ANTI-DIABETIC POTENTIAL OF *HYPHAENE THEBAICA* FRUIT IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

MUHAMMAD MUBARAK DAHIRU[1, 2 *], MARGRET SAMUEL NADRO [1]

[1] Department of Biochemistry, Modibbo Adama University, Yola, Nigeria.
[2] Department of Science Laboratory Technology, Adamawa State Polytechnic Yola. Nigeria.
* Department of Science Laboratory Technology, Adamawa State Polytechnic Yola. Nigeria. mubaraq93@gmail.com. +2348036508768.

Abstract

Complications of Diabetes mellitus caused by persistent hyperglycemia is a challenge in management of the disease as synthetic drugs used are often expensive, specific and associated with side effects. This study was aimed to determine the anti-diabetic potential of mesocarp extract of *Hyphaene thebaica* fruit in streptozotocin-induced Diabetic rats. The hypoglycemic effects of methanol, aqueous and ethylacetate extract of *Hyphaene thebaica* fruit oral glucose tolerance test (OGTT) were determined followed by the effects of the ethylacetate extract on body weight, blood glucose, liver and kidney. Glucose was fed to rats the 30 minutes after pretreatment with extract, followed by measurement of blood glucose levels at 0, 30, 60, 90, 120, and 240 minutes to assess the effect of the extracts on blood glucose levels of the glucose loaded animals. The rats were also divided into six groups and treated ethylacetate extract daily by intragastric tube for four weeks. The result revealed the ethylacetate extract was more hypoglycemic in OGTT than the other extracts. There was a significant ($p < 0.05$) improvement in body weight of rats administered ethylacetate extract at 400 mg and 200 mg/kg body weight with significant ($p < 0.05$) decrease in blood glucose, AST, GGT, creatinine, and urea levels. Conclusively, *H. thebaica* can be used in the management of diabetes evident to its hypoglycemic effects and significant improvement in weight and hepato-renal function. **Key words –** anti-diabetic, *Hyphaene thebaica*, streptozotocin, mayobelwa.

INTRODUCTION

The term Diabetes describes a group of metabolic disorders characterized and identified by the presence of hyperglycemia in the absence of treatment. The heterogeneous aetio-pathology includes defects in insulin secretion, insulin action, or both, and disturbances of carbohydrate, fat and protein metabolism, American Diabetes Association [ADA] (2021a). The long-term specific effects of diabetes include retinopathy, nephropathy and neuropathy, among other complications, World Health Organization [WHO] (2019). Insulin directs the uptake of glucose by cells of the body specifically muscles and adipose tissues. However in its absence the cells are unable to take up glucose and utilize it properly (Victor *et al.*, 2018). In the other scenario where there is resistance, insulin is available, however the tissues are insensitive to its action.

Diabetes mellitus is diagnosed through four important tests; measurement of fasting plasma glucose, 2-hour post-load plasma glucose after a 75g oral glucose tolerance test (OGTT), glycosylated hemoglobin (HbA1c), and a random blood glucose test in the presence of signs and symptoms of diabetes. People with fasting plasma glucose values of ≥ 126 mg/dl, 2 h post-load plasma glucose ≥ 200 mg/dl, HbA1c ≥ 6.5%; or a random blood glucose ≥ 200 mg/ dl in the presence of signs and symptoms are considered diabetic (WHO, 2019).

Worldwide, an estimation 9.3% of adults aged between 20-79 years, which is a staggering 463 million people are living with diabetes globally, which is expected to rise to 578 million in 2030, International Diabetes Federation [IDF] (2019). A hyperglycemic level of 180mg/dl is considered diabetic, often associated with symptoms such as polyuria, polydipsia etc. (Vasudevan *et al.*, 2018). Acute complications of diabetes include keto acidosis which is common in type 1 diabetes, hyperosmolar nonketotic coma and lactic acidosis.

