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Investigation of antidiabetic and antilipidemic effect of fruit extract of *Spondias pinnata* (Amra) in alloxan induced hyperglycemic rats

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Abstract
Diabetes and hyperlipidemia are two major threats to human health. Importantly, these two events coexist in most of the case studies. Our objective of the study was to investigate antidiabetic and antilipidemic effect of fruit extract of *Spondias pinnata* in Alloxan induced hyperglycemic rats. Thirty six Sprague Dawley rats were divided into six groups. Thirty of them were induced diabetes by IP administration of Alloxan monohydrate at a single dose of 150 mg/kg BW. Group I, II and III were given methanol extract of fruits of *Spondias pinnata* at a daily single dose of 250 mg/kg, 500 mg/kg and 1000 mg/kg BW, respectively for 21 days. Group IV was given Metformin HCl as standard drug at a daily single dose of 100 mg/kg BW for 21 days. Group V was kept as diabetic control group. Group VI rats were not induced hyperglycemia and served as normal control group. The fasting blood glucose levels were lower in Group I, II, III and IV compared to diabetic control group. On 21st day, sacrificing the rat's blood was collected and prepared for further tests. The lipid parameters such as serum Triglycerides, Total Cholesterol, Low Density Lipoprotein and Very Low Density Lipoprotein were significantly reduced, whereas High Density Lipoprotein (HDL) level was significantly increased in Group I, II, III and IV compared to the diabetic control group. Atherogenic index and Coronary risk index were also lower in Group I, II and III compared to diabetic control group. Thus it can be claimed that this fruit has a mild antidiabetic property and a good prospect for discovery of some lead compounds against hyperlipidemia. Finally, it might be concluded that this fruit can be a good choice for the control of hyperlipidemia and thereby many other relevant consequences like diabetes and hypertension.

Keywords: Hyperglycemia, hyperlipidemia, antilipidemic, alloxan

1. Introduction
Diabetes Mellitus (DM) is a disease characterized by high blood glucose, altered carbohydrates, lipid and protein metabolism and an elevated risk of cardiovascular complications [1]. The prime reason behind Diabetes Mellitus is either absolute or relative deficiency of insulin; a hormone produced in the islets of Langerhans in pancreas [2]. Around 170 million people are suffering from DM all over the world and this number is expected to be double by 2030 [3]. Like many developing countries, prevalence of DM in Bangladesh increased alarmingly from 4% in 1990 to 10% in 2011 and is expected to reach 13% by 2030 [4]. A significant change in the composition and concentration of lipid occurs in diabetic patients. Alteration in lipid structure and metabolism also occurs in diabetes [5]. It is also related to high cardiovascular risks caused by altered metabolism of cholesterol which in turn leads to hyperlipidemia [6]. Hyperlipidemia is a metabolic anomaly specifically characterized by elevated concentration of total cholesterol (TC), serum triglycerides (TG), very low density lipoprotein cholesterol (VLDL-C), low density lipoprotein cholesterol (LDL-C) and with a concurrent decrease in the concentration of high density lipoproteins (HDL-C) in systemic circulation [7]. It increases the chance atherosclerosis and many conditions associated with atherosclerosis such as ischemic cerebrovascular disease, coronary heart disease and peripheral vascular disease [8]. According to World Health Organization (WHO) elevated serum cholesterol causes approximately 56% cases of cardiovascular complications all over the world and causes about 4.4 million deaths every year [9]. Management techniques of hyperlipidemia include physical exercise, diet control and medication therapy [10]. Though many effective lipid lowering synthetic medicines are available in the market, none is perfectly efficacious in all lipoprotein disorders and those agents are associated with various adverse drug reactions. So, it is necessary to search other medications from natural sources those are less toxic, less costly and capable to provide higher safety and efficacy on a long term usage [11]. Over last few

Over last few decades, medicinal plants were considered as a primary tool in the preservation of public health and disease management. Because, the usage of synthetic drugs produce detrimental adverse effects which are comparatively lower in drugs of natural origin e.g. plant origin [12].

2. Materials and methods

2.1 Plant materials

The fresh fruits of the plant species, *Spondias pinnata* (Family: Anacardiaceae) was selected for chemical and biological investigation.

2.2 Collection of plant materials

The fresh fruits of *Spondias pinnata* (Local name 'Amra') were collected from the local market of Savar, Dhaka, Bangladesh in February, 2016.

2.3 Preparation of plant materials

At first the collected fruits were water washed and cleaned with a brush to remove any adhering dirt. Then fruits were cut carefully and sun dried for few days. Afterwards they were dried in an oven for 24 hours at considerably low temperature for better grinding. The dried fruits were ground into coarse powder by a grinding machine in Department of Pharmacy, Jahangirnagar University.

