids. Pharmacol. Ther. 90:157-

177.
Chung KT, Wong TY, Wei Y, Huang YW, Lin Y (1998). Tannins and human health. A review. Crit. Rev. Food Sci. Nutr. 8:421-464.
Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12:564-582.
Dewan SMR, D Abhijit (2013). Investigation of in vitro thrombolytic potential and phytochemical nature of *Crinum latifolium* leaves growing in coastal region of Bangladesh. Int. J. Biol. Pharm. Res. 4(1):1-7.
Evan WC (2002). Trease and Evans pharmacognosy. London: WB Saunders. P 585.
Geissman TA (1963). Flavonoid compounds, tannins, lignins and related compounds. In: Florkin M and Stotz EH (eds). Pyrrole Pigments, Isoprenoid Compounds and Phenolic Plant Constituents, New York, USA: Elsevier Press. P 265.
Greenberg ER, Baron JA, Tosteson TD (1994). A clinical trial of antioxidant vitamins to prevent colorectal cancer. N. Engl. J. Med. 331:141-147.
Half E, Arber N (2009). Colon cancer: Preventive agents and the present status of chemoprevention. Exp. Opin. Pharmacother. 10:211-219.
Islam MK, Etil Z, Chowdury JA (2009). Cytotoxic Studies on Two Meliaceae plants: Chukrasia Tabularis and Aglaia Roxburghiana. J. Sci. Res. 1(2):399-403.
Kintzios SE, Barberaki MG (2004). Plants that fight cancer. Boca Raton: CRC Press.
Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV (2006). Biological Screening of Medicinal Plants Collected from Eastern Ghats of India Using *Artemia salina* (Brine Shrimp Test). Int. J. Appl. Sci. Eng. 4(2):115-125.
Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL (1982). Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45:31-34.
Michaud DS, Feskanich D, Rimm EB (2000). Intake of specific carotenoids and risk of lung cancer in two prospective U.S. cohorts. Am. J. Clin. Nutr. 72:990-997.
Middleton JE (1996). Biological properties of plant flavonoids: An overview. Int. J. Pharmacogn. 34:344-348.
Mukherjee A, Basu S, Sarkar N, Ghosh A (2001). Advances in cancer therapy with plant based natural products. Curr. Med. Chem. 12:1467-1468.
Naibi RA, Sukumar Sethuraman V, Suluchana N, Sadique J (1985). Satellite symposium on traditional medicine as Asian congress of Pharmacology. Tamil University of Thanjavur. 140.
Opler A, Mizell R, Robert A, Cervantes-Cervantes M, Kincaid D, Kennelly EJ (2002). Use of a brine shrimp assay to study herbal teas in the classroom. Am. Biol. Teach. 64: 8596-8604.
Oyedapo OO, Sab FC, Olagunju JA (1999). Bioactivity of fresh leaves of *Lantana camara*. Biomed. Lett. 59:175- 183.
Omale J, Okafor PN (2008). Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata*. Afr. J. Biotechnol. 7:3129-3133.
Prabhu S, Britto SJ, Thangavel P, Raj L (2011). Antibacterial and Antioxidant Activity of Leaves of *Canavalia Mollis* Wight and Arn. (Horse Bean). Int. J. Pharm. Sci. Drug Res. 2(1):95.
Pratibha N, Sushma D, Gupta, Rajinder K (2012). Screening for Antioxidant and Antibacterial potential of common medicinal plants in the treatment of Acne. Int. J. Drug Dev. Res. 4:65-71.
Rajendran V, Lakshmi KS (2008). In vitro and In vivo anti-inflammatory activity of leaves of *Symplocos cochinchnensis* (Lour) Moore ssp laurina. Bangladesh J. Pharmacol. 3(2):121-124.
Runyoro D, Matee M, Olipa N, Joseph C, Mbwambo H (2006). Screening of Tanzanian medicinal plants for anti-Candida activity. BMC Complement Altern. Med. 6:11.
Scalbert A (1991). Antimicrobial properties of tannin. Phytochemistry 30:3875-3883.
Shahidi BH (2004). Evaluation of antimicrobial properties of Iranian medicinal plants against *Micrococcus luteus, Serratia marcescens, Klebsiella pneumonia and Bordetella bronchoseptica*. Asian J. Plant Sci. 3:82-6.
Ueda J, Tezuka Y, Banskota AH, Tran Q, Harimayay, SaikiI (2002). Antiproliferative activity of vietnamese medicinal plants. Biol. Pharm. Bull. 25:753-60.