There are different strategies for management/treatments of diabetes depending on the type of diabetes, however diet and exercise are often recommended (ADA, 2021b). Synthetic drugs of different types are also used in lowering insulin demand, stimulation of endogenous insulin secretion, enhancing insulin action at the target cells and the inhibition of degradation of oligo and disaccharides (Mohd *et al.*, 2017). Some of the drugs used in conventional management of diabetes targets different enzymes through inhibition of their action. α-amylase and glucosidase which break down disaccharides and oligosaccharides to monosaccharide, serves as a target of inhibition by anti-hyperglycemic drugs (Mohd *et al.*, 2017). The overall function of these drugs is to delay the breakdown of carbohydrate, thus absorption during digestion there by preventing high level of blood glucose.

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The side effects associated with the use of synthetic anti-diabetic drugs and complications of diabetes mellitus led to prospects into plant medicinal sources which are natural, less expensive and effective in management of complications of diabetes such as oxidative stress. The medicinal importance of plants in management of diseases is due to their high antioxidant properties contributed by their phytochemical constituents. Phytochemicals from plants such as anti-inflammatory substances, constitutes the bioactive substances of different efficacy used in disease managements (Mengjie *et al.,* 2021). Some of the medicinal plants used in the management of diabetes include *Coccinia indica, Moringa oleifera, Eugenia jambolana, Tinospora cordifolia, Zingiber officinale, Aegle marmelos, Cinnamomum tamala, Trichosanthes cucumerina, Leptadania hastata, Anisopus mannii, Opium sanctum, Hyphaene thebaica* etc. (Han *et al.,* 2019).

Hyphaene thebaica (Doum palm fruit) is a desert palm tree with edible oval fruit native to the Nile valley, which is a member of the palm family, Arecaceae, and a source of potent antioxidants (Ghada *et al.,* 2020). The fruit contains flavonoids (quercetin, hesperetin and naringin), steroids, terpenes and tannins, carbohydrates, cardiac glycosides, terpenes and terpenoids besides various metals (Hossam *et al.,* 2018). Doum palm fruit have significant antimicrobial activities which were attributed to the presence of flavonoids. Also, the aqueous extract of doum fruits showed an antioxidant activity; this is due to the substantial amount of their water-soluble phenolic contents (Ghada *et al.,* 2020). *Hyphaene thebaica* fruit also have hypolipidemic activity as the administration of the fruit decoction significantly lowers blood cholesterol, glucose, triglycerides and total lipids (Bayad, 2016). Thus, this study considered the use of *H. thebaica* fruit for its antidiabetic potential, as it is applied in traditional management of diabetes.

MATERIALS AND METHOD

MATERIALS

Plant material

Hyphaene thebaica fruit was collected from Mayo-belwa Local Government Area of Adamawa state, Nigeria. Mayo-Belwa has a latitude of 9°3'10.38"N and a longitude of 12°3'27.17"E (Google, n.d). The plant was authenticated by a Botanist with the Department of Plant Sciences, Modibbo Adama University, Yola. The fruit pulp was dried and the mesocarp was removed and grounded into powder using mortar and pestle.

Drugs

Streptozotocin (STZ): Powder (InvivoChem, USA), was used for induction of diabetes.

Metformin**:** Diabetmin® tablets (Hovid Pharmaceuticals Ltd, Nigeria) was used as a reference drug.

Chemical and reagents

Methanol, Chloroform and Ethyl acetate. All other chemicals and reagents were of AnarlaR.

Equipment

Incubator/oven: UNISCOPE SM9053, Portable glucometer: SD CodeFree™ (SD Biosensor, Inc., Korea), Centrifuge [Spectrafuge™ (Corning Life Sciences, USA)].

Experimental Animals

Male Wistar albino rats weighing 172 g ±10 were used for the study. The rats were obtained from the Animal house of Gombe State University, Nigeria. They were maintained under standard condition of light (12-hour light) and fed standard diet (Finisher pellet, ECWA feed Nigeria Ltd, Jos) and water *ad libitum.* All animal experimental procedures were conducted according to the ethical guidelines of the National Committee for Research Ethics in Science and Technology (NENT) 2018, (NNREC, 2019).

