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Effect of different concentration of *Moringa oleifera* leaves on the serum profile and organs of the induced diabetic rats

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Abstract
Background: *Moringa oleifera* (MO), a medicinal, multifunctional and nutritious Asian herb, used as therapy for large number diseases including diabetes mellitus (DM). This study reports the anti-diabetic potential of MO aqueous leaf extract in the streptozotocine (STZ) induced (180mg/kg) DM in rats.
Methods: 32 Sprague Dawley female rats were divided into four weight matched groups (4/group). All the groups were STZ induced, one of which was diabetic control and the remaining 3 groups were treated with 100, 200 and 300mg/kg b.w. of MO leaf extract for four weeks. The body weight and blood glucose were calculated weekly. After four weeks organs weight, total protein (TP), albumin, creatinine, alaninie amino-transferase (ALT), aspartate amino-transferase (AST), lactate dehydrogenase (LDH), high density lipoprotein (HDL), low density lipoprotein (LDL), alkaline phosphatase (ALP), triglyceride (TG) and total cholesterol (TC) in the serum and organs were calculated and compared with diabetic control.
Results: The induced DM was reduced with the administration of MO leaf extract. There was a significant (P<0.05) increase in the level of TP, albumin and HDL concentration, while significant (P<0.05) decrease was observed in blood glucose, organ weight, creatinine, ALT, AST, LDH, ALP, TG, TC and LDL concentration. A non-significant (P<0.05) decrease was observed in the level of body weight of the STZ induced diabetic rats.
Conclusion: The results demonstrated that MO extract (200mg/kg) caused a considerable hypoglycemic potential against diabetic rats. The results thus, showed that MO holds hepatoprotective effects and may be safer in preventing DM induced damage to the rats.
Significant finding of the study: MO extract showed a potential against DM and exerted a considerable hypoglycemic activity. Further study needs to be accomplished for identification of specific components present in MO for its observed experimental effects.
What this study adds: To the best of our information there has been no reported broad study to assess the anti-diabetic activity of the aqueous MO leaf extract. The present study adds the hepatoprotective effect in injury to the organs of diabetic animals.

Keywords: *moringa oleifera*, diabetes mellitus, streptozotocine, serum profile, organs

1. Introduction
Moringa oleifera (MO), is an Asian herb also called Drumstick tree [1] which have high medicinal value and can be cultivated in tropical and subtropical regions of the world. [2] It is usually grown and cultivated in Pakistan and is also known as Sohanjna [3]. Maximum height of its tree is up to 10m [4]. Its leaves, seeds, pods and roots are also used in treating lung diseases, hypertension and skin infection [3]. MO leaves contain vital proteins, essential amino acids, trace elements, phenolic compounds, antioxidants, bio active compounds, tannins that make it ideal against certain health problems [5]. It is also rich in vitamins and minerals, that mostly resides in leave parts in form of vitamin B6, provitamin A as β-carotene, magnesium, calcium, [6] phosphorous, potassium, vitamin A and D as well as various antioxidants as vitamin C and flavonoids [1]. The antioxidant and antidiabetic activity of aqueous extract of MO leaves have emerged as a potent antidiabetic in streptozotocin induced diabetic albino rats [7]. DM is one of the crucial health problems all over the world and the community is mostly in trouble [8]. It is more common in developed countries; even though there is an increase in its prevalence in Asia and Africa. WHO projects revealed that there is a continuously increase of DM patients throughout the world. This trend is more evident that more than 70% people may be affected by DM by the year 2025 in developing countries, in contrast with 62% in 1995 [9]. Keeping in view the ethanopharmacological potential of MO and high incidence of DM, this study was planned to establish the scientific uses of MO with reference to its anti-diabetic effect and examine the influence of oral administration on some of commonly used biochemical assays and activities of various enzymes in organs of STZ induced diabetic rats.

2. Methods
2.1 Plant Collection and Extraction Process
The naturally grown MO leaves were collected from Pir Mehr Ali Shah Arid Agriculture University Rawalpindi. The plants were recognized through their local name and further identified by the Department of Forestry and Range Management. For extraction purposes, aqueous cold extraction process was adopted. The leaves (50g) were washed, dried, pulverized, macerated in distilled water (500ml), placed in a stoppered container and allowed to mix on shaking water bath at 40°C. The mixture was strained, the marc was pressed and the liquid was filtered. The filtrate was then preserved in a refrigerator at a temperature of 4±1°C [10].