academicJournals

Vol. 10(37), pp. 656-661, 3 October, 2016
DOI: 10.5897/JMPR2016.6245
Article Number: 59CE36B60808
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Acute oral toxicity study of *Mystroxylon aethiopicum* root bark aqueous extract in albino mice

Mhuji Kilonzo, Patrick A. Ndakidemi and Musa Chacha*

School of Life Sciences and Bio-Engineering, Nelson Mandela African Institution of Science and Technology, P. O. Box 447, Arusha-Tanzania.

Received 31 August, 2016; Accepted 19 September, 2016

Acute oral toxicity of *Mystroxylon aethiopicum* root bark aqueous was evaluated in albino mice of either sex. In this study, five groups of mice were orally treated with doses of 1000, 2000, 3000, 4000 and 5000 mg/kg body weight of crude extract. The mortality, signs of toxicity and body weights were observed individually for two weeks. At the end of the two weeks study, all animals were sacrificed and the hematological and biochemical parameters as well as organ weights relative to body weight of each animal were determined. No mortality, signs of toxicity and abnormalities in vital organs were observed in the entire period of study for both treated and control groups of mice. Additionally, there were no significant changes ($p>0.05$) in the blood hematology and biochemical analysis. However, the body weights of all mice increased significantly. The *M. aethiopicum* root bark aqueous extract were found to have a high safe margin when administered orally. Hence, the extract can be utilized for pharmaceutical formulations.

Key words: *Mystroxylon aethiopicum,* acute oral toxicity, albino mice.

INTRODUCTION

The use of medicinal plants has received great attention in the world as an alternative to conventional drugs and the demand for these remedies has recently increased (Phani and Kumar, 2014). Plant based medicines have been used by traditional healthcare in most parts of the world for thousands of years (Newman et al., 2000). According to the World Health Organization, 80% of the populations in developing countries rely on traditional medicines for their healthcare (WHO, 2007). Some of these traditional medicines involve the use of crude plant extracts in the form of infusion, decoction or tincture which may contain an extensive diversity of molecules, often with indefinite biological effects (Olowa and Nuñeza, 2013).

The *Mystroxylon aethiopicum* (Celastraceae) is a small to medium sized evergreen tree that grow up to 12 m high (Pope, 1995). The plant grows in a wide range of habitats including the forest margins, evergreen forests, open woodland, riverine fringes and also on termite mounds and rocky ridges (Burrows and Willis, 2005). In Africa, the plant is widely distributed in Ethiopia, Sudan, South Africa, Namibia, Angola, Cameroon, Madagascar, Seychelles and Comoro (Curtis and Mannheimer, 2005). In Tanzania, the species is found in highlands of Arusha

*Corresponding author. E-mail: musa.chacha@nm-aist.ac.tz. Tel: +255 753458177.

and Kilimanjaro regions, where it locally known as "*Oldonyanangui*" in Maasai language (Kokwaro, 1993). Traditionally, the plant is consumed by many ethnic groups in Africa for the management of hemorrhagic diarrhea, stomach, respiratory tract infections, coughs and anemia (Boera et al., 2005: Iwu, 2014). In Kenya, root bark extract of this plant is reportedly to be used in making a kind of tea which is drank as a stomach medicine, particularly by children (Kokwaro, 1993). Despite the wide use of *M. aethiopicum* in the management of different diseases, there is lack of scientific studies regarding the prevalence of the toxicological profile of this plant. This study hypothesize that extracts of *M. aethiopicum* is safe for usage in traditional medicine since it has been used by many communities in Africa as an alternative to conventional drugs. The aim of this paper therefore was to determine the safety profile of the aqueous root bark extracts of *M. aethiopicum* in albino mice.