METHODS

Extract Preparation

H. thebaica fruit pulp powder (500 g) was macerated with 1.5 L of distilled water, methanol and ethylacetate in a glass jar for 2 days at room temperature. The extract was filtered, concentrated to dryness in Oven (Trease and Evans, 2009).

Induction of diabetes

The rats were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of STZ (50 mg/Kg body weight) in normal saline. Rats with blood glucose levels >200 mg/dl were considered diabetic (Al-Hariri, 2012).

Oral glucose tolerance test (OGTT)

The Oral Glucose Tolerance Test (OGTT) was performed on STZ-induced diabetic rats. Extracts (200 and 400 mg/Kg body weight) and Metformin (80 mg/Kg body weight) were administered to the groups of rats, respectively. Glucose (2 g/Kg body weight) was fed to rats 30 minutes after pretreatment. Blood glucose levels were measured at 0, 30, 60, 90, 120, and 240 minutes after glucose load to assess the effect of the extract on blood glucose levels of the glucose loaded animals. The blood glucose level was measured using blood glucose test strips and Glucometer.

Experimental design

The rats were divided into six groups and treated with ethylacetate extract daily by intragastric tube for four weeks.

Group 1: Normal (control group).

Group 2: Normal rats treated with 400 mg/kg body weight of ethylacetate extract of *H. thebaica* fruit.

Group 3: Rats injected with STZ without treatment (STZ-induced diabetic group).

Group 4*:* STZ-induced diabetic rat treated with 80 mg/kg body weight Metformin.

Group 5: STZ-induced diabetic rats treated with 400 mg/Kg body weight of ethylacetate extract of *H. thebaica* fruit.

Group 6: STZ-induced diabetic rats treated with 200 mg/Kg body weight ethylacetate extract of *H. thebaica* fruit.

The blood glucose level was measured by Glucometer initially and after the end of treatment whereas body weight was recorded on 0, 7, 14, 21 and 28 day of the study.

Biochemical assay

Blood samples were collected from the heart through cardiac puncture and centrifuge at 3000 rpm for 15 minutes to separate serum from cells to analyze for biochemical parameters.

Determination of Blood glucose

Blood glucose was determined according to (Richardson, 1977).

Determination of Aspartate aminotransferase (AST)

AST was determined by spectrophotometric method as described previously (Reitman and Frankel, 1957).

Determination of Gamma Glutamyl Transpeptidase (GGT)

GGT was determined by spectrophotometric method as described previously (Szasz, 1969).

Determination of Albumin concentration

Albumin was determined by spectrophotometric method as described previously (Grant *et al.*, 1987).

Determination of Urea concentration

Urea concentration was determined by spectrophotometric method as described previously (Chaney and Marbach, 1962).

Determination of Creatinine concentration

Creatinine concentration was determined by spectrophotometric method as described previously (Bartels and Bohmer, 1972).

Statistical analysis

Data was expressed as mean ± standard error of mean (± SEM). Differences among treatment group means were assessed by One-way analysis of variance (ANOVA) followed by Tukey multiple comparison test. Group means were considered to be significantly different at $p < 0.05$. Data was statistically evaluated using Statistical Package for the Social Sciences (SPSS) version 22 Software.

RESULTS AND DISCUSSION

The Hypoglycemic effect of the aqueous, methanol and ethylacetate extract of *H. thebaica* fruit Oral glucose tolerance test (OGTT) in STZ-induced diabetic rats is presented in Table 1. The result revealed a significant difference ($p < 0.05$) between all the extracts and the normal control. The methanol and aqueous extract (200 mg/Kg b.wt.) were significantly higher ($p < 0.05$) than the standard control (Metformin), with a significant difference ($p < 0.05$) between the ethylacetate extract (400 mg/Kg b.wt.) and methanol extract (200 mg/Kg b.wt.). The ethylacetate extract (400 mg/Kg b.wt.) showed a significant decrease ($p < 0.05$) in blood glucose level after oral administration of glucose to diabetic rats compared to all other extracts.

