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AK Azad

Department of Basic Medical Sciences, Faculty of Pharmacy, International Islamic University Malaysia (IIUM), Jalan Sultan Ahmad Shah, 25200 Indera Mahkota, Kuantan, Pahang, Malaysia.

MK Rahman

Faculty of Business and Accountancy, University of Malaya, Kuala Lumpur, Malaysia.

NK Sunzida

Gonoshasthaya Samaj Vittik Medical College, Faculty of Health Sciences, Gono University, Dhaka, Bangladesh.

Acute oral toxicity Study on Malaysian traditional herb: *Lagerstroemia speciosa* L. (Banaba)

AK Azad, MK Rahman, NK Sunzida

Abstract

To evaluate the toxicity impact of ethanol concentrates of Banaba. This study utilized as a part of *in vivo* methodologies. Harmfulness of Banaba concentrates was assessing through *in vivo* with diverse dosages. Thirty male grown-up Sprague-Dawley rats (10 weeks) measuring (180 to 200gm) separated into Control group I (Control-10% ordinary saline) and Treatment groups (500, 1000, 2000 and 3000 mg/kg), n=6 for every gathering individually. The Banaba concentrates was offered orally to every rodent and disregard at 4 and 6 hourly for any physical change. *In vivo* biochemical analysis for the treated animal' serum qualities were like control animal. Histological results demonstrated no periportal rot of the hepatocytes and no aggravation of lymphocytes and macrophages in both gatherings. No distinction was found in glomeruli or some other section of kidney tubules to that of control typical rats. Discoveries of the present study propose the Banaba are non-dangerous and suitable for further study.

Keywords: Histology, Banaba, Toxicity, LDL, HDL, Kidney, Liver

1. Introduction

Banaba (*Lagerstroemia speciosa* L., crepe myrtle) has been used as a folk medicine to treat diabetes in various parts of the world, primarily Southeast Asia. Banaba, identified scientifically as *Lagerstroemia*, is a flowering tree that is native to the Philippines and India. The Banaba plant is also well-known as the crepe myrtle and is prominent for its elegant flowers and dark bark, which sheds away in big patches throughout the year. As per Medical Health Guide report, banaba can grow up to 30 feet high, producing pink and lavender flowers and leaves that are 3 inches wide by 7 inches in length. The plant can be grown in almost any climate [1].



Fig 1: Mature Banaba tree with leaves, flowers, bark, stem and fruits

Banaba in the Tagalog name, *Lagerstroemia speciosa* L., has been used as a folk medicine for a long time among diabetics in the Philippines [2]. Extracts from Banaba leaves have been reported to reduce diabetic symptoms in genetically diabetic mice (Type II, KK-Ay). In the present study, female mice of the same strain showing remarkable body weight gain were used to examine the anti-obesity effect of dietary banaba extract [3].

Correspondence**AK Azad**

Department of Basic Medical Sciences, Faculty of Pharmacy, International Islamic University Malaysia (IIUM), Malaysia.
Email: azad2011iium@gmail.com

Banaba (*Lagerstroemia speciosa* L.) extracts have been used for many years in folk medicine to treat diabetes, with the first published research study being reported in 1940. Pure corosolic acid has been reported to decrease blood sugar levels within 60 min in human subjects. Corosolic acid also exhibits anti-hyperlipidemic, anti-oxidant, anti-inflammatory, anti-fungal, antiviral, anti-neoplastic and osteoblastic [4-5]. The *Lagerstroemia speciosa* L. Banaba) acts by increasing insulin secretion, enhancing glucose uptake by adipose and skeletal muscle tissues, inhibiting intestinal glucose absorption and inhibiting hepatic glucose production [6].

Corosolic acid (CA), contained in the leaves of the Banaba plant (*Lagerstroemia speciosa* L.), is a pentacyclic triterpene, and has hypoglycemic effects. It has some direct effects on the cholesterol absorption process in the small intestine. It may inhibit the activity of cholesterol acyltransferase, which acts in the re-esterification of cholesterol in the small intestine, in type2 diabetes [1].

Corosolic acid (CA), a constituent of Banaba leaves, has been reported to have anti-inflammatory and hypoglycemic activities. Treatment with CA lowered blood pressure, which was elevated in control animals, by 10% after 8 weeks, and serum free fatty acids by 21% after 2 weeks [7-8].