2.4 Extraction of the fruits

The coarse powders were extracted with Methanol by Soxlet extraction apparatus. The mixtures obtained from Soxlet apparatus was recycled by a rotary evaporator under reduced pressure at 50^0C temperature to obtain the pure concentrated methanol extract.

2.5 Induction of diabetes:

Diabetes was induced by using Alloxan monohydrate at a single IP dose of 150 mg/kg BW. [13].

2.6 Standard Drug

Metformin HCl was used as standard drug at a dose of 100 mg/kg BW [14].

2.7 Experimental design

Thirty six Sprague Dawley rats were divided into six groups. Thirty of them were induced diabetes by IP administration of Alloxan monohydrate at a single dose of 150 mg/kg BW. These 30 diabetic rats were divided into 5 classes for clinical experiment. Group I, II and III were given methanol extract of fruit of *Spondias pinnata* (SPFM) at different doses.

Group I (SPFM 250): Alloxan 150 mg/kg IP + SPFM 250 mg/kg BW orally once daily.

Group II (SPFM 500): Alloxan 150 mg/kg IP + SPFM 500 mg/kg BW orally once daily.

Group III (SPFM 1000): Alloxan 150 mg/kg IP + SPFM 1000 mg/kg BW orally once daily.

Group IV (MET 100): Alloxan 150 mg/kg IP + Metformin 100 mg/kg BW orally once daily.

Group V (Diabetic Control): Alloxan 150 mg/kg IP.

Group VI: Normal control group.

Rats were given a single IP dose of Alloxan. Study was conducted for 21 days after the induction of diabetes. Metformin HCl was used as standard drug.

2.8 Determination of antidiabetic effect of methanol extract of *S. pinnata* fruit (SPFM)

Blood glucose level of all the 36 experimental rats were measured with On Call EZ II glucometer on Day 1, Day 7, Day 14 and Day 21.

2.9 Determination of Antilipidemic effect of methanol extract of *S. pinnata* fruit (SPFM)

On day 21, the rats were sacrificed and blood was collected from inferior vena cava. Ketamine (Popular Pharma, Bangladesh) was used as anesthesia before sacrificing the rats. Serum was collected by centrifuging the blood sample in 2000 rpm for 10 minutes. Effects of SPFM on serum lipid parameters such as Triglycerides (TG), Total Cholesterol (TC) and High Density Lipoprotein (HDL) compared to the diabetic control group were investigated by QCA mini Discrete Random Access Analyzer.

Serum Low Density Lipoprotein (LDL) was calculated by the following formula: [15].

LDL = TC – HDL – TG/5

Serum Very Low Density Lipoprotein (VLDL) was calculated by the following formula: [15].

VLDL = TG/5

Atherogenic index (AI) was calculated by the following formula: [16].

$$\text{Atherogenic index (AI)} = \frac{\text{LDL}-\text{cholesterol}}{\text{HDL}-\text{cholesterol}}$$

Coronary risk index (CRI) was calculated by the following formula: [16].

$$\text{Coronary Risk Index (CRI)} = \frac{\text{Total cholesterol}}{\text{HDL cholesterol}}$$

2.10 Statistical Analysis

The results were expressed as Mean ± SEM. The statistical analysis involving all six groups were carried out by one way Analysis Of Variance (ANOVA) followed by LSD test, where P value < 0.05 is considered as statistically significant.

3. Results and discussions

Hyperlipidemia is one of the major health threat now-a-days and it is highly associated with diabetes. In type-2 diabetes, serum lipid profile is elevated due to metabolic syndrome [17]. Elevated lipid level appears as a health risk and leads to atherosclerosis, MI, stroke etc. The leaf extract of *S. pinnata* shows potent Alpha-amylase and Lipase inhibitory activity [18]. So, antidiabetic and antilipidemic effects of fruit extract of *Spondias pinnata* in diabetic rats was assessed in this study.

3.1 Determination of antidiabetic effect:

Blood glucose level of all the 36 experimental rats were measured with On Call EZ II glucometer on Day 1, Day 7, Day 14 and Day 21. The result is shown as Mean±SEM in Table 1. The data was analyzed by SPSS 14 and result was obtained by one way ANOVA followed by Dunnet's multiple comparison test.

Table 1: Blood glucose level in experimental rats (Mean ± SEM).