2.2 Animal Husbandry
Thirty two Sprague Dawley female rats divided into four, weight matched groups (30-40g), eight animals (n=8) in each group. Animals were taken from the animal house of NIH, Islamabad. Thereafter, these rats were housed in specially designed cages where water and food were provided adlibitum. These cages were maintained under standard conditions of temperature (23±1ºC) with 12 hour light and 12 hour dark cycle [10].

2.3 Experimental protocol and treatments
Rats were fasted complete night and DM was induced in animals by injecting the STZ solution (180mg/kg body weight) dissolved in the 0.3 ml solution of 1M citrate buffer [11]. The treatments were started on the 2nd day after STZ injection. These animals were kept for 30 days and treated with different doses of MO leaves extract and usual diet but control group are only treated with usual diet for 4 weeks as described below.

DC: Induced DM and not treated (control).
D1 : Induced DM and 100mg/kg b.w. of the MO leaf extract.
D2 : Induced DM and 200mg/kg b.w. of the MO leaf extract.
D3 : Induced DM and 300mg/kg b.w. of the MO leaf extract.

2.4 Sample collection and Serum preparation
Blood were obtained from the tail of rats on weekly basis and blood glucose concentration was measured**.** Body weights of rats were determined weekly while the food and fluid intake were monitored daily. At the end, all the animals were anesthetized with chloroform and samples of serum, kidney and liver were harvested and the organ weight was taken [10].

2.5 Serum, liver and kidney homogenate preparation
For serum preparation one side of rat carotid and jugular vein were cut and the collected blood was centrifuged at 3000 rpm for 20 min. Kidney and liver tissue were immediately removed; cut into small pieces and washed with phosphate-buffered saline solution. Kidney and liver were minced and homogenized in a homogenizer and centrifuged at 3000 rpm for 20 min. The collected supernatant was then analyzed for biochemical assays [12].

2.6 Biochemical Analysis
The blood glucose concentration was analyzed by using glucometer [13]. Total protein (TP) concentrations were analyzed by photometric test (biuret method) through commercially available kit Ecoline [14]. Albumin concentration was determined by colorimetric method using commercially available kit BIO RAYS (CAT# 1405) [15]. Creatinine and Alkaline phosphatase (ALP) activities were measured by modificated Jaffe's method [16] and DGKC method [17], respectively, through commercially available kit, I-TRON, France. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined by the method of Reitman and Frankel [18] through commercially available kit, Cat No CZ902C GPT, CRESCENT Diagnostics. Triglyceride (TG) and total cholesterol (TC) were determined by enzymatic, liquid, Colorimetric test by GPO-PAP method (CAT# CS611) and CHOD/PAP method (CAT# CS603), respectively through commercially available kit, CRESCENT Diagnostics [19, 20]. Lactate dehydrogenase (LDH) was determined by kinetic ultraviolet method through commercially available kit (SPECTRUM Germany) [21]. High density lipoprotein cholesterol (HDL-C) was assayed by liquicolor test through commercially available kit (HUMAN, Germany) [22]. Low density lipoprotein cholesterol (LDL-C) was calculated by Iranian formula from TC, HDL and TG concentrations [23].

2.7 Statistical Analysis
All the data was analyzed by using analysis of variance (ANOVA) at 5% level of significance (P<0.05) followed by factorial design and the LSD was calculated by using computer software Statistix 8.1 [24].

3. Results
3.1 Effect of MO on blood glucose
Blood glucose levels in diabetic control animals and in experimental groups varied significantly after 1st week (Table 1). But after 2nd and subsequent weeks dose level of 200 and 300mg/kg body weight (b.w.) showed a non significant effect on glucose level of studied animals. With passage of time, increase in glucose level was observed in untreated diabetic control group of 14.96%, 22.28% and 40.06% after 2nd, 3rd and 4th weeks, respectively. Contrary to this, decrease in blood glucose was professed with the increase in the concentration of MO leaf extract at all intervals. The percent decrease in blood glucose level in treated animals over control increased with increase in dose of MO extract. Maximum decrease in blood glucose was perceived in D3 by the administration 300 mg/kg b.w. of MO of 18.35%, 23.33% and 48.26% after 2, 3 and 4 weeks, respectively. Similarly when compared with untreated diabetic control (within columns), percent decrease in blood glucose in treated samples was elevated with increase in the interval of time at all concentrations of MO leaf extract. After 1st and 4th week the percent decrease in blood glucose level in treated animals was observed as 4.05% to 57.43%, 7.07% to 64.83% and 10.68% to 67.01% by the administration of 100, 200 and 300mg/kg b.w of MO leaf extract. This dose dependency further enhanced with advancement of the intervals suggesting a carryover effect of MO over period of time.