It is suggested that the hot-water extract HWE, especially, ethanol eluent of partial fraction adsorbed on HP-20 resin (HPME), obtained from Banaba leaves have beneficial effects on control of the level of plasma glucose in non-insulin dependent diabetes mellitus.

Materials and Methods

Plant material collection and authentication

The fresh and matured leaves of *Lagerstroemia speciosa* (*L. speciosa*) (15-20 cm) are collected from IIUM Kuantan Campus areas, Indera Mahkota, Kuantan-25200, Malaysia, in the month of February-March, 2012. It was authenticated by Department of Pharmaceutical Chemistry of Pharmacy Faculty of IIUM with a voucher specimen no. PIIUM:0423.

Processing and extraction of samples

The fresh leaves are collected for the microscopic analysis. For the extraction process, matured leaves of *L. speciosa* are identified, collected and shade dried up to 7-12 days; care taken to avoid direct sunlight contact⁹. Then, the leaves are

crushed and grinded into fine powder by a mixer or blender. The sieving was done repeatedly to remove the coarse parts. Defatting is done by immersing the leaf powder into petroleum ether (Pet-ether) for more than 12 hours by regular shaking and stirring. The defatted leaf powder was used for extraction. The ethanol extract was concentrated in vacuo (temperature at 45 °C, 175mbar and rotation 80-85rpm) using a rotary vacuum evaporator (BUCHI R-205) to a final corrected volume of 500 ml. This was further frozen at -70 °C and shifted instantly to three weeks successive freeze drying at -50 °C using bench top freeze dryer (ALPHA 1-4LD-2), to give a ultimate yield [10-11]

Experimental design of acute toxicity study

The experimental procedures in the present study were approved by the local ethics committee (Institutional Animal Care and Use Committee, IACUC-IIUM, IIUM/IACUC Approval/2014/ (3) (8)), Ref. No. IIUM/519/14/3/IACUC and conformed to International guidelines for the use and care of laboratory animals (OECD). The animals (SD male rats) were divided into seven groups of six animals each [12-14]. The control group received normal saline (2 mL/kg body weight p.o.) while other groups received 500, 1000, 2000 and 3000 mg/kg of the test extract orally. Immediately after dosing, the animals were observed continuously for the first 4 h for any behavioral changes [15-16]. They were then kept under observation for 14 days after drug administration to find out the mortality if any. Observations were made twice daily, one at 7 a.m. and again at 7 p.m.

Statistical Analysis

The data generated were statistically processed using SPSS 17.2 statistical software. Analysis of variance (ANOVA), with Tukey post-hoc test to identify the variable(s) with very significant differences *vis-a-vis* the control group, was used to analyse the data from the evaluation of body weight and biochemical parameters.

Results

The body weights of those animals from the day of dosing with Banaba 500, 1000, 2000 and 3000 mg/kg to before sacrifice in this present study has been shown in the Table1. It was noted that there were no significant differences between control and treatment groups.

Table1: Effects on body weight of experimental rats after treatment with ethanol extracts of Banaba at various doses for 2 weeks

Dose (mg/kg)	Day0	Day3	Day7	Day10	Day14
Control	270±4.2	286±3.8	309±3.1	328±2.5	346±4.2
500	290±2.7*	310±2.4*	335±2.3**	348±2.1**	365±2.6**
1000	263±6.3*	278±4.2*	290±3.9*	318±5.3**	350±3.6**
2000	283±3.9*	300±3.4**	322±2.5*	348±4.9*	369±4.5**
3000	256±4.5*	280±2.7**	296±3.8*	322±4.3**	356±6.2*

Significantly different from control group ($P < 0.05$, ** $P < 0.01$), $n = 6$.

Serum Biochemistry of Experimental Rats

In biochemical perceptions, Table 3 demonstrated the renal profile, lipid profile and liver profile for the treated contrasted with control creatures. It was noticed that treated creature serum glucose level was somewhat diminished contrasted with control bunch.