MATERIALS AND METHODS

Plant materials and preparation of extracts

The plant materials were collected from Imbibya village in Arusha rural district, Tanzania. Plant species were identified by Mr. Gabriel Laizer, a botanist from the Tropical Pesticide Research Institute (TPRI) and voucher specimen coded MA-001 was kept at Nelson Mandela African Institution of Science and Technology (NM-AIST). Root barks were harvested without affecting the plant, air dried under the shade and pulverized into fine particles using electric blender. Pulverized materials (250 g) were added to a 1 L of distilled water maintained at 30°C for 4 h and allowed to cool to room temperature. The extracts were sieved and centrifuged at 5000 rpm for 10 min. The supernatant was collected and filtered using Whatman No. 1 filter paper and dried by freezing to eliminate water by sublimation. The extracts were stored in a deep freezer at -20°C for further activities.

Experimental animals

Albino mice of both sexes, weighing between 19 and 20 g and aged 3 to 4 weeks were randomly obtained from the Plant Protection Division of the Tropical Pesticides Research Institute (TPRI) Arusha, Tanzania. The animals were allowed to stay in cages with sawdust litters in a controlled temperature environment of about 23°C. Lighting was controlled to supply 12 h of light and 12 h of darkness for each 24 h period.

Ethical consideration

Prior to the experimental work, an ethical clearance with notification number NIMR/HQ/R.8a/Vol. IX/2145 was given by the National Health Research Ethics Sub-Committee (NatHREC) of the National Institute for Medical Research (NIMR) in Tanzania.

Experimental design

Acute oral toxicity test was done according to OECD guideline number 425 of 2001 (OECD, 2001). The mice were acclimatized for 7 days before experimentation. Before dosing with extract, the mice were starved for 4 h with access to adequate drinking water only. The mice were divided into a control group and five experimental groups with six mice each (3 males and 3 females). Body weights of the mice were determined and the dose was calculated in accordance with their body weights. The control group received 1% tween 80 in normal saline only by the oral route (5 ml/kg body weight), whereas the animals in the treatment group were administered oral doses of 1000, 2000, 3000, 4000 and 5000 mg/kg body weight, respectively, of crude extract that was dissolved in 1% Tween 80 in normal saline. Food was withheld for 1 h after administration of the extract but not water. The mice were observed regularly for mortality and any sign of toxicity such as change in skin and fur, eyes, respiratory effects, mucus membrane, diarrhea and sleep.

By the 14th day, all mice were weighed and blood samples were collected by cardiac puncture into two vacutainer tubes for each animal. The first vacutainer tube contained anti-coagulant substance (EDTA) and the second vacutainer tube was plain. Hematological parameters including red blood cell (RBC), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), white blood cell (WBC), lymphocyte and hemoglobin concentration (Hb) were determined using the blood samples contained in the EDTA tubes. The blood samples contained in plain vacutainer tubes were centrifuged at 4000 rpm for 10 min and the serum obtained were subjected to biochemical parameters which included cholesterol, protein, bilirubin and alkaline phosphate (ALP). After blood collection, all mice were sacrificed and dissected for macroscopic organ analysis. The internal organs such as liver, heart, lungs, spleen and kidneys were carefully removed and weighed.

Statistical analysis

The student's t-test was employed to compare mean body weight between day zero and day fourteen for both control and treated groups of mice while one way analysis of variance (ANOVA) was used in multiple comparisons of the means for organ weight, hematological and biochemical data between the control and treated groups of animals. All statistical analysis was performed using STATISTICA software version 8 (StatSoft, 2007) with the level of significance set at $p< 0.05$

RESULTS

Findings from this study indicate that there were no signs of toxicity in mice of both control and treated groups up to a dose level of 5000 mg/kg body weight. All animals were normal throughout the study period and they survived until the end of the 14th day of experimentation.

Body weight changes

The body weights of the control and treated mice with aqueous extract of root barks of the plant are shown in Table 1. Results from this study revealed that there were a gradual increase in body weights of both control and treated groups of mice.

Hematological parameters

The results of hematological parameters of control and

Table 1. Body weight (g) values of control and mice treated with *M. aethiopicum* root bark aqueous extract measured during the acute toxicity study.