3.2 Effect of MO on body weight
Body weights in untreated diabetic control varied significantly (P<0.05) with the treated experimental animals after 2nd, 3rd and 4th week (Table 2). Contrary to this a small but non significant (P<0.05) difference was observed in body weight after 1st week in treated and untreated groups. Animals in diabetic control group showed body weight loss of 8.78%, 18.43 and 23.02% after 2nd 3rd and 4th week, respectively of STZ induction. However, bodyweight was gained in treated groups and this gain is elevated non-significantly with the increase in time interval. Administration of MO extract 200 and 300mg/kg b.w. revealed bodyweight gain up to 41.33% and 40.96%, respectively after 4 weeks. The nutrients dependency in MO was sustained with the time interval conveying encapsulating effect.

3.3 Effect of MO on organs weight
This study shows that higher organ weight was examined in the STZ induced DM group (Table 2). On the contrary to this, the groups treated with MO leaf extract at the dose 300mg/kg b.w significantly decreased up to 24.25% and 26.36% in

kidney and liver, respectively compared to STZ induced diabetic animals. Greater reduction in liver weight was observed than organ weight. Reduction in the organs weight of animals treated with 100 and 200mg/kg b.w. was also observed (6.43%, 16.04%) in liver and (16.87% and 23.94%) kidney, respectively, but not significant. Over all, dose dependent effect of MO was significant in both organs; increase in dose was effective in reducing the weight of both organs.

3.4 Effect of MO on Biochemical parameters

A significant (P<0.05) increase in total protein (TP) concentration was observed among all the four groups of serum and kidney with the administration of various doses of MO. (Table 3). However, in liver, TP concentrations in three groups (Diabetic control, 100 and 200mg/kg b.w of MO extract adminidtration) were significant, but with 300mg/kg b.w TP was not significant with 200mg/kg b.w. Lower values of TP were observed in diabetic animals as 3.61, 3.93 and 3.83g/dl in serum, kidney and liver, respectively. MO extract (300mg/kg b.w.) wielded maximum increase in TP observed in serum kidney and liver was 66.48%, 23.4%, and 29.5%, respectively.

Similarly, significant (P<0.05) increase in the albumin concentrations with the increase in MO leaf extract (100 and 200 mg/kg b.w.) was observed in both serum and organs (kidney and liver) but non-significant effect was observed between 200 and 300mg/kg b.w (Table 3). Lower values calculated in serum, kidney and liver were 1.92, 1.69 and 2.47g/dl, respectively in diabetic control group. In the contrast, MO extract (300mg/kg b.w.) exerted a maximum increase of albumin 61.45%, 60.35% and 51.01% in serum, kidney and liver, respectively.

ANOVA table predicted significant (P<0.05) decrease in creatinine concentration was observed in serum, kidney and liver (Table 3). Groups of diabetic control, 100 and 300mg/kg b.w. were significant among each other in serum and liver, but non-significant variation was examined in 200mg/kg b.w. However in case of kidney 100 and 200mg/kg b.w. was significant among each other but 300mg/kg b.w. of MO exerted no significant difference. Higher values of creatinine were observed in diabetic group (DC) as 0.74, 0.77 and 0.80mg/dl in serum, kidney and liver, respectively. By the administration of 300mg/kg b.w. of MO extract, maximum decrease in creatinine concentration was observed as 32.43%, 38.96% and 27.5% in serum, kidney and liver, respectively.

It is evident from results that a significant (P<0.05) decrease was observed in ALT concentration of kidney by the administration of MO at various doses (Table 3). However, in liver the dose at 100 and 200mg/kg b.w. showed a non significant effect. Unlike this, in AST, significant (P<0.05) variation was observed in kidney with the administration of various doses of MO. Unlike this, non-significant effect was observed at doses of 200 and 300mg/kg b.w. of MO extract in serum and liver samples. Higher values in ALT and AST in serum kidney and liver of diabetic groups were observed as 75.29, 74.35, 78.41units/ml and 59.02, 92.31, 71.71units/ml, respectively. MO leaf extract (300mg/kg b.w.) exerted maximum decrease in ALT and AST in serum, kidney and liver were observed as 51.97%, 57.91%, 62.49% and 49.61%, 62.48%, 51.78%, respectively.

In LDH-C concentration, significant (P<0.05) decrease was predicted by ANOVA table in serum, kidney and liver with the administration (100, 200 and 300mg/kg b.w.) of MO leaf extract (Table 3). However, significant variation were observed in kidney and liver within diabetic group (DC), 100 (D1) and 200mg/kg b.w. (D2) but non-significant with 300mg/kg b.w. (D3). Unlike this, in serum DC, 100 and 300mg/kg b.w. were significant (P<0.05) but 200mg/kg b.w. revealed a non-significant variance. Maximum values of LDH-C in diabetic group (DC) were found to be as 1543.4, 1235.8 and 1219.6U/L in serum, kidney and liver respectively. By administration of MO extract (300mg/kg b.w.) maximum decrease observed in LDH-C of 25.17%, 41.04% and 41.59% in serum, kidney and liver, respectively.

