3. Results

TABLE 1: Effects of *Spondias mombin* Aqueous Extracts root on Blood Glucose Levels (mg/dl)

	DAYS				
Group	**0**	**7**	**14**	**21**	**28**
Normal control	**62.16 ± 14.37[a]**	**58.83±6.11[a]**	**61.33± .86[a]**	**67.33 ± 14.58[a]**	**60.83 ± 0.65[a]**
Diabetic control	**227.33 ± 17.16[c]**	**257.16± 9.12[d]**	**263.16± 29.32[d]**	**265.66 ±25.15[d]**	**206.00 ± 4.08[c]**
Standard Drug(0.6mg/bw)	**233.50 ± 28.11[c]**	**200.50± 2.53[c]**	**196.50 ± 13.18[b]**	**149.00 ± 15.38[a]**	**125.66 ± 8.15[d]**
200mg/kg	**224.33 ±19.81[b]**	**207.16± 4.72[b]**	**205.00 ± 15.46[b]**	**198.16 ±11.53[b]**	**165.50 ±04.78[a]**
400mg/kg	**229.83 ± 24.49[c]**	**231.16± 7.13[c]**	**226.50 ± 24.83[c]**	**226.16 ± 20.65[c]**	**161.00 ± 8.39 [a]**
600 mg/kg	**236.33 ±22.10[d]**	**220.00± 6.74[c]**	**216.33 ± 17.87[b]**	**215.33 ± 17.50[c]**	**186.00 ± 8.92 [c]**

Values are mean ± SD for n=6. Values in the same row bearing the same letter of the alphabets are not significantly different (P >0.05) from each other.

The result of the effect of aqueous extract of *Spondias mombin* root on blood glucose level of streptozotocin – induced diabetic rats is presented in Table 1. The result revealed that the streptozotocin significant increased (P<0.05) the blood glucose level of the animals when compared to the normal control at days 7, 14, 21 and 28. Amaryl and plant extract slightly (P<0.05) at different concentration reversed the effect at different days. However, there was significant (P>0.05) difference in the blood glucose level of the animal between the normal and Diabetic control.

Table 2: The effect of aqueous extract of *Spondias monbin* root on red blood cells and the differentials in STZ induced diabetic rats (*n* = 6, mean ± SD).

Parameters	Control group	Diabetes group	Amaryl (glimepiride) group	Extract at 200 Mg/Kg Bw	Extract at 400 mg/kg bw	Extract at 600 mg/kg bw
RBC ($\times 10^{12}$/L)	8.94 ± 0.04	7.50 ± 0.60a	8.15 ± 0.40b	8.13 ± 0.35^b	8.82 ± 1.23^b	7.65 ± 0.24
Hb (g/dL)	15.03 ± 0.06	13.43 ± 1.02a	15.33 ± 1.03b	15.10 ± 0.84^b	16.20 ± 1.97^b	12.03 ± 0.05
PCV (L/L)	0.50 ± 0.02	0.36 ± 0.03a	0.48 ± 0.02b	0.56 ± 0.02^b	0.60 ± 0.07^b	4.70 ± 0.08
MCV (fl)	55.83 ± 1.11	52.13 ± 0.38a	62.65 ± 1.02b	68.30 ± 2.52^b	68.50 ± 0.14^b	50.33 ± 0.10
MCH (pg)	16.93 ± 0.35	15.20 ± 0.40a	17.80 ± 0.46b	18.60 ± 0.69^b	18.30 ± 0.21^b	20.50 ± 0.32
MCHC (g/dL)	30.27 ± 0.82	18.23 ± 0.83a	28.50 ± 1.25b	27.30 ± 0.66^b	26.80 ± 0.35^b	31.48 ± 0.32
RCDW (%)	13.63 ± 0.90	12.43 ± 0.55a	15.13 ± 1.20b	14.50 ± 3.13^b	13.50 ± 2.47^b	10.98 ± 0.10

a: *P* <0.05 *vs* control group; b: *P* <0.05 *vs* diabetes group.