This present study that the ordinary treated rats indicated insignificant varieties in couple of biochemical parameters in correlation to typical control, which were measurably no contrast between the medicines creatures contrasted with controls ($p > 0.05$)

Table2: Serum urea biochemistry parameter for kidney function after 14 days repeated dose

Groups	Creatinine	Urea
Control	30.3±2	0.84±0.1
500	28.3±1.3	0.73±0.1
1000	22.4±2.2	0.70±0.09
2000	24.5±1.2	0.68±0.1
3000	23.7±1.6	0.71±0.2

*Significantly different from control group ($P<0.05$, $**P<0.01$), $n=6$

Table3: Blood biochemistry parameter for lipid function after 14 days repeated dose

Groups	Cholestrol	LDL	HDL	VLDL	TG
Control	72.5±0.5	23.2±0.3	11.5±0.7	16.8±0.3	66.6±0.9
500	69.2±0.4	27.4±0.2	12±0.5	14.7±0.3	70.5±0.7
1000	67.7±0.7	21.8±0.2	11±0.5	18±0.1	72.6±0.4
2000	70.6±0.2	26.3±0.6	10.7±0.1	16.3±0.4	65.8±0.9
3000	73±0.4	23.4±0.5	13.9±0.8	15.7±0.5	69.4±0.6

*Significantly different from control group ($P<0.05$, $**P<0.01$), $n=6$

Table4: Blood biochemistry parameter for liver function after 14 days repeated dose

Groups	ALT	AST	ALP	Albumin	Globulin	T. Prot.
Control	55.4±12.3	129.8±17.6	156.2±21.5	38.2±1.6	38.78±2.9	76.2±4.4
500	53.8±7.8	122.6±18.8	148.6±20.6	33.1±1.8	37.4±4.5	74.6±3.2
1000	55.2±6.4	125.3±16.8	155.2±12.4	36.9±1.2	38.1±1.9	75.2±5.4
2000	57.8±9.2	128.4±14.3	162.7±16	38.4±2.8	36.5±3.2	72.6±7.2
3000	53.4±11.2	123.5±18	153.6±13.6	34.6±2.4	37.7±2.7	77.6±6.5

*Significantly different from control group ($P<0.05$), $n=6$

Necropsy of Experimental Rats

In the treated groups with Banaba (500, 1000, 2000 and 3000 mg/kg/b.w) there was no critical histological modification in the glomeruli or whatever other fragment of kidney tubule taking after concentrates treatment under the present

exploratory convention. All the constituent structures of the kidney tubule in (Banaba) treated groups seemed, by all accounts, to be very much kept up. Different areas of the kidney tubules had all the earmarks of being ordinary with no progressions in mesangial grid.

