Antihyperlipidemic activity of aqueous and ethanolic extracts of fruits of *Kigelia africana* (Lam.) Benth. in Triton X-100 induced hyperlipidemic rats

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Summary

The plant *Kigelia africana* (Lam.) Benth. is used in traditional medical practices of Africa and India to treat hepatic diseases. The present study is designed to evaluate the effect of *K. africana* fruit extract (KAFE) on lipid profiles in Triton X-100 induced hyperlipidemia in male wistar rats. The aqueous and alcoholic extracts of the fruits when tested for antihyperlipidemic potential, exhibited activity in albino rats when compared to standard drugs. The activity was assessed by studying the lipid profiles of serum and liver of the control and standard/extract-treated animals. The aqueous and alcoholic extract significantly increased ($p < 0.0001$) plasma High Density Lipoprotein (HDL)-cholesterol and decreased plasma Total Cholesterol (TC), Low Density Lipoprotein (LDL)-cholesterol and Triglyceride (TG) levels as compared to hyperlipidemic control.

Keywords: *Kigelia africana*, lipid profile, Triton X-100.

Introduction

Hyperlipidemia is defined as an elevation of one or more of the plasma lipids, including cholesterol, cholesterol esters, triglycerides and phospholipids. An elevation of plasma lipids may be caused by a primary genetic defect or secondary to diet, drugs or diseases. Despite of differences in lipoprotein distribution and metabolism between humans and rats, hyper-lipidemic rat models are extensively used in lipid research**.** The solution of Triton X-100 has successfully been used to induce hyperlipidemia in rats in previous studies and it was chosen as the hyperlipidemic model due to its convenience, reproducibility and availability[1].

Kigelia Africana (*Lam.*) *Benth*. is a tropical African plant widely grown and distributed in South, Central and West Africa. It belongs to the family of *Bignoniaceae* and commonly called the Sausage tree because of its huge fruits. The sausage tree has a long history of use by rural African communities especially for its medicinal properties. The fruits are believed to be a cure for a wide range of ailments including rheumatism, snakebites, evil spirits and venereal diseases like syphilis. The fruits are a popular source of traditional medicine throughout Africa and beyond. *Kigelia africana* fruit pulp and extracts have been exploited in a variety of ways; traditional/folklore, dietary/herbal supplement, cosmeceutical, nutraceutical and pharmaceutical purposes. It has strong anti-oxidative effects against hepatotoxicity induced by paracetamol toxicity. It is speculated that the antioxidant activity is attributed to the caffeic acid derivative and compounds unique to *Kigelia*. Other notable bioactivities include it's antimicrobial action against sexually transmitted diseases, Antiprotozoal activity against *Plasmodium falciparium*, *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major*, anti-amoebic activity against *E. histolytica* anti-diarrhoeal activity, anti-inflammatory/ analgesic activity and anticancer activity. The *Bignoniaceae* family is noted for the occurrence of iridoids, naphthoquinones, flavonoids, terpenes, tannins, steroids, saponins and caffeic acid in the fruits, stem, leaves and roots. The anti-oxidant actions of *Kigelia africana* have been attributed to the abundance of flavonoids and saponins in the fruits. *Kigelia africana* is reported to have low acute systemic toxicity[2].

Based on these findings and others, this work investigates the effect of administration of *Kigelia africana* fruit extract on Triton X-100 induced hyperlipidemia in wistar albino rats.

Materials and methods

Plant material

The fruits of *Kigelia africana* (Lam.) Benth. were collected from Forest Research Institute (FRI), Dehradun (Uttarakhand) campus, identified and authenticated by taxonomist Mr. S. K. Srivastava, Botanical Survey of India (BSI) Dehradun (Uttarakhand). A specimen sample of the same was preserved in the herbarium section of the Botanical Survey of India (BSI) Dehradun, with the Acc. No. 113498 for future reference.

Preparation of extracts

Alcoholic extract

Shade dried and coarsely powdered fruit (200g) was extracted exhaustively with 95% ethanol by cold percolation method (3×72 h). The solvent was distilled off over heating mantle and the extract so obtained was dried in a vacuum desiccator till free from moisture (yield: 3.78%). It was then stored in glass bottles and labeled for further use.