Table 3 shows the levels of serum WBC, basophils, neutrophils, eosinophils, lymphocyte and monocytes. The level of WBC was slightly increased after oral administration of the extract at 200 mg/kg while the dose of 400 and 600 mg/kg did not have any effect as compared with the diabetic groups. The plant extract significantly increased the level of lymphocyte, eosinophils, monocytes and platelet at both dosages while the best result was observed at the lower dose (200 mg/kg) (*P*<0.05). The significant effect depicted at 200 mg/kg compared favourably well whereas that of 200 mg/kg significantly boosted the level of neutrophils (Table 3). The extracts at dosages 200,400 and 600 did not have any beneficial effect on the level of basophils.

Table 3: The effect of aqueous extract of *Spondias mombin root* on white blood cells and its differentials in STZ induced diabetic rats (n = 6, mean ± SD).

Parameters	Control	Diabetes group	Amaryl (glimepiride) group	Extract at 200mg/kg bw	Extract at 400mg/kg bw	Extract at 600 mg/kg bw
WBC ($\times 10^9$/L)	17.00 ± 3.20	2.53 ± 0.93^a	6.00 ± 5.60^b	6.96 ± 2.03^b	3.16 ± 3.72^b	30.32± 0.99
Neutrophils (%)	25.37 ± 1.17	2.59 ± 0.51^a	23.30 ± 0.16^b	19.80 ± 0.01^b	40.60 ± 0.30^b	35.86 ± 0.05
Monocytes (%)	17.46 ± 6.11	4.69 ± 1.00^a	19.00 ± 0.64^b	14.70 ± 0.41^b	9.60 ± 0.01^b	33.14 ±0.78
Lymphocyte (%)	65.40 ± 6.86	5.36 ± 0.33^a	61.40 ± 5.10^b	60.90 ± 2.20^b	47.30 ± 3.10^b	29.41 ±0.16
Eosinophil (%)	5.70 ± 1.18	1.03 ± 0.78^a	1.20 ± 0.32^b	4.50 ± 0.22^b	2.50 ± 0.60^b	38.75 ±0.37
Basophils (%)	0.53 ± 0.21	0.03 ± 0.03^a	0.25 ± 0.15^b	0.10 ± 0.02^b	0.10 ± 0.03^b	8.20±0.35
Platelets ($\times 10^9$)	851.00±78.58	55.00±31.11^a	176.00±55.20^b	201.00±68.00^b	90.00±20.00^b	7.91± 0.17

a: P <0.05 *vs* control group; b: P <0.05 *vs* diabetes group.

Group	CATALASE (IU/L)	PEROXIDASE (I U/L)	THIOBARBITURIC ACID REDUCING SUBSTANCE (TBARS) (μM/L) (MDA activity)
Control	0.0074 ± 0.007	0.0081 ± 0.009	0.181 ± 0.108
Diabetic group	0.0010 ± 0.001	0.0013 ± 0.002	0.291 ± 0.013
Amaryl (glimepiride) group	0.0076 ± 0.003*	0.0096 ± 0.003*	0.125 ± 0.013 *
Extract at 200mg/kg bw	0.0061 ±0.001*	0.0074 ± 0.002 *	0.148 ± 0.098 *
Extract at 400mg/kg bw	0.0047 ± 0.006*	0.0056 ± 0.003 *	0.155 ± 0.054 *
Extract at 600mg/kg bw	0.0052 ±0.004*	0.0054 ± 0.003 *	0.165 ± 0.083 *

TABLE 4: The effect of aqueous extract of *Spondias mombin root* on *invivo* antioxidant properties in STZ induced diabetic rats

Values are the mean ± Standard deviation (SD) n=6, *The mean difference is significant the p<0.05 level from the controls group.

There was significant increase in catalase (IU/L), peroxidase (IU/L) and significant decrease in thiobarbituric acid reducing substance (TBARS) (μM/L) (MDA Activity) in animals in group 3 (three) to 6 when compared to animals in group 2