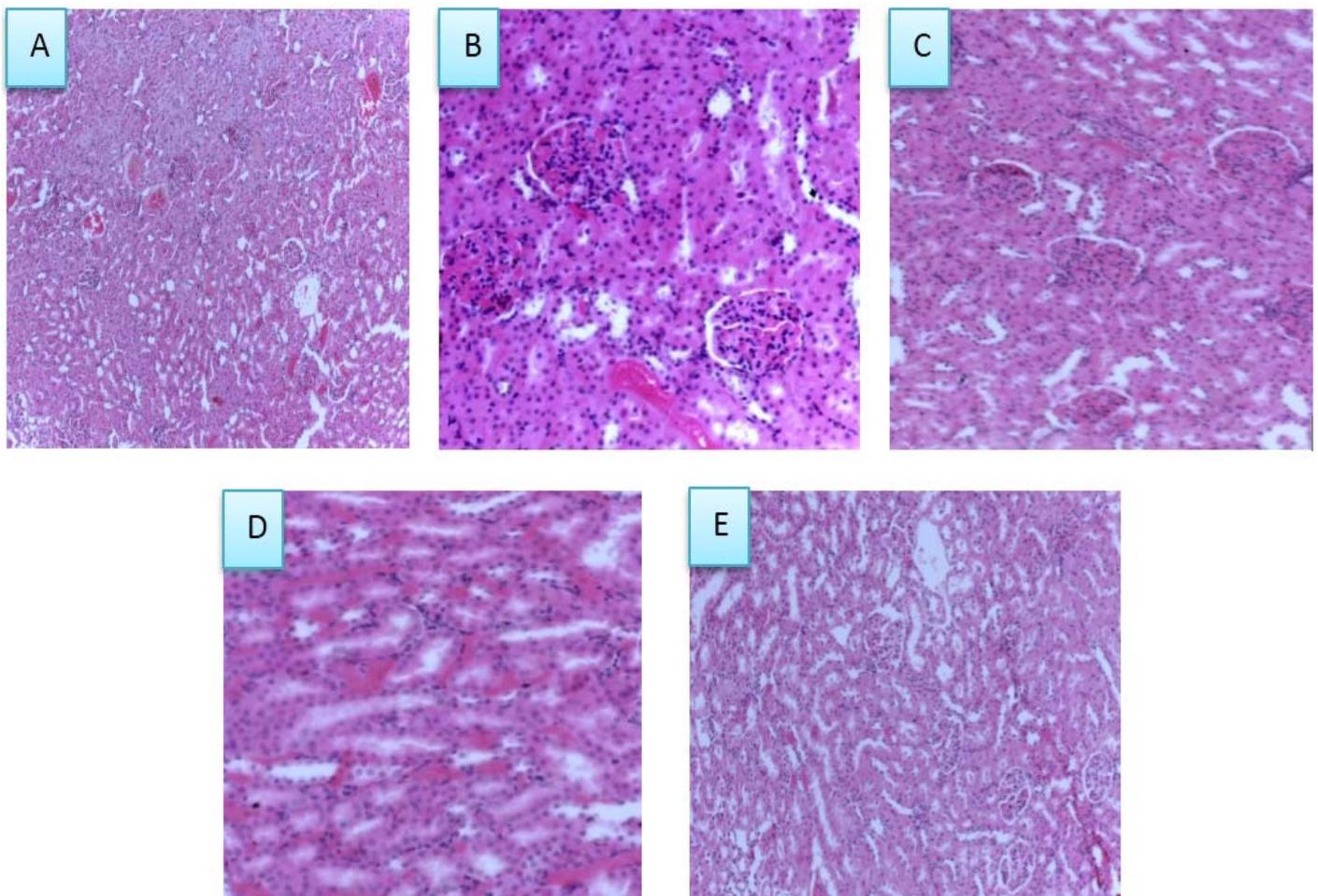


Fig 1: The Light microscopic image from the kidney of a healthy rats and ethanol extract of Banaba 500 (B), 1000 (C), 2000 (D) and 3000 (E) mg/kg treated rats.