Table 1: Hypoglycemic effects of the Methanol, Aqueous, and Ethylacetate extracts of *H. thebaica* Fruit OGTT in STZ-induced Diabetic rats after 2 g/Kg b. wt. glucose load.

Groups (Treatments Per Kg b. wt.)		Glucose concentration (mg/dl) Time after glucose administration in Minutes 0	30	60	90	120	240
N.C		103.8 ±3.1	156.6 ±5.8	136.8 ±7.7	117.0 ±4.6	123.3 ±5.6	129.6 ±8.1
D.C		441.2 ±6.8^{a}	514.1 ±7.5^{a}	543.6 ±6.3^{a}	550.4 ±4.4^{a}	576.8 ±6.6^{a}	581.6 ±7.2^{a}
S.C	(80 mg)	443.2 ±3.5^{a}	468.0 ±8.1^{ace}	421.0 ±5.2^{ac}	373.0 ±6.1^{ac}	344.8 ±4.3^{ac}	321.4 ±9.3^{ac}
M.E	(400 mg)	428.0 ±6.1^{a}	484.4 ±7.1^{ace}	468.2 ±6.1^{acdef}	432.0 ±6.3^{acdef}	411.3 ±5.1^{acde}	381.6 ±7.9^{acde}
	(200 mg)	433.8 ±6.8^{a}	489.6 ±5.6^{acde}	476.4 ±5.8^{acdef}	450.8 ±5.2^{acdef}	424.8 ±6.2^{acde}	398.4 ±8.6^{acde}
E.E	(400 mg)	440.6 ±5.6^{a}	426.6 ±6.4^{ac}	414.0 ±5.9^{ac}	401.4 ±5.3^{acd}	368.1 ±7.4^{acd}	328.8 ±6.1^{ac}
	(200 mg)	429.2 ±7.1^{a}	415.8 ±6.9^{ac}	409.8 ±6.1^{ac}	402.0 ±5.7^{acd}	392.4 ±6.5^{acd}	355.6 ±7.8^{acd}
A.E	(400 mg)	435.6 ±6.6^{a}	465.2 ±6.1^{ace}	419.4 ±6.7^{ac}	408.6 ±5.5^{ac}	420.7 ±6.8^{acde}	401.8 ±8.8^{acde}
	(200 mg)	438.2 ±5.8^{a}	468.6 ±7.1^{ace}	431.8 ±8.1^{ac}	411.0 ±6.4^{ac}	432.9 ±4.4^{acde}	408.8 ±9.5^{acde}

N.G = Normal control (non-diabetic).
D.C = Diabetic control
S.C = Standard control [(Metformin) diabetic + Metformin)].
M.E = Methanol Extract (diabetic + Extract per Kg b. wt.).
E.E = Ethylacetate Extract (diabetic + Extract per Kg b. wt.).
A.E = Aqueous Extract (diabetic + Extract per Kg b. wt.)
Values are express as mean ± SEM: n = 5.
All values in the same column with ^{a} superscript were significantly ($p < 0.05$) higher than Normal control.
Values in the same column with ^{c} superscript were significantly ($p < 0.05$) lower than Diabetic control.
Values in the same column with ^{d} superscript were significantly ($p < 0.05$) higher than Standard control.
Values in the same column with ^{e} superscript were significantly ($p < 0.05$) higher than E.E.
Values in the same column with ^{f} superscript were significantly ($p < 0.05$) higher than A.E.