Group	Glucose level of rats (mmol/L) (Mean ± SEM)			
	Day-1	Day-7	Day-14	Day-21
SPFM 250	18.65 ± 3.45	24.07 ± 3.62	26.17 ± 2.81	23.52 ± 2.82
SPFM 500	20.02 ± 3.36	24.42 ± 3.41	26.07 ± 3.73	21.12 ± 3.24
SPFM 1000	19.52 ± 3.64	23.68 ± 3.05	21.72 ± 3.75	20 ± 4.38
MET100	22.05 ± 2.55	23.17 ± 1.62	25.77 ± 0.48	20.08 ± 4.29
DC	20.22 ± 1.51	25.33 ± 1.48	27.17 ± 1.33	24.83 ± 0.83
NC	4.6 ± 0.22	4.45 ± 0.21	4.47 ± 0.22	4.67 ± 0.24

NC: Normal Control; DC: Diabetic Control; SPFM250: Methanol Extract of *Spondias pinnata* fruit 250 mg/kg BW; SPFM500: Methanol Extract of *S. pinnata* fruit 500 mg/kg BW; SPFM1000; Methanol extract of *S. pinnata* fruit 1000 mg/kg BW; MET100: Metformin HCl 100 mg/kg BW. Result is shown as Mean ± SEM. *p<0.05: Significant compared to DC group.

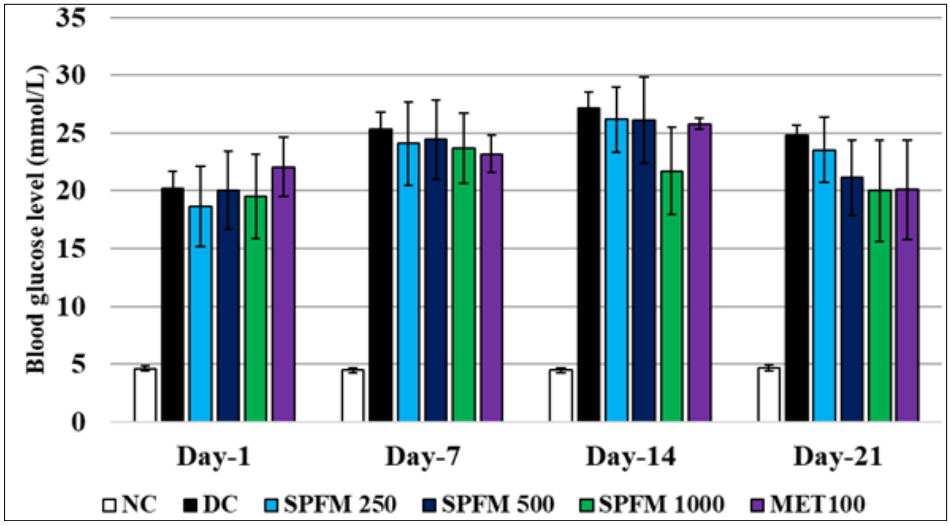

Fig 1: Blood glucose level of the experimental rats in (mmol/L).

The result shows that the blood glucose level was lower in the extract groups compared to the diabetic control group. On 21st day blood glucose level in group V was 24.83 ± 0.83mmol/L whereas that in group III was 20 ± 4.38 mmol/L. Blood glucose level in group I, II, III and IV were lower compared to group V but the glucose level is still much higher than normal level. This indicates that the plant may have a mild antidiabetic effect.

3.2 Determination of antilipidemic effect

Serum levels of all the lipid parameters were determined by QCA mini random access analyzer and the result is shown as Mean ± SEM in Table 2. The data was analyzed by SPSS 14 and result was obtained by one way ANOVA followed by LSD test.

Table 2: Lipid profile in normal rats and diabetic rats and antilipidemic effects of fruit extract of *Spondias pinnata* on those rats.

Lipid Profile	TG (mg/dL)	TC (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	AI	CRI
NC	73.67±4.44	48±2.22	27.47±1.91	5.8±1.58	14.73±0.89	0.22±0.06	1.77±0.1
DC	91.67±15.06	52.17±3.38	20.47±1.14	13.37±2.16	18.33±3.01	0.69±0.14	2.59±0.22
SPFM250	59.33±1.94*	46.5±1.59*	25.52±0.98*	9.12±1.0*	11.87±0.39**	0.36±.05	1.83±0.07*
SPFM500	65.5±2.69*	48.33±1.15	26.98±0.84**	8.25±0.73*	13.1±0.54*	0.31±0.03*	1.79±0.04*
SPFM1000	57.33±2.91*	44.5±0.89**	25.330.36*	7.7±0.69*	11.47±0.58**	0.3±0.02*	1.76±0.02**
MET100	76±2.8	45±0.58*	22.98±0.27	6.82±0.56**	15.2±0.56	0.3±0.03**	1.96±0.02*

NC: Normal Control; DC: Diabetic Control; SPFM250: Methanol Extract of *Spondias pinnata* K fruit 250 mg/kg BW; SPFM500: Methanol Extract of *S. pinnata* K fruit 500 mg/kg BW; SPFM1000; Methanol extract of *S. pinnata* K fruit 1000 mg/kg BW; MET100: Metformin HCl 100 mg/kg BW. Result is shown as Mean±SEM. *p<0.05: Significant compared to DC group. **p<0.01: Significant compared to DC group.