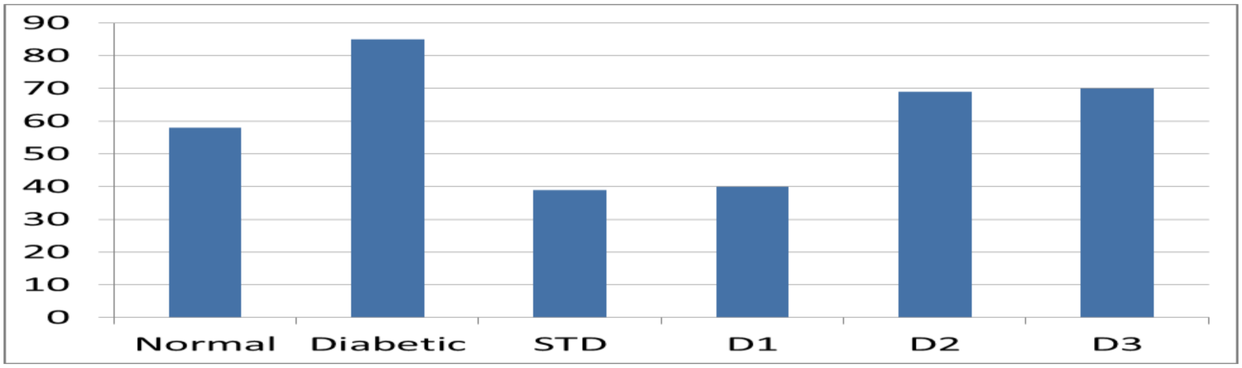

Figure 1.:**The effect of aqueous extract of *Spondias mombin* root on the feed intake of diabetic rats.**

Values are mean ± SD of 6 rats in each group. Normal: Control., Diabetic: without treatment., STD: Standard Drugs [Amaryl (glimepiride)] D1 = Diabetic + *Spondias mombin* (200 mg/kg), D2 = Diabetic + *Spondioas mombin* (400 mg/kg). D3 = Diabetic + *Spondias mombin* (600 mg/kg).