Similarly, in ALP a significant (P<0.05) decrease was observed in serum and kidney with the administration of various doses of MO extract (Table 3). However, in liver, significant decrease observed in diabetic control, 100 and 200mg/kg b.w. but 200 and 300mg/kg b.w. exerted a non-significant effect. Higher values of ALP were observed in diabetic group, 128.34, 134.78 and 143.52 U/L in serum, kidney and liver, respectively. With the administration of 300mg/kg b.w. of MO maximum decrease observed in ALP was 39.78%, 42.32% and 28.20% in serum, kidney and liver, respectively.

A significant (P<0.05) increase in HDL-C concentration in serum with the administration of 100, 200 and 300mg/kg b.w. of MO extract (Table 3), dose of 200 and 300mg/kg b.w. reveals a non-significant increase in kidney and liver samples. In kidney there was significant variation among the groups diabetic control (DC), 100mg/kg b.w. (D1) and 300mg/kg b.w (D3) but non-significant with 200mg/kg b.w. of MO extract. Unlike this, in liver, significant variation was observed in DC, D1 and D2 but not in D3. Lower values of HDL-C were observed in diabetic group, 27.25, 30.19 and 31.67mg/dl in serum, kidney and liver, respectively. MO extract (300mg/kg b.w.) exerted a maximum increase in HDL-C of 41.46%, 114.61%, and 94.79% in serum, kidney and liver, respectively.

A significant (P<0.05) decrease is evident in triglyceride (TG) concentration in serum with the administration of 100, 200 and 300mg/kg b.w of MO extract (Table 3), but 200 and 300mg/kg b.w. revealed a non-significant decrease in kidney and liver samples. Values of TG concentration were higher in diabetic group, 154.02, 185.82 and 158.62mg/dl in serum, kidney and liver, respectively. MO at 300mg/kg b.w. showed maximum decrease in triglyceride, 45.52%, 47.42%, and 41.54% in serum, kidney and liver, respectively.

Similarly, a significant (P<0.05) decrease was observed in total cholesterol (TC) concentration in serum with the administration of MO extract at various doses. However, in kidney and liver, doses of 200 and 300mg/kg b.w. showed a non-significant variance (Table 3). Higher values of TC were examined in diabetic group, 187.55, 152.21 and 148.59mg/dl in serum, kidney and liver, respectively. MO extract (300mg/kg b.w.) revealed maximum decrease in TC, 8.99%, 30.87% and 29.46% in serum, kidney and liver, respectively.

Table 3 shows significant (P<0.05) decrease in LDL-C concentration in serum and kidney with the administration of different doses of MO extract. While in liver, diabetic control group (DC), 100mg/kg b.w. (D1), and 200mg/kg b.w. (D2) were significant among eachother, but not significant with 300mg/kg b.w. (D3). Higher values of LDL-C were examined in diabetic group (DC), 175.89, 160.26 and 141.57mg/dl in

serum, kidney and liver, respectively. MO extract (300mg/kg b.w.) revealed maximum decrease in LDL-C was 34.87%, 47.42% and 41.54% in serum, kidney and liver, respectively.

Table 1: Effect of MO leaf extract on glucose concentration of STZ induced diabetic rats after four weeks of treatment

Blood Glucose (mg/dl)	W1	W2	W3	W4
DC	262.92±1.48a	302.25±8.12a	321.50±5.65a	368.25±5.27a
D1	252.25±3.59b	237.12±3.23b	207.24±7.72b	156.75±6.48b
D2	244.33±2.85c	197.06±3.56c	190.75±8.05c	129.50±1.30c
D3	234.84±2.17d	191.75±2.71c	180.05±7.59c	121.50±1.69c

Values are articulated as the means ± S.D of 8 rats, n=8. [a b c d] within columns represents the significant (P<0.05) difference among the groups when compared with DC (Diabetic Control). D1, D2 and D3 indicate 100, 200 and 300mg/kg, respectively of MO leaf extract. W1, W2, W3 and W4 indicate week 1, week 2, week 3 and week 4, respectively.

Table 2: Body weight and organs weight of STZ induced diabetic rats after treatment with *M. oleifera* leaf extract for four weeks.