Dose (mg/kg BW)	Sex	Mean at day 0	Mean at day 14	Mean difference	p-value
Control	M	20.355 ± 0.078	24.67 7 ± 0.400	4.322	0.000448
	F	19.316 ± 0.065	23.416 ± 0.219	4.100	0.000057
1000	M	20.321 ± 0.047	24.217 ± 0.062	3.896	0.000001
	F	19.248 ± 0.044	23.310 ± 0.047	4.062	0.000000
2000	M	20.220 ± 0.060	24.309 ± 0.089	4.089	0.000003
	F	19.311 ± 0.145	23.288 ± 0.079	3.977	0.000018
3000	M	20.292 ± 0.115	23.738 ± 0.357	3.446	0.000782
	F	19.149 ± 0.050	23.260 ± 0.119	4.111	0.000006
4000	M	20.393 ± 0.072	24.147 ± 0.260	3.754	0.000154
	F	19.437 ± 0.100	23.502 ± 0.248	4.065	0.000108
5000	M	20.223 ± 0.052	23.643 ± 0.106	3.42	0.000008
	F	19.141 ± 0.064	23.202 ± 0.137	4.061	0.000011

Values are expressed as mean ± SEM, M = male, F = female, BW = body weight.

treated mice are shown in Table 2. These results show that there were no significant changes ($p>0.05$) in hematological parameters, both in the control and treated groups of mice after 14 days of treatment with *M. aethiopicum* root bark aqueous extract. All values of hematological parameters such as red blood cell, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, white blood cell, lymphocyte and hemoglobin concentration remained within normal limits throughout the experimental period.

Biochemical parameters

In this study, no significant changes ($p>0.05$) were observed at all doses in alkaline phosphate (ALP) and cholesterol levels between the control and treated group of mice as shown in Table 3. Furthermore, the values of protein and bilirubin did not differ significantly ($p>0.05$) in the treated mice as compared to the control group.

Macroscopic examination

The macroscopic examination of the internal organs of animals revealed no difference between the control and treated mice groups after administration even with higher dose of 5000 mg/kg body weight.

Organ weight

The organ weights relative to body weights of the animals were determined and results are summarized in Table 4. Findings from this study indicated that there were no significant differences ($p>0.05$) in weight changes of each organ between the control and treated mice at all doses.

DISCUSSION

Medicinal plants have been used worldwide for thousands of years in the form of crude drugs such as tinctures, teas, poultices, powders and other herbal formulations (Gurib, 2006). *M. aethiopicum* is among the medicinal plant which is known for many traditional applications in humans (Schmidt et al., 2002). The root bark extract has been commonly used by many populations in Africa for medicinal purposes (Burkil, 2004). Despite the usage of this plant as traditional medicine, there are few studies on the safety evaluations. Therefore, experimental screening methods using animal models are essential to ascertain the safety of this plant.

In this study, the *M. aethiopicum* root bark aqueous extract did not affect the body weight of the treatment mice as compared to the control mice. The gradual body weight gain shown by animals against the extract, provide circumstance evidence that the administration of the crude extract has negligibly level of toxicity on the growth of the animals. According to Raza et al. (2002) and Teo et al. (2002), the reduction in gain body weight is a sensitive indicator of toxicity after exposing the animals to the toxic substances and it is usually significant if such losses are more than 10% of the initial weight. Findings from this study are in agreement with previous study conducted by Ndukui et al. (2014) who reported weight gain in albino rats treated with aqueous leaf extract of *M. aethiopicum* in Uganda.

Blood parameters analysis is relevant to risk evaluation as the hematological system has a higher predictive value for toxicity in humans when assay involves animals (Olson et al., 2000). Blood is an important index of physiological and pathological status in both animals and humans and the parameters usually measured are red

Table 2. Hematological values of control and mice treated with *M. aethiopicum* root bark aqueous extract measured during the acute toxicity study.