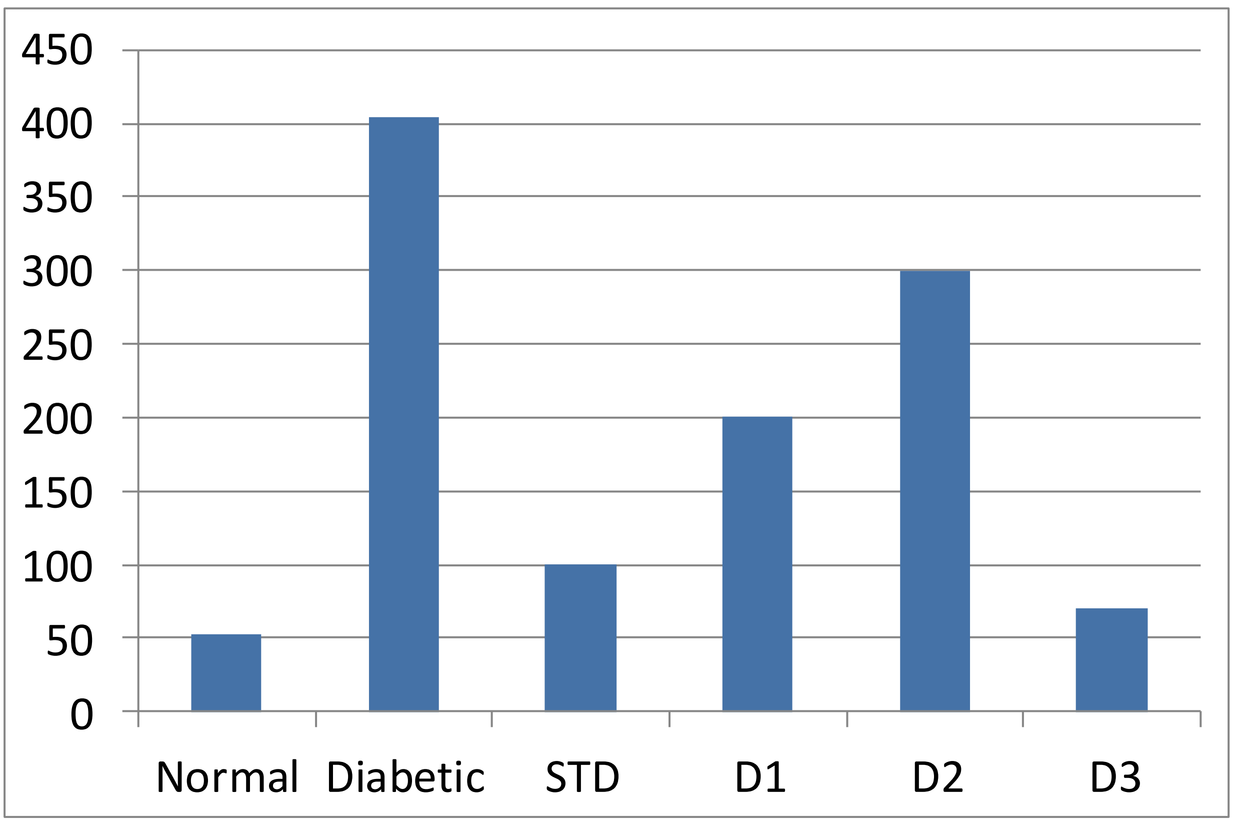

Figure 2.:**The effect of aqueous extract of *Spondias mombin root* extract on the water intake of diabetic rats.**

Values are mean ± SD of 6 rats in each group..Normal: Control., Diabetic: without treatment., STD: Standard Drugs [Amaryl (glimepiride)] D1 = Diabetic + *Spondias*

***mombin* (200 mg/kg), D2 = Diabetic + *Spondioas mombin* (400 mg/kg). D3 = Diabetic + *Spondias mombin* (600 mg/kg).**

A significant decrease in the body weights (28-33 g) of diabetic animals was observed 10 days after induction of streptozotocin into the animals. The oral administration of plant extract markedly increased the body weight of the animals but the effect was not dose related (Figure 3). The percentage increase in the body weight at 400 mg/kg was 8.82% while that of 200 mg/kg did not show any significant difference as compared with the initial body weight.