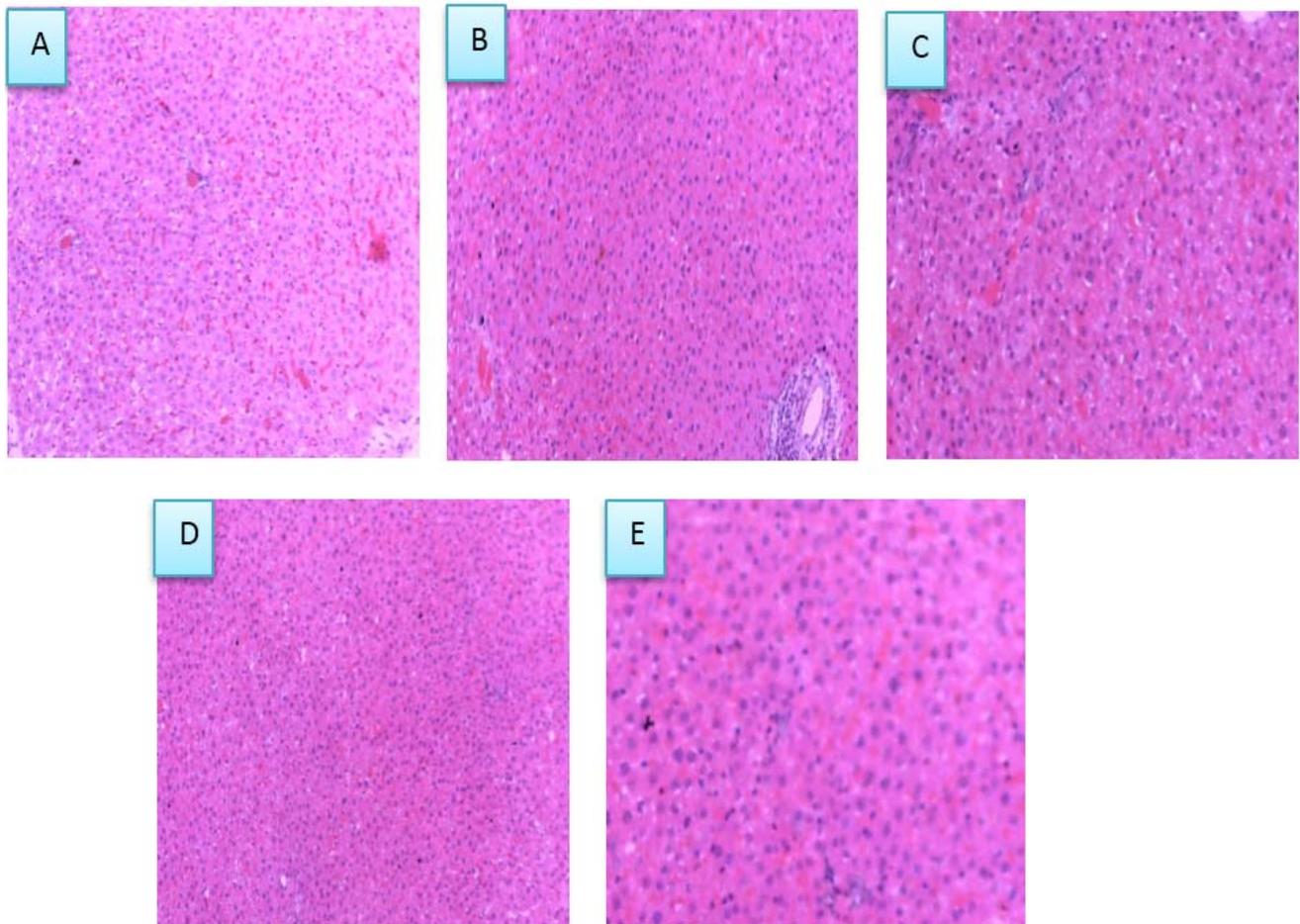


Fig 2: The Light microscopic image from the liver of a healthy rats (A) and ethanol extract of Banaba 500 (B), 1000 (C), 2000 (D) and 3000 (E) mg/kg treated rats.

The histological arrangement of liver and kidney from control rats demonstrated that the different portions of liver and kidney parts were very much saved. (Figure 1 &2). The liver tissue of the control rats were analyzed for basic changes under the light magnifying instrument utilizing hematoxylin and eosin recoloring.

The liver of control and treated gatherings rats had all the earmarks of being separated into the traditional hepatic lobules; each was shaped of ropes of hepatocytes emanating from the focal vein to the lobule's fringe. The cell strings were isolated by thin blood Sinusoids. Hepatocytes were in concentric game plan around the focal vein. The cells are vast in size with pretty much halfway set noticeable core. The hepatic cells are hexagonal in nature (Figure 2).

Discussion

In the writing there were numerous studies guaranteeing therapeutic properties of Banaba, deductively known as *Lagerstroemia speciosa*. This present studies were demonstrated the vicinity of numerous at optional metabolites which conceivably adds to the guaranteed restorative. In any case, treatment with Banaba at a given measurements is protected and not destructive to liver or kidney.

In this present study, our findings showed that the Banaba has no effect on body weight after oral administration. We consistently showed that there were neither toxic sign nor symptoms nor any significant behavioral changes related to dosing of the Banaba fruits.

Discoveries in this present learn at entire life form level were further upheld by the biochemical profile for those treated contrasted with control creature bunches which demonstrates no confirmation of renal, lipid and liver profile variation from the norm.

Keeping in mind the end goal to bolster the present biochemical discoveries, we treated creatures with Banaba (500, 1000, 2000 and 3000 mg/kg/b.w) to research for liver and kidney harmfulness impact. This present study found that, there were no huge histological changes on the liver of treated contrasted with control.

The present concentrate additionally demonstrated that there was no periportal corruption of the hepatocytes and no aggravation of lymphocytes and macrophages in both control and treated gatherings. Moreover, when contrasted control with treated, kidney of treated creature gathering did not demonstrate interruption of glomerular storm cellar film. Those discoveries give the proof that the Banaba were not dangerous to liver and kidney. In this manner we recommend that the Banaba was sheltered at entire creature level.

Conclusion

The present study suggested that the crude ethanol extract of Banaba is non-toxic and well tolerated at tested dose levels. It could be used for further experiment in animal study or cell line study.

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