Aqueous extract

Shade dried and coarsely powdered fruit (100 g) was extracted exhaustively with distilled water (1 lt.) by cold percolation method (3×72 h) followed by sonication. The solvent was distilled off over boiling water bath and the extract so obtained was dried in a vacuum desiccator till free from moisture (yield: 22%). It was then stored in glass bottles and labeled for further use.

Animals

Wistar male albino rats (150–200 g) maintained in the Animal House facility of the Faculty of pharmacy, Dehradun institute of technology, Dehradun were used for the experiments. The animal house facility is registered under CPCSEA (Reg.-1156/ac/07/CPCSEA). They were housed at a room temperature of 25±2^{0}C, relative humidity of 75±5% and 12 h dark–light cycle and provided basal diet in the form of pellets supplied by M/s. Hindustan Lever Ltd., Bangalore and water *ad libitum*. Necessary permission from the Institutional Ethical Committee was obtained for the study and the experiments were conducted in accordance with the principles prescribed for laboratory animal use.

Acute toxicity test (LD50)

Animals (mice-20 – 25 g) of either sex were obtained from the Animal House of Indian Veterinary Research Institute, Bareilly (U.P.). They were randomly divided into groups. The animals were fed with mice pellets and had free access to drinking water but starved for 12 h prior to testing.

Alcoholic extract

66 mice (20 – 25 g) of either sex were randomly divided into eleven (11) groups of six (6) mice each. The first group was given distilled water (10 ml/kg) and second to eleventh, ten groups were orally administered with 500, 1000, 1500, 2000, 3000, 3500, 4000, 5000, 6000 and 8000 mg/kg of alcoholic extract, respectively. General symptoms of toxicity and mortality were observed for 24 h, for any sign of delayed toxicity and observed for 7 days more[3].

Aqueous extract

20 mice (20 – 25 g) of either sex were randomly divided into five (5) groups of four (4) mice each. The first group was given distilled water (10 ml/kg) and second to fifth, four groups were orally administered with 2000, 4000, 6000 and 8000 mg/kg of aqueous extract, respectively. General symptoms of toxicity and mortality were observed for 24 h for any sign of delayed toxicity[3].

Antihyperlipidemic activity

Rats were divided into five groups each consisting of six animals. The group I received only the vehicle, viz. water. While the group II was hyperlipedemic control- received a single dose of Triton X-100 **(**150mg /kg, I. p.). Group III was given standard drug, fenofibrate (65 mg/kg) after 72 hrs of Triton X-100 injection. Groups IV and V received a daily dose of K. africana fruits aqueous and alcoholic extracts (100mg/kg, p.o.), respectively for 7 days, after inducing hyperlipedemia i.e. after 72 hrs of Triton X-100 injection. The experiment was continued for 7 days. On the 8^{th} day, the animals were sacrificed and the blood was withdrawn by cardiac-puncture method. Serum was separated by centrifugation and refrigerated. The vital organ liver were removed quickly, washed with normal saline to remove the extraneous matter and weighed prior to storing in deep freezer. The liver was homogenized in cold 0.15M KCl and extracted with $CHCl_3$; CH_3OH (2% v/v). This serum and lipid extracts were then used for the estimation of lipid parameters[4].

Biochemical studies

The lipid profiles such as total cholesterol, triglycerides, High-density lipoprotein (HDL), Low-density lipoprotein (LDL), Very low-density lipoprotein (VLDL), of serum and liver extracts were studied by standard methods using Erva diagnostic's detection kits[5,6,7,8,9].

Statistical analysis

The results of the study were subjected to analysis of variance (one way ANOVA) followed by Dunnett's *t*-test for multiple comparisons. Values with $P < 0.05$ were considered to be significant.