Ethylacetate mesocarp extract of *H. thebaica* fruit prevented the early spike in blood glucose observed in the other extracts during the early hours of glucose administration and continuously decreased the blood glucose level after 240 minutes following oral administration glucose. Hyperglycemia is considered a challenge in the management of diabetes coupled with the complications it manifest when persistent. One of methods of preventing hyperglycemia especially post-prandial hyperglycemia is through the inhibition of the key enzymes (α-amylase and α-glucosidase) that degrade carbohydrate, thereby decreasing the influx of glucose into the blood (Mohammed *et al.,* 2013). The ability of the Ethylacetate extract to prevent the hyperglycemia after oral glucose load might be due to the ability of the extract to inhibit the enzymes involved in the digestion of carbohydrates (Eman *et al.,* 2019).

Bayad (2016), reported oral administration of aqueous extracts of *H. thebaica* fruits significantly decreased blood glucose levels after 1 and 2 months. Salah *et al.,* (2011) reported the administration of flavonoids extracts to diabetic rats significantly increased adiponectin levels which stimulate the hypoglycemic action of insulin without altering the concentration of insulin in blood. The high hypoglycemic effect of Ethylacetate mesocarp extract might be due the high amount of flavonoids present in the extract. The flavonoid Luteolin inhibits alpha-glucosidase and alpha-amylase, resulting in the decrease of postprandial hyperglycemia, which decrease rise in blood glucose and significantly decreases blood glycosylated hemoglobin levels (Salib *et al.,* 2013). This might thus, contribute the hypoglycemic effect of the Ethylacetate mesocarp extract of *H. thebaica*, which contains high amount of flavonoid.

Inhibition of carbohydrate hydrolyzing enzymes are a therapeutic approach to decrease hyperglycemia (Soeng *et al.,* 2015). Inhibition of glucosidase activity regulates blood sugar level by postponing sugar breakdown (Yin *et al.,* 2014). Auwal *et al.,* (2012) reported oral administration of aqueous mesocarp extract of *H. thebaica* experimentally caused a significant decrease in blood glucose level in wistar albino rats, at 12 to 18 h post administration. Shehu *et al.,* (2014) observed a dose dependent reduction in fasting blood glucose of diabetic rats with a dose administration of 400 mg/dl methanol extract of *H. thebaica* fruit having the highest (28.4%) decrease which was almost 2 folds higher than that observed in glibenclamide treated group. The present study correlated with the reports by Auwal *et al.,* (2012), Shehu *et al.,* (2014), and Bayad (2016).

Table 2 show the effect of ethylacetate extract of *H. thebaica* fruit on the body weight of STZ-induced diabetic rats. There was a significant ($p < 0.05$) change in the body weight of all the groups after three weeks. At the end of week four, the normal (8.1%) and baseline (8.0%) control showed a significantly ($p < 0.05$) increased body weight than the negative control (-20.5%). A significant ($p < 0.05$) increase in the weight of diabetic rats in groups 5 (400 mg/Kg b. wt.) and 6 (200 mg/Kg b. wt.) were

observed from week three which were up to 9.6% and 7.9% respectively after week four were significantly ($p < 0.05$) higher than the negative control.

Table 2: Effect of Ethylacetate extract of *H. thebaica* Fruit on the body weight of STZ-induced Diabetic rats.

Groups (Treatments per Kg b. wt.)	Body weight (g)					Change (%)
	Weeks					
	0	One	Two	Three	Four	
1 (Normal)	172 ±8.6	174 ±7.1	178 ±5.3	181 ±6.0^{c}	186 ±4.6^{c}	+ 8.1
2 (Normal + 400 mg extract)	174 ±3.2	175 ±2.5	177 ±5.5	183 ±8.6^{c}	188 ±6.1^{c}	+ 8.0
3 (Diabetic without treatment)	176 ±4.8	171 ±5.1	167 ±7.8	146 ±6.2	140 ±5.6	- 20.5
4 (Diabetic + 80 mg Metformin)	180 ±3.7	182 ±4.1	180 ±6.9	178 ±7.1^{c}	184 ±6.2^{c}	+ 2.2
5 (Diabetic + 400 mg extract)	177 ±6.3	181 ±4.1	184 ±6.7	187 ±6.1^{c}	192 ±3.4^{c}	+ 9.6
6 (Diabetic + 200 mg extract)	177 ±6.4	182 ±4.3	183 ±3.5	185 ±3.5^{c}	191 ±4.5^{c}	+ 7.9

Values are express as mean ± SEM: n = 5.