Parameter	Sex	Dose (mg/kg BW)						
		Control	1000	2000	3000	4000	5000	p-value
RBC (m/mm^3)	M	8.099 ± 0.265	8.058 ± 0.145	7.948 ± 0.071	7.978 ± 0.234	8.311 ± 0.114	8.308 ± 0.091	0.525153
	F	7.323 ± 0.064	7.283 ± 0.015	7.250 ± 0.021	7.287 ± 0.038	7.373 ± 0.056	7.377 ± 0.187	0.864938
MCV (fl)	M	47.167 ± 0.186	46.867 ± 0.203	47.057 ± 0.030	46.860 ± 0.146	46.910 ± 0.124	47.070 ± 0.032	0.542368
	F	49.130 ± 0.214	49.083 ± 0.020	49.313 ± 0.041	48.993 ± 0.219	49.017 ± 0.284	48.700 ± 0.062	0.308154
MCH (pg)	M	18.800 ± 0.321	19.043 ± 0.159	18.233 ± 0.120	18.333 ± 0.088	18.733 ± 0.285	19.033 ± 0.176	0.073604
	F	19.000 ± 0.190	18.790 ± 0.114	19.327 ± 0.059	19.217 ± 0.164	18.893 ± 0.131	19.233 ± 0.103	0.085373
MCHC (g/dl)	M	34.600 ± 0.404	34.190 ± 0.107	34.700 ± 0.379	34.467 ± 0.186	34.303 ± 0.170	35.087 ± 0.297	0.325877
	F	33.157 ± 0.230	32.930 ± 0.268	33.293 ± 0.091	32.850 ± 0.189	33.370 ± 0.151	33.360 ± 0.064	0.249899
WBC (m/mm^3)	M	9.433 ± 0.376	8.890 ± 0.137	9.273 ± 0.057	9.303 ± 0.126	9.933 ± 0.234	9.323 ± 0.026	0.060657
	F	8.177 ± 0.127	7.943 ± 0.217	7.953 ± 0.159	8.327 ± 0.039	8.070 ± 0.192	8.253 ± 0.052	0.383882
LYM (%)	M	74.533 ± 0.338	74.300 ± 0.058	74.200 ± 0.115	75.133 ± 0.120	74.520 ± 0.321	74.907 ± 0.254	0.094817
	F	73.693 ± 0.059	73.670 ± 0.176	73.640 ± 0.052	73.653 ± 0.035	73.583 ± 0.043	73.373 ± 0.028	0.144279
Hb (g/dl)	M	14.967 ± 0.233	14.965 ± 0.120	15.167 ± 0.088	15.100 ± 0.208	14.967 ± 0.233	15.010 ± 0.116	0.942121
	F	14.390 ± 0.021	14.383 ± 0.032	14.363 ± 0.028	14.417 ± 0.034	14.413 ± 0.054	14.393 ± 0.048	0.919693

Values are expressed as mean ± SEM, M = Male, F = Female, BW = Body weight, RBC = Red blood cell, MCV = Mean cell volume, MCH = Mean cell hemoglobin, MCHC = Mean cell hemoglobin concentration, WBC = White blood cell, LYM = Lymphocyte, Hb = Hemoglobin concentration

Table 3. Clinical biochemical values of control and mice treated with *M. aethiopicum* root bark aqueous extract measured during the acute toxicity study.

Parameter	Sex	Dose (mg/kg BW)						
		Control	1000	2000	3000	4000	5000	p-value
Cholesterol (mg/dl)	M	86.975 ± 0.187	86.993 ± 0.136	87.079 ± 0.269	86.996 ± 0.092	87.019 ± 0.164	87.105 ± 0.192	0.993597
	F	88.068 ± 0.145	88.246 ± 0.309	87.742 ± 0.387	88.048 ± 0.100	88.150 ± 0.108	87.896 ± 0.995	0.974879
Total protein (g/dl)	M	5.073 ± 0.136	5.184 ± 0.052	4.883 ± 0.150	5.228 ± 0.042	4.840 ± 0.192	5.197 ± 0.278	0.428318
	F	6.191 ± 0.238	5.993 ± 0.239	6.180 ± 0.138	5.841 ± 0.157	6.412 ± 0.111	6.546 ± 0.174	0.147521
Direct bilirubin (mg/dl)	M	0.602 ± 0.036	0.588 ± 0.026	0.629 ± 0.008	0.614 ± 0.010	0.592 ± 0.019	0.622 ± 0.008	0.670220
	F	0.705 ± 0.017	0.678 ± 0.038	0.728 ± 0.015	0.682 ± 0.024	0.695 ± 0.050	0.736 ± 0.016	0.660283
ALP (U/L)	M	71.449 ± 0.410	71.777 ± 0.190	72.048 ± 0.201	71.613 ± 0.163	71.680 ± 0.154	70.855 ± 0.189	0.058343
	F	71.865 ± 0.321	71.593 ± 0.332	72.190 ± 0.053	72.155 ± 0.085	71.976 ± 0.226	72.255 ± 0.093	0.322070