Results

Effect of K.africana fruit extract on body and liver weights

After 7 days of study, the body weight of the Triton X-100 treated animals in group II increased significantly when compared to normal group ($P < 0.001$). Treatment with standard drug (fenofibrate) as well as KAFE (65 and 100 mg/kg) appreciably decreased the gain in the body weight ($P < 0.01$). The liver weight of the animals in groups I–V were 3.5±0.13, 4.8±0.12, 3.8±0.10, 3.9±0.12 and 3.7±0.16 g, respectively.

Table 1: The liver weight of animals in various treatment groups.

Group-	I	II	III	IV	V
Liver weight-	3.5 ± 0.13g	4.8 ± 0.12g	3.8 ± 0.10g	3.9 ± 0.12g	3.7 ± 0.16g

Acute toxicity test (LD_{50})

Alcoholic extract

The LD50 was estimated from a log-dose response curve (Figure 1) as 4395mg/kg. The extract was well tolerated by the animals as no signs of acute toxic effects like restlessness, dizziness or seizures were observed after the administration at 1 – 3 g/kg. However at 3.5 g/kg, the animals showed signs of toxicity like writhes and jerks, with 33% death. At 4 g/kg there was 50% death and at 6g/kg, 83% deaths. While at 8 g/kg, there was 100% death[3].

Table 2. Acute toxicity effect of the ethanolic extract of *Kigelia africana* fruits.

Treatment (mg/kg)	**Log dose**	**Percentage mortality (%)**	**Corrected % mortality**	**Probit value**
Control	--	0	-	-
500	2.698	0	-	-
1000	3.000	0	-	-
1500	3.176	0	-	-
2000	3.301	0	-	-
3000	3.477	0	4.16	3.27
3500	3.544	33	33	4.56
4000	3.602	50	50	5.00
5000	3.698	66	66	5.41
6000	3.778	83	83	5.95
8000	3.903	100	95.83	6.86

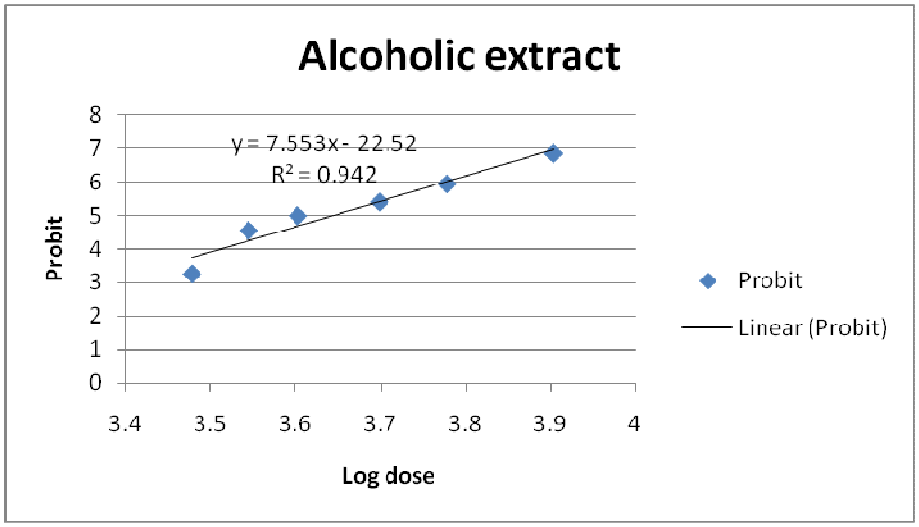

Fig 1. Log dose response curve of the ethanolic extract of *Kigelia africana* fruit.

Aqueous extract

The LD50 was estimated from a log-dose response curve (Figure 2) as 4395mg/kg. The extract was well tolerated by the animals as no signs of acute toxic effects like restlessness, dizziness or seizures were observed after the administration at 2 g/kg. However at 4 g/kg, the animals showed signs of toxicity like writhes and jerks, with 25% death. At 6 g/kg there was 75% death. While at 8 g/kg, there was 100% death[3].

Table 3. Acute toxicity effect of the aqueous extract of *Kigelia africana* fruits.