Values with ^{c} superscript in the same column were significantly ($p < 0.05$) higher than Negative control.

Streptozotocin Induction of experimental diabetes have been observed to lead to severe body weight loss in animal models (Deeds *et al.*, 2011; Zhang *et al.*, 2016). Many studies revealed severe hyperglycemia is associated with decreased body weight after STZ treatment (Deeds *et al.*, 2011). The present study agreed that STZ-induction of diabetes lead to a significant body weight loss in the diabetic control which might be due to increased wasting of muscle, and tissue proteins (Chikhi *et al.*, 2014). Triglyceride-enriched HDL particles easily undergo catabolism, while the Triglyceride-enriched LDL particles undergo subsequent hydrolysis via hepatic lipase or lipoprotein lipase resulting in reduced LDL particle size (Wu and Parhofer, 2014). The 400 mg/Kg dose was effective in significantly ($p < 0.05$) improving the body weight of the diabetic rats and not baseline control, as administration of the extract at the dose didn't lead to a significant ($p > 0.05$) weight gain in the baseline control. The improved body weight might be due to marked improvement in the islets form of Langerhans cells reverting back to normal acinar cells and normal histological structure of pancreatic tissue as observed by El Halim *et al.*, (2020).

The effect of ethylacetate extract of *H. thebaica* fruit on blood glucose level in STZ-induced diabetic rats is shown in Table 3. The result revealed that the negative control had a significantly ($p < 0.05$) higher (514 mg/dl ±8.6) final blood glucose compared to all other groups. Group 5 [400 mg/Kg b. wt. extract (164 mg/dl ±18.6)] and 6 [200 mg/Kg b. wt. (177 mg/dl ±10.0)] showed a significantly ($p < 0.05$) lower final blood glucose compared the Standard (Metformin) but significant ($p < 0.05$) higher than normal and baseline control.

Table 3: Effect of Ethylacetate extract of *H. thebaica* Fruit on Blood Glucose Level in STZ-induced Diabetic rats.

Groups (Treatments per Kg b. wt.)	Blood Glucose concentration (mg/dl)		Change (%)
	Initial	Final	
1 (Normal)	104 ±3.1	105 ±3.2	+ 0.96
2 (Normal + 400 mg extract)	103 ±2.0	102 ±3.6	- 0.97
3 (Diabetic without treatment)	426 ±12.0^{ab}	514 ±8.6^{ab}	+ 20.65
4 (Diabetic + 80 mg Metformin)	423 ±10.2^{ab}	194 ±12.1^{abc}	- 54.14
5 (Diabetic + 400 mg extract)	432 ±13.8^{ab}	164 ±11.6^{abcd}	- 62.03
6 (Diabetic + 200 mg extract)	427 ±12.0^{ab}	177 ±10.0^{abc}	-58.54

Values are express as mean ± SEM: n = 5.
Values in the same column with ^{a} superscript were significantly ($p < 0.05$) higher than Normal control.
Values in the same column with ^{b} superscript were significantly ($p < 0.05$) higher than Baseline control.
Values in the same column with ^{c} superscript were significantly ($p < 0.05$) lower than Negative control.
Values in the same column with ^{d} superscript were significantly ($p < 0.05$) lower than Standard control (Metformin).

Treatment of the STZ-diabetic rats with ethylacetate extract of *H. thebaica* at the dose of 400 mg/Kg b. wt. led to significant ($p < 0.05$) reduction in the serum glucose levels after four weeks when compared with the diabetic group. Thus, the anti-diabetic effect of the extracts of *H. thebaica* could be attributed to the presence of the phytochemical components in the extracts, which could act synergistically and/or independently to promote the activity of insulin and glycolytic enzymes. This agrees with the report of Salib *et al.*, (2013), which showed diabetic rats treated with fraction water soluble fractions (acetone, methanol and ethylacetate) of *H. thebaica* showed highly significant ($p < 0.05$) decrease in serum glucose level as compared to diabetic ones. El Halim (2020), reported the administration of *H. thebaica* extract caused significant decreases in blood glucose levels and insulin in rats as compared to diabetic rats.