The result shows that lipid parameters such as Serum Triglycerides, Total Cholesterol, LDL and VLDL were Increased and HDL was decreased significantly in diabetic control group compared to normal rats. The lowering of atherogenic index and coronary risk index were significant compared to the diabetic control group. Oral administration of SPFM (250, 500 & 1000 mg/kg BW) reduced blood glucose level and significantly improved all the lipid parameters compared to the diabetic control group. Metformin HCl was used as standard drug. The antilipidemic effect of SPFM groups were better than that of Metformin group.

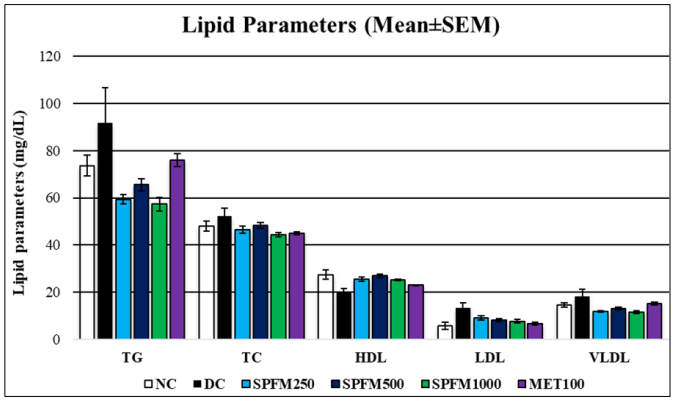

Fig 2: Antilipidemic effect of fruit extract of *Spondias pinnata* compared to the diabetic control group.

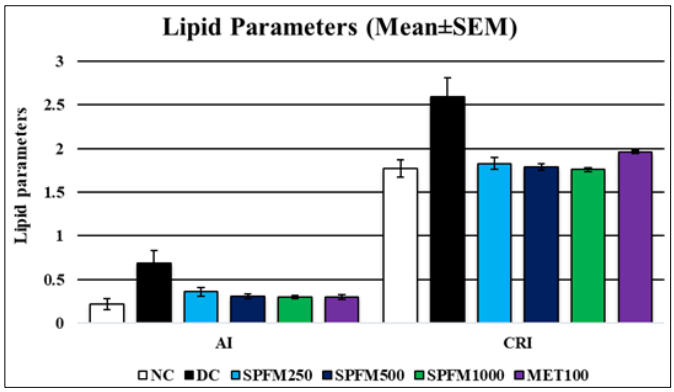

Fig 3: Effect of *Spondias pinnata* on atherogenic index and coronary risk index compared to diabetic control group.

4. Conclusion

The use of medicinal plants for the treatment of different diseases is becoming popular day by day due to the side effects of synthetic medicines. In Bangladesh, fruits of different medicinal plants such as *Coccinia indica* (Local name: Telakucha), *Momordica charantia* (Local name: Korola), *Musa sapientam* (Kola), *Phyllanthus emblica* (Amloki) etc. are being used for the treatment of diabetes [19]. *Spondias pinnata* is a well-known plant indigenous to South East Asian countries. In Bangladesh it is locally calls as "Amra". The fruit of this plant is sour and delicious. This fruit is widely consumed in Bangladesh. It can be consumed green or in cooked form. It is widely used to prepare pickles. The plant has been used intensively in many traditional herbal medicines across the globe [20]. Former studies on this plant show that different parts of this plant possess antioxidant, antimicrobial, thrombolytic [21], ulcer-protective, anti-cancer, anti-diarrheal, anthelmintic, cytotoxic, diuretic, laxative [22] anti-diabetic, nephroprotective and hepatoprotective [23] activities. The bark extract of this plant produced hypoglycemic effect whereas leaf extract possesses amylase and lipase inhibitory activity [24]. Phytochemical study of the plant shows that it contains a large amount of flavonoids, tannins, saponins and terpenoids. The result obtained from this study indicates that *Spondias pinnata* possesses antidiabetic and antilipidemic activities. The methanol extract of *S. pinnata* fruit reduced blood glucose level in Alloxan induced diabetic rats compared to diabetic control group. The lipid parameters such as Serum triglycerides, total cholesterol, LDL, VLDL were reduced significantly ($p < 0.05$) where serum HDL level was increased. Atherogenic index and Coronary risk index were also lower in the extract group compared to diabetic control group. This could provide a rationale for the use of the fruits of this plant in hyperglycemia and hyperlipidemia disorders in folk medicine. Further investigations are anticipated to identify the active components and lead to their further clinical use.

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