Values are expressed as mean ± SEM, M = Male, F =Female, BW = Body weight, ALP = Alkaline phosphate

study has shown that acute oral ingestion of M. aethiopicum root bark aqueous extract did not lymphocyte and hemoglobin concentration (Vaghasiya et al., 2011). The normal range of these parameters can be altered by the intake of some toxic plants (Ajagbonna et al., 1999). This

Table 4. Organ-body weight values of control and mice treated with *M. aethiopicum* root bark aqueous extract measured during the acute toxicity study.

Organ	Sex	Organ-body weight index (%)						
		Dose (mg/kg BW)						
		Control	1000	2000	3000	4000	5000	p-value
Liver	M	4.658 ± 0.181	4.630 ± 0.087	4.502 ± 0.116	4.547 ± 0.219	4.534 ± 0.185	4.601 ± 0.057	0.973370
	F	4.441 ± 0.008	4.432 ± 0.005	4.413 ± 0.001	4.419 ± 0.007	4.348 ± 0.043	4.405 ± 0.015	0.055842
Heart	M	0.533 ± 0.110	0.446 ± 0.050	0.444 ± 0.062	0.411 ± 0.067	0.319 ± 0.149	0.468 ± 0.083	0.720114
	F	0.348 ± 0.002	0.341 ± 0.006	0.345 ± 0.004	0.334 ± 0.006	0.342 ± 0.005	0.343 ± 0.005	0.427091
Lungs	M	0.696 ± 0.033	0.661 ± 0.048	0.611 ± 0.011	0.598 ± 0.026	0.611 ± 0.018	0.678 ± 0.074	0.446295
	F	0.535 ± 0.004	0.535 ± 0.002	0.534 ± 0.003	0.533 ± 0.006	0.528 ± 0.003	0.535 ± 0.001	0.699071
Spleen	M	0.638 ± 0.013	0.626 ± 0.015	0.612 ± 0.026	0.630 ± 0.007	0.574 ± 0.028	0.608 ± 0.019	0.289455
	F	0.438 ± 0.008	0.434 ± 0.004	0.434 ± 0.001	0.431 ± 0.003	0.432 ± 0.004	0.421 ± 0.005	0.293800
Kidney (R)	M	0.634 ± 0.012	0.606 ± 0.006	0.625 ± 0.012	0.614 ± 0.008	0.618 ± 0.009	0.599 ± 0.007	0.166221
	F	0.543 ± 0.002	0.534 ± 0.003	0.538 ± 0.004	0.542 ± 0.003	0.537 ± 0.002	0.538 ± 0.003	0.352114
Kidney (L)	M	0.620 ± 0.013	0.559 ± 0.023	0.590 ± 0.022	0.583 ± 0.026	0.602 ± 0.026	0.570 ± 0.023	0.484740
	F	0.543 ± 0.001	0.541 ± 0.001	0.542 ± 0.001	0.542 ± 0.001	0.541 ± 0.001	0.542 ± 0.000	0.665622

Values are expressed as mean ± SEM, M = male, F = female, BW = body weight, R = right, L = left.

study has shown that acute oral ingestion of *M. aethiopicum* root bark aqueous extract did not cause any change in hematological parameters for both control and treated mice verifying the nontoxic nature of the extract. Findings from this study validate the safety nature of the extract through oral administration. This explains why there is no reported case of intoxication due to the use of this plant among the Maasai communities in Tanzania (Kokwaro, 1993).