The results of the present study agrees with these results and results reported by Bayad (2016). In their research on ameliorative potential of *Hyphaene thebaica* on streptozotocin-induced diabetic nephropathy in rats, AbdEl-moniem *et al.*, (2015) reported an increased glucose levels with a decrease in insulin levels, which was due to the fact that type 2 diabetes mellitus was linked to reduced insulin release or insulin resistance, impaired glucose, lipid metabolisms, also as activation of oxidative stress indices. El Halim *et al.*, (2020) observed a marked improvement in the islets form of Langerhans cells reverting back to normal acinar cells and normal histological structure of pancreatic tissue.

The effect of ethylacetate extract of *H. thebaica* fruit on AST, GGT, and Albumin in STZ-induced diabetic rats are presented in Table 4. The result showed the AST levels of the negative control (122.45 IU/L ±5.57) was significantly ($p < 0.05$) higher than all other groups. Groups 5 and 6 had a significantly ($p < 0.05$) higher AST levels compared to the normal control (91.21 IU/L ±3.45) and baseline control (89.36 IU/L ±4.88) with the latter being significantly ($p < 0.05$) higher than the Standard control (96.41 IU/L ±4.40). The result also revealed the GGT level of the negative control (98.61 IU/L ±4.84) was significantly ($p < 0.05$) higher than all other groups. Group 5 (46.32 IU/L ±5.33) and 6 (49.54 IU/L ±4.88) were significantly ($p < 0.05$) higher than normal control with the latter being significantly ($p < 0.05$) higher than baseline and standard control. A slight but no significant ($p > 0.05$) difference was observed between the Albumin levels of all the groups.

Table 4: Effect of Ethylacetate extract of *H. thebaica* Fruit on serum levels of AST, GGT, and Albumin in STZ-induced Diabetic rats.

Groups (Treatments per Kg b. wt.)	AST (IU/L)	GGT (IU/L)	Albumin (g/L)
1 (Normal)	91.21 ±3.45	34.44 ±4.76	38.66 ±3.85
2 (Normal + 400 mg extract)	89.36 ±4.88	35.16 ±3.82	39.33 ±2.45
3 (Diabetic without treatment)	122.45 ±5.57^{abd}	98.61 ±4.84^{abd}	27.00 ±2.73
4 (Diabetic + 80 mg Metformin)	96.41 ±4.40	38.12 ±4.0	37.38 ±3.60
5 (Diabetic + 400 mg extract)	105.21 ±5.52^{abc}	46.32 ±5.33^{ac}	36.33 ±3.45
6 (Diabetic + 200 mg extract)	108.12 ±4.15^{abcd}	49.54 ±4.88^{abc}	34.33 ±2.33

Aspartate Amino Transferase (AST), Gamma-Glutamyl Transferase (GGT).
Values are express as mean ± SEM: n = 5.
Values in the same column with ^{a} superscript were significantly ($p < 0.05$) higher than Normal control.
Values in the same column with ^{b} superscript were significantly ($p < 0.05$) higher than Baseline control.
Values in the same column with ^{c} superscript were significantly ($p < 0.05$) lower than Negative control.
Values in the same column with ^{d} superscript were significantly ($p < 0.05$) higher than Standard control (Metformin).