Treatment (mg/kg)	Log dose	Percentage Mortality (%)	Corrected % mortality	Probit value
Control	--	0	-	-
2000	3.301	0	6.25	3.46
4000	3.602	25	25	4.33
6000	3.778	75	75	5.67
8000	3.903	100	93.75	6.52

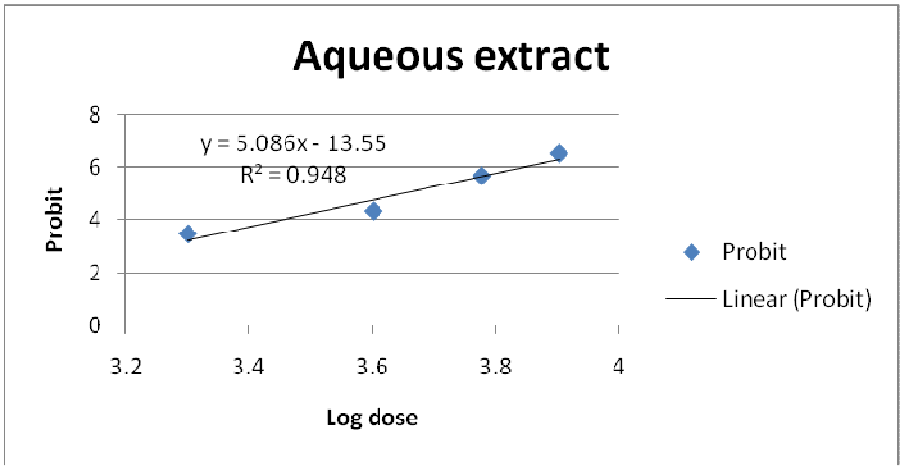

Fig 2: Log dose response curve of the aqueous extract of *Kigelia africana* fruits.

Antihyperlipidemic activity

Injection of Triton X-100 (150 mg/kg) has successfully induced hyperlipidemia in rats by increasing the serum TC, TG, LDL-c and VLDL levels. Treatment with standard drug (fenofibrate) or KAFE altered this elevation to different degrees. In animals that were treated with Triton X-100 (group II), the total lipid level was increased in serum and liver while the increase was prevented in animals that received standard drug (Table 4-5). The effect of KAFE on serum and liver lipid profile levels shown in (Table 4-5). Treatment with aqueous and ethanolic extracts of *K. africana* fruits at the doses of 100mg/kg significantly ($P < 0.0001$) reduced the serum TC, TG, LDL-c and VLDL levels and increased the serum HDL-c levels when compared to the hyperlipidemic (HL) control group. The change in lipid levels in groups of IV and V were comparable with group of fenofibrate treated rats.

The aqueous and alcoholic extracts demonstrated anti-hyperlipedemic activity and not much difference was observed in readings. The aqueous extract reduced the elevated total cholesterol level and increased HDL level more significantly ($p < 0.001$) than alcoholic extract. The alcoholic extract reduced the elevated LDL, VLDL and triglyceride level more significantly ($p < 0.001$) than the aqueous extract.

Table 4: Effect of *K. africana* fruit extracts on Blood lipid profile of Triton-induced hyperlipedemia in rats.

Group No.	Sample	T.C. ± SD	T.G. ± SD	LDL-c ± SD	HDL-c ± SD	VLDL ± SD
1.	Normal Control	70.23 ± 2.17	67.64 ± 2.01	50.04 ± 1.37	37.045 ± 9.45	14.022 ± 0.29
2.	Triton control	177.75 ± 1.263****	128.83 ± 19.56****	122.15 ± 3.46****	25.80 ± 0.56***	25.765 ± 3.91****
3.	Fenofibrate(Std.) control	55.99 ± 5.845***	52.77 ± 10.01****	46.40 ± 6.14***	52.373 ± 11.14***	10.554 ± 2.00****
4.	Aqueous extract	68.5 ± 1.767***	73.62 ± 9.63***	70.724 ± 10.41**	97.58 ± 16.433****	14.724 ± 1.926***
5.	Alcoholic extract	73.84 ± 2.107***	57.95 ± 1.909***	57.19 ± 6.745***	50.167 ± 9.27****	11.59 ± 0.38***

N = 6; **** p < 0.0001, *** p < 0.001, ** p < 0.01 *Vs* Group-2
Group-2 values were compared with the Group-1.