Disease or response to toxic substances is indicated by alterations in the biochemical parameters which are the sensitive indicators of organ function or metabolic defects (Reddy et al., 2013). Liver plays a major role in the metabolism and detoxification of compounds that reach the liver and hence it serves as a prime target organ for drugs and toxic substances (Reddy et al., 2013). A liver function test such as alkaline phosphate (ALP) is therefore useful in determining the extent of damage (Shah et al., 2011). In the same perspective, liver is the major site for cholesterol synthesis or disposal in mammals (Gautam and Goel, 2014). In this study, there were no significant changes in ALP and cholesterol levels for both control and treated mice, suggesting that *M. aethiopicum* root bark aqueous extract had no effects on the liver function and cholesterol metabolism of the mice and therefore strengthen the safety utilization of the plant in traditional medicine. Safety nature of the root bark extract of the plant is also indicated by other biochemical parameters such as protein and bilirubin which did not differ significantly as compared to control group of mice. These results support the reported use of *M. aethiopicum* by traditional healers in Uganda for treating various diseases with no severe adverse effect (Ndukui et al., 2014).

Macroscopic examination of internal organs of the experimental animals in this study did not reveal any abnormalities, presence of lesions or changes in the color for both control and treated group and therefore suggest that *M. aethiopicum* root bark aqueous extract is potentially safe for human consumption. In toxicological studies, internal organs such as liver, hearts, lungs, spleen and kidneys are primarily affected by metabolic reactions caused by the toxicants (Dybing et al., 2002).

Organ weight is an important index to diagnose whether the organ was exposed to the injury or not (Jothy et al., 2011). In this study, the weights of internal organs were not statistically significantly in both control and treated group of mice, indicating that the extract is virtually nontoxic. The non-toxicity shown by *M. aethiopicum* root bark aqueous extract towards albino mice, ratify the safety profile of the aqueous root bark extract of the plant. Results from this study collaborate with the previous cytotoxicity investigation study of the same plant growing in Uganda, which did not show significant changes

in organ weight of albino rats (Ndukui et al., 2014).

Conclusion

This study presents evidence of nontoxic effects of *M. aethiopicum* root bark aqueous extract in animal models. No mortality or any sign of toxicity was observed in mice treated with the extract and therefore establishing its safety in use. The hematological and biochemical analysis showed no adverse effects between control and treated groups of mice. Furthermore, the plant extract did not induce any damage to the vital body organs and therefore considered as relatively safe for utilization especially in rural communities where conventional drugs are unaffordable due to their high costs. However, a detailed experimental analysis of sub-acute toxicity remains unveiled to complete the safety profile of this plant.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to the Government of Tanzania through the Commission for Science and Technology (COSTECH) for financial support. Herbalists from Imbibya village are appreciated for availing us ethnomedical information of medicinal plants. Last but not the least, the authors wish to thank Mr. Alfred Mwanyika of the Department of Physiology, Pharmacology and Toxicology at the Sokoine University of Agriculture for his technical support.

REFERENCES

Ajagbonna OP, Onifade KI, Suleiman U (1999). Hematological and biochemical changes in rats given extract of *Calotropis procera.* Sok. J. Vet. Sci. 1(1):36-42.

Boera HJ, Koola A, Broberg A, William R, Mziray WR, Hedberg I, Levenfors JJ (2005). Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. J. Ethnopharmacol. 96:461-469.

Burkil HM (2004). The useful plants of west tropical Africa. Publisher Royal Botanic Gardens; Kew. pp. 25-28.

Burrows JE, Willis CK (2005). Plants of the Nyika Plateau: an account of the Nyika National Parks of Malawi and Zambia. Southern African Botanical Diversity Network Report No. 31. SABONET, Pretoria.

Curtis BA, Mannheimer CA (2005). Tree Atlas of Namibia National Botanic Research Institute. Windhoek. pp. 406-407.

Dybing E, Doe J, Groten J, Kleiner JO, Brien J (2002). Hazard characterization of chemicals in food and diet: dose response, mechanism and extrapolation issues. J. Food Chem. Toxicol. 40(2):237-282.