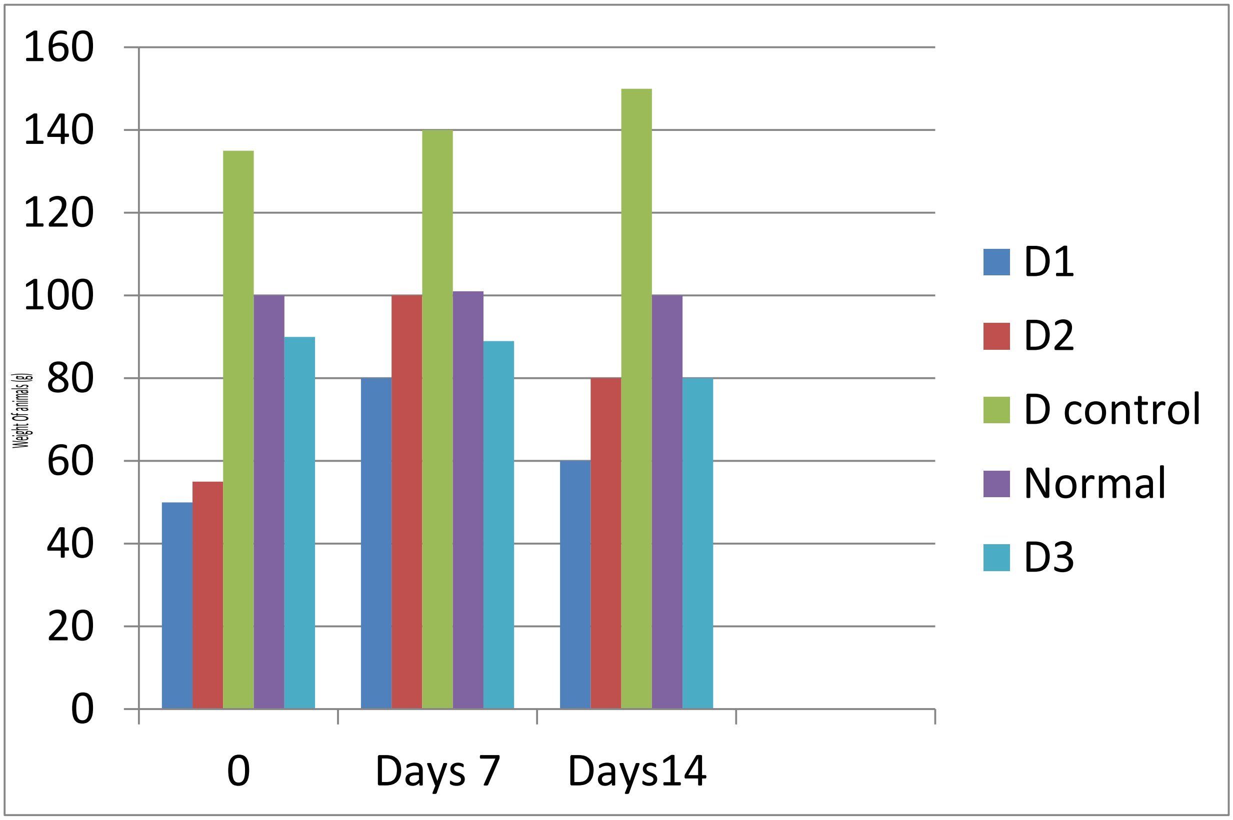

Figure 3.:The effect of aqueous extract of *Spondias mombin root* extract on the Body Weight of diabetic rats. Values are mean ± SD of 6 rats in each group.

4 Discussion

Diabetes is induced by streptozotocin (STZ), a glucosamine–nitrosourea compound derived from *Streptomyces achromogenes* that is used clinically as a chemotherapeutic agent in the treatment of pancreatic β cell carcinoma. STZ damages pancreatic β cells, resulting in hypoinsulinemia and hyperglycemia[17,18]. STZ can induce a diabetic state in 2 ways, depending on the dose. The selectivity for β cells is associated with preferential accumulation of the chemical in β cells after entry through the GLUT2 glucose transporter receptor: chemical structural similarity with glucose allows STZ to bind to this receptor. The mode of action has best been demonstrated in mouse studies. At high doses, typically given singly, STZ targets β cells by its alkylating property corresponding to that of cytotoxic nitrosourea compounds[19]. At low doses, generally given in multiple exposures, STZ elicits an immune and inflammatory reaction, presumably related with the release of glutamic acid decarboxylase autoantigens. Under this condition, the destruction of β cells and induction of the hyperglycemic state is associated with inflammatory infiltrates including lymphocytes in the pancreatic islets[20]. STZ has well-known adverse side effects, which include hepatotoxicity and nephrotoxicity [21,22].

Streptozotocin is used as an agent to induce diabetes mellitus by selective cytotoxicity effect on pancreatic beta cells. Thus it affects endogenous insulin release and as a result increases blood glucose level. The continuous administration of aqueous extract of *Spondias mombin a*queous root extract at 200,400 and 600 mg/kg or amaryl (glimepinde) for 14 days significantly reduced the blood glucose concentration in STZ induced diabetic rats. The plant extract (200,400 and 600 mg/kg) showed a comparable activity with the amaryl treated groups.

Amaryl is an oral blood sugar-lowering drug that is used to treat patients with type 2 diabetes. It contains the active ingredient glimepiride and belongs to the sulphonylurea class of diabetes medicines, which work by boosting the secretion of natural insulin and increasing the body's sensitivity to the blood sugar-regulating hormone. The probable mechanisms of action of the plant extract at higher dose could be linked to potentiation of insulin from beta cells or by increasing peripheral glucose uptake [21,22].

The assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds including plant extracts on the blood constituents of animals. They are also used to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, haematology, normal functioning and histomorphology of the organs [23,24].

The occurrence of anaemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of RBC membrane proteins [15]. Oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBC [27]. In this study, the RBC membrane lipid peroxide levels in diabetic rats were not measured. However, the red blood cells parameters such as Hb, MCHC, MCH, PCV, MCV and RCDW were studied to investigate the effect of *Spondias mombin root* extract on the anaemic status of the diabetic rats. The levels of RBC, Hb, haematocrit, LUC and MCHC in the diabetic animals were drastically reduced which may be attributed to the infections on the normal body systems. This observation agrees with report of Baskar *et al*[20] who reported antihyperglycemic activity of aqueous root extract of *Rubia cordifolia* in streptozotocin-induced diabetic rats. The alterations of these parameters are well known to cause anaemic condition in man [28]. Following plant extract administration, the level of RBC and its related indices were appreciably improved especially at 400 mg/kg. This gives an indication that the plant extract may contain some phytochemicals that can stimulate the formation or secretion of erythropoietin in the stem cells of the animals. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells [29]. The stimulation of this hormone enhances rapid synthesis of RBC which is supported by the improved level of MCH and MCHC [30]. These parameters are used mathematically to define the concentration of haemoglobin and to suggest the restoration of oxygen carrying capacity of the blood. Though, the action mechanism of this plant is not investigated in this study. However, it may be attributed to the ability of plant extract to lower lipid peroxidation level that causes haemolysis of erythrocytes [31]. Previous study on this plant revealed the presence of flavonoids, proanthocyanidins, tannins, phenols and flavonols in this plant. These compounds have been reported to possess strong antioxidant capacity[32], therefore, could inhibit peroxidation of polyunsaturated fatty acids in the cell membrane and haemolysis of red blood cells in the diabetic animals reported by Torell and Faure *et al* [32]. Streptozotocin is a well known chemical that suppresses the immune system by damaging WBC and certain organs in the body [9]. The intraperitoneal injection of streptozotocin into rats significantly reduced the WBC count and its differentials such as basophils, monocytes, eosinophils, lymphocytes and neutrophils. The reduction of these parameters could be linked to suppression of leucocytosis from the bone marrow which may account for poor defensive mechanisms against infection [33]. Consequentially, they might have effects on the immune system and phagocytic activity of the animals [32]. The white blood counts and its related indices were significantly restored to near normal after plant extract administration at both doses. The presence of some phytochemicals with ability to stimulate the production of white blood count in the extract could be responsible for the observed result in the treated rats [34]. The extract at both dosages significantly improved the levels of WBC, monocytes, lymphocytes, eosinophils and neutrophils as compared with glibenclamide treated group. However, the extract did not have any significant effect on basophils in this study.