Table 5: Effect of *K. africana* fruit extracts on Liver lipid profile of Triton-induced hyperlipedemia in rats.

Group No.	Sample	T.C. ± SD	T.G. ± SD	LDL-c ± SD	HDL-c ± SD	VLDL ± SD
1.	Normal Control	75.60 ± 9.76	70.7 ± 3.719	26.283 ± 1.107	35.185 ± 10.67	14.135 ± 0.742
2.	Triton control	159.31 ± 11.479****	174.065 ± 15.222****	107.45 ± 5.21****	17.045 ± 1.223****	34.815 ± 5.044****
3.	Fenofibrate(Std.) control	69.066 ± 6.660***	85.68 ± 12.126***	20.94 ± 5.612***	36.78 ± 6.616***	12.27 ± 3.569***
4.	Aqueous extract	75.13 ± 8.188***	64.485 ± 3.514***	18.14 ± 5.515***	44.09 ± 3.372***	12.895 ± 0.700***
5.	Alcoholic extract	86.1 ± 7.394***	60.825 ± 1.096***	23.13 ± 5.147***	50.805 ±12.027****	12.165 ± 0.219***

N = 6; **** p < 0.0001, *** p < 0.001, ** p < 0.01 *Vs* Group-2
Group-2 values were compared with the Group-1.

Discussion and conclusion

The main causative factor for atherothrombotic diseases is the disturbances occurring in lipid metabolism. Though there are a large class of hypolipidemic drugs used in the treatment, none of the existing ones available worldwide is fully effective, absolutely safe and free from side effects [10]. Hence efforts are being made to find out safe and effective agents that may be beneficial in correcting the lipid metabolism and preventing cardiac diseases. Amongst the natural resources, medicinal plants hold promise in the discovery of new drugs. *Kigelia africana* is a drug-plant used mainly in the treatment of hepatic disorders in the Indian and African traditional medical

systems. As the traditional practitioners has used this plant to treat hyperlipidemic conditions and hence, it was considered worthwhile to investigate the claim in experimentally induced hyperlipidemia. Single administration of *K. africana* fruit extracts (KAFE) in various doses (50–400 mg/kg) did not produce any changes in the autonomic and behavioral responses in rats and no mortality was observed even after 7 days. This indicated the extract's safety and absence of toxicity in the doses studied.

A single dose of Triton X-100 (150mg/kg; I.P.) to group II animals resulted in the elevation of various parameters of lipid profile. A significant increase in the body weight was also noticed in these animals during the study period. Treatment with the standard drug fenofibrate effectively prevented the increase in body weight to a large extent ($P < 0.01$).

Biochemical assay of various lipid profiles in serum and liver of the animals revealed that KAFE reduced the elevated levels of total lipids, total cholesterol, triglycerides, LDL-c and VLDL, a significant activity was observed. This observation corroborates the use of *K. africana* fruit in the traditional practices in the treatment of hyperlipidemia.

Oral administration of *K.africana* fruit extracts (KAFE) significantly reduced the elevated lipid levels in rats possibly by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues. It is widely accepted that reduction in plasma HDL is a risk factor for developing atherosclerosis. HDL facilitates the translocation of cholesterol from the peripheral tissue, such as arterial walls to liver for catabolism. The increase in HDL may slow down the atherosclerotic process. Increased levels of HDL (cardio protective lipid) after administration of KAFE concluded that the extract is a potent cardio protective agent and this effect may be due to the increase in the activity of Lecithin: cholesterol acyl transferase (LCAT), which play a key role in incorporating the free cholesterol into HDL and transferring back to Very Low Density Lipoproteins (VLDL) or Intermediate Density Lipoproteins (IDL), which is taken back by the liver cells. Several studies show that an increase in HDL-C is associated with a decrease in coronary risk. High levels of Total Cholesterol and LDL-C are major coronary risk factors. Administration of KAFE lowered both total and LDL cholesterol in hyperlipidemic rats. This lowering of Total Cholesterol and LDL-cholesterol would reduce the incidence of coronary events[11].

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