Ndukui JG, Mugisha K, Owiny D, Waako P (2014). Effects of crude aqueous leaf extracts of *Citropsis articulata* and *Mystroxylon aethiopicum* on sex hormone levels in male albino rats. Int. J. Pharm. Sci. Inven. 3(1):5-17.

Gautam MK, Goel RK (2014). Toxicological study of *Ocimum sanctum* Linn Leaves: Hematological, biochemical and histopathological studies. J. Toxicol. 9:312-342.

Gurib-Fakim A (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol. Aspect. Med. 27(1):1-93.

Iwu MM (2014). Handbook of African medicinal plants. Second edition: CRC Press, Taylor & Francis Group. pp. 28-29.

Jothy LS, Zakaria Z, Chen Y, Ling Lau Y, Yoga Latha L, Sasidharan S (2011). Acute oral toxicity of methanolic seed extract of *Cassia fistula* in mice. Mol. 16(6):5268-5282.

Kokwaro JO (1993). Medicinal plants of East Africa. Second edition: Published and printed by Kenya literature bureau, Nairobi Kenya. pp. 176-177.

Newman DJ, Cragg GM, Snader KM (2000). The influence of natural products upon drug discovery. Nat. Prod. Rep. 17(3):215-234.

Olowa LF, Nuñeza OM (2013). Brine shrimp lethality assay of the ethanolic extracts of three selected species of medicinal plants from Iligan City, Philippines. Int. Res. J. Biol. Sci. 2(11):74-77.

Olson HG, Betton D, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Van Deun K, Smith P, Berger B, Heller A (2000). Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul. Toxicol. Pharm. 32(1):56-67.

Organization for Economic Cooperation and Development (OECD) (2001). Guidelines for testing of chemicals. Health effects - Test No. 425: Acute oral toxicity: Up-and-Down procedure. https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl425-508.pdf

Phani K, Kumar AR (2014). Toxicity studies of combined extracts of *Vitex leucoxylon*, *Vitex negundo* and *Vitex trifolia.* J. Chem. Pharm Sci. 7(1):54-58.

Pope GV (1995). Flora Zambesiaca: Mozambique, Malawi, Zambia, Zimbabwe, Botswana. Flora Zambesiaca Management Committee.

Raza M, Al-Shabanah OA, El-Hadiyah TM, Al-Majed AA (2002). Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice. J. Sci. Pharm. 70(2):135-145.

Reddy RR, Lokanatha O, Ratnam K, Reddy CS, Raju IN, Reddy CD (2013). Acute and sub-acute toxicity of *Moringa oleifera* stem bark extract in Swiss albino mice. Int. J. Sci. Biotech. Pharm. Res. 2(4):74-82.

Schmidt E, Lotter M, McCleland W (2002). Trees and shrubs of Mpumalanga and Kruger national park. Jacana Media. pp. 356-359.

Shah R, Parmar S, Bhatt P, Chandra S (2011). Evaluation of hepatoprotective activity of ethyl acetate fraction of *Tephrosia purpurea*. Pharmacology 3:188-194.

StatSoft Inc. STATISTICA (data analysis software system), version 8.0 (2007). http://www.statsoft.com/textbook

Teo SD, Stirling S, Thomas A, Kiorpes A, Vikram K (2002). A 90-day oral gavage toxicity study of D-methylphenidate and D, L methylphenidate in Sprague-dawley rats. Toxicology 179(3):183-196.

Vaghasiya YK, Shukla VJ, Chanda SV (2011). Acute oral toxicity study of *Pluchea arguta* boiss extract in mice. J. Pharmacol. Toxicol. 6(2)113-123.

World Health Organization (WHO) (2007). Department of technical cooperation for essential drugs and traditional medicine. Guidelines for assessing quality of herbal medicines with reference to contaminants and residues, Geneva, Switzerland.

Journal of
Medicinal Plant Research

Related Journals Published by Academic Journals

- *African Journal of Pharmacy and Pharmacology*
- *Journal of Dentistry and Oral Hygiene*
- *International Journal of Nursing and Midwifery*
- *Journal of Parasitology and Vector Biology*
- *Journal of Pharmacognosy and Phytotherapy*
- *Journal of Toxicology and Environmental Health Sciences*

academicJournals