Platelet aggregation ability has been shown in diabetic patient with long term poor glycaemic control due to lack or deficiency of insulin [35]. Platelets known as thrombocytes help to mediate blood clotting, which is a meshwork of fibrin fibres. The fibres adhere to any vascular opening and thus prevent further blood clot. It plays a crucial role in reducing blood loss and repairing of vascular injury[36]. The reduction of platelets levels in diabetic rats induced with streptozotocin was confirmed in this study in relation to the normal control rats. Long term reduction of this parameter may result in internal and external haemorrhage and finally leads to death. However, after plant extract administration, the level of platelet was improved markedly especially at the dose of 200 mg/kg while that of 400 mg/kg did not have strong effect as compared with diabetic untreated rats. This effect indicated the ability of the plant extract to stimulate the biosynthesis of clotting factors[23] due to the presence of active compounds that might help to precipitate blood coagulation or clotting, especially during severe bleeding or haemorrhage [32].

Also in this work there was increased levels of peroxidases, catalases and decrease level of thiobarbyturic acid reducing substances (levels of MDA) at $p < 0.05$ when compared to the control groups. The increase in free radicals as described by (Kim *et al., [37*] is associated with oxidative processes. The feed and water intake of the diabetic rats were significantly increased as compared with the normal rats. These symptoms are well known markers of type 2 diabetes in both human and animal models which are direct consequence of insulin deficiency[1]. The feed intake was significantly reduced after administration of *Spondias mombin* root extract. The dose of 200 mg/kg showed higher activity than 400 mg/kg which was also reflected in water intake. The water intake of diabetic animals was significantly higher than the diabetic treated rats. The dose of 200 mg/kg was significantly lower than the group treated with 200 mg/kg. These results were similar to the report of Oyeluma *et al.*, [19,37] who demonstrated the effect of *leonotis heorman* in controlling the desire for food and water intake under diabetic condition. A significant decrease in the body weights (28-33 g) of diabetic animals was observed 10 days after induction of diabetes in the animals. The loss in the body weight of the animals agrees with the finding of Oyedemi *et al.* [19] who observed similar effect on streptozotocin-induced diabetic animals. This reduction has been linked to degradation of structural proteins and muscle wasting. Oral administration of plant extract at all doses was able to improve the body weight of the animals. The result indicated that extract of *Spondias mombin* root extract possessed the ability of managing glucose level as well as controlling muscle wasting and induced adipogenesis[21].

Conclusion

From the data obtained in the present study we can conclude that the *Spondias mombin* root extract possesses antihyperglycemic properties. In addition, the extract could prevent various compications of diabetes as well as improving some haematological parameters. The study also demonstrated improvement in the *in vivo* antioxidant property of *Spondias mombin* root extract showed marked increase in the levels of catalase and peroxidases and a decrease in the levels of Thiobarbituric acid reactive substances (the malondialdehyde levels). Further experimental investigation is also needed to exploit its relevant therapeutic effect to substantiate its ethnomedicinal usage.

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