Liver enzymes levels are important vital indicators for detecting liver toxicity, thus, the increased levels of serum enzyme such as AST and GGT indicates necrosis or damage to the liver cells (Hasan, 2011). In the present study, a significant ($p < 0.05$) increase in in diabetic rats liver enzymes was observed which might be due to excess deposition of fat in liver (Williams, 2015). The increment in transaminases AST and GGT may be explained by oxidative stress from reactive lipid peroxidation, peroxisomal beta-oxidation and recruited inflammatory cells (Mandal *et al.* 2018). Cellular GGT has a central role in glutathione homeostasis by initiating breakdown of extracellular glutathione, a critical antioxidant defense for the cell (Lushchak, 2012). Increase in GGT activity might be attributed to oxidative stress, making transport of glutathione into cells (Kunutsor, 2016).

Oral administration of ethylacetate extract of *H. thebaica* fruit for four weeks led to a significant decrease in the in serum liver enzymes which indicated a significant improvement in liver function with no significant effect on Albumin levels. The present study is in concurrence with the results reported by AL-amer *et al.* (2012), and Tohamy *et al.*, (2013) that the treatment of diabetic rats with *H. thebaica* resulted in significant ($p < 0.05$) reduction in AST and GGT compared to untreated diabetic control. Thus, administration of the extract can improve liver functions.

The effect of ethylacetate extract of *H. thebaica* fruit on creatinine and urea levels in STZ-induced diabetic rats are shown in Table 5. There was a significantly lower creatinine level of all the groups compared to negative control (139 µM/L ±3.52) (Table 4). No significant ($p > 0.05$) difference in the creatinine levels were observed between the normal control (115 µM/L ±1.73) and the other groups. A significantly ($p < 0.05$) lower levels of urea were observed in all the groups compared to negative control (7.00 mM/L ±0.47) with no significant ($p > 0.05$) difference between the other groups.

Table 5: Effect of Ethylacetate extract of *H. thebaica* Fruit on Creatinine and Urea levels in STZ-induced Diabetic rats.

Groups (Treatments per Kg b. wt.)	Creatinine (µM/L)	Urea (mM/L)
1 (Normal)	115.00 ±1.73^{c}	5.17 ±0.15^{c}
2 (Normal + 400 mg extract)	114.67 ±2.03^{c}	5.01 ±0.12^{c}
3 (Diabetic without treatment)	139.00 ±3.52	7.00 ±0.47

4 (Diabetic + 80 mg Metformin)	115.22 ±0.67^c	5.13 ±0.15^c
5 (Diabetic + 400 mg extract)	118.65 ±4.63^c	5.50 ±0.46^c
6 (Diabetic + 200 mg extract)	119.37 ±6.69^c	5.60 ±0.89^c

Values are express as mean ± SEM: n = 5.
Values in the same column with ^{c} superscript were significantly ($p < 0.05$) lower than Negative control group.

Destruction of the capillary lumen of the kidney associated with mesangial expansion, leads to the reduction of the renal filtration area, and consequently, glomerular function cessation (Quezada *et al.,* 2013). Salib *et al.,* (2013) reported a significant improvement of kidney functions in response to treatment with *H. thebaica* fruit extract extracted with acetone followed by fractionation with methanol and ethylacetate, with parallel reduction in the concentration of both urea and creatinine levels in serums, which were high, significantly dropped.

The present study partially agrees with this report as the creatinine level significantly decreased, however there was no significant reduction in the urea level at four weeks of treatment with the extract. AbdEl-moniem *et al.,* (2015) reported the administration of ethyl alcohol extract of *H. thebaica* led to improved renal function, which was similar to the findings of Wang *et al.* (2011) with luteolin, one of the active flavonoids found in *H. thebaica*, which prevented the development of diabetic nephropathy by reducing levels of urea and creatinine. The present study agreed with the report of AbdEl-moniem *et al.,* (2015), which was a significant ($p < 0.05$) decrease in blood glucose, and creatinine following oral administration of ethyl alcohol extract of *H. thebaica* fruit to STZ-induced diabetic rats.

CONCLUSION

The aqueous, methanol and ethylacetate extracts of *H. thebaica* fruit showed good hypoglycemic potential. The decrease in the serum levels of AST, GGT, Creatinine, and Urea is evident to the potential of *H. thebaica* in the improvement of hepato-renal functions.

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