	Body weight(g)				Organs weight(g)	
	W1	W2	W3	W4	Kidney	Liver
DC	35.74±0.70a	32.6±1.18b	29.16±0.92b	27.51±0.94b	0.268±0.022a	1.612±0.097a
D1	36.05±0.66a	36.89±0.78a	37.32±0.53a	38.24±0.28a	0.251±0.008ab	1.340±0.072b
D2	36.50±0.08a	37.06±0.80a	37.95±0.38a	38.88±0.15a	0.225±0.012bc	1.226±0.077bc
D3	36.49±0.55a	37.04±0.53a	37.88±0.86a	38.78±0.66a	0.203±0.020c	1.187±0.077c

Values are articulated as the means ± S.D of 8 rats, n=8. [a b c d] within columns represents the significant (P<0.05) difference among the groups when compared with DC (Diabetic Control). D1, D2 and D3 indicate 100, 200 and 300mg/kg, respectively of MO leaf extract. W1, W2, W3 and W4 indicate week 1, week 2, week 3 and week 4, respectively.

Table 3: Effect of MO leaf extract in serum, kidney and liver biochemical parameters of STZ induced diabetic rats

Parameters		Experimental Groups			
		DC	D1	D2	D3
TP g/dL	Serum	3.61±0.22d	4.81±0.27c	5.68±0.07b	6.01±0.10a
	Kidney	3.93±0.11d	4.49±0.08c	4.68±0.13b	4.85±0.11a
	Liver	3.83±0.12c	4.35±0.04b	4.70±0.25a	4.96±0.07a
Albumin g/dL	Serum	1.92±0.06c	2.63±0.07b	2.98±0.06a	3.1±0.07a
	Kidney	1.69±0.18c	2.04±0.07b	2.47±0.12a	2.71±0.12a
	Liver	2.47±0.31c	3.06±0.31b	3.41±0.24ab	3.73±0.18a
Creatinine mg/dL	Serum	0.74±0.03a	0.62±0.03b	0.53±0.02bc	0.5±0.03c
	Kidney	0.77±0.05a	0.66±0.03b	0.61±0.03b	0.47±0.07c
	Liver	0.80±0.03a	0.69±0.06b	0.63±0.05bc	0.58±0.05c
ALT/GPT U/ml	Serum	75.29±0.91a	65.72±0.66b	45.72±0.25c	36.16±0.50d
	Kidney	74.35±5.32a	58.55±6.27b	44.05±5.44c	31.29±2.62d
	Liver	78.41±5.44a	52.60±6.66b	44.19±6.58b	29.41±7.19c
AST/GOT U/ml	Serum	59.02±1.09 a	47.36±3.11 b	33.31±2.29 c	29.74±5.77c
	Kidney	92.31±4.38a	75.37±6.44b	55.00±4.18c	34.63±3.22d
	Liver	71.72±3.93a	53.47±5.60b	42.13±6.08bc	33.98±6.72c
LDH-C U/L	Serum	1543.4±28.42a	1314.1±12.36b	1270.9±14.02b	1154.9±12.36c
	Kidney	1235.8±40.74a	1030.7±52.04b	760.9±32.38c	728.5±48.57c
	Liver	1219.6±37.39a	1003.7±48.57b	755.53±24.73c	712.36±58.37c
ALP U/L	Serum	128.34±7.17a	99.36±8.28b	88.32±4.78c	77.28±4.78d
	Kidney	134.78±5.75a	105.80±4.44b	88.78±3.47c	77.74±2.87d
	Liver	143.52±6.02a	131.10±2.39b	106.72±3.47c	103.04±4.44c
HDL- C mg/dL	Serum	27.25±0.32d	30.49±0.08c	36.88±0.47b	38.55±0.07a
	Kidney	30.19±4.51c	46.21±10.96b	53.12±3.42ab	64.79±6.63a
	Liver	31.67±3.67c	43.33±5.29b	57.38±7.94a	61.69±5.18a
TG mg/dL	Serum	154.02±1.75a	144.45±1.32b	88.13±1.76c	83.91±1.15d
	Kidney	185.82±9.64a	135.63±13.05b	111.88±12.4bc	97.70±12.8c
	Liver	158.62±9.41a	121.07±13.07b	105.36±9.64bc	92.72±4.79c
TC mg/dL	Serum	187.55±2.51a	182.33±0.69b	174.3±1.84c	170.68±0.69d
	Kidney	152.21±3.03a	138.15±3.87b	112.45±3.87c	105.22±1.84c
	Liver	148.59±5.02a	134.94±4.34b	108.84±4.56c	104.82±2.09c
LDL-C mg/dL	Serum	175.89±2.11a	163.52±0.68b	121.32±2.59c	114.54±1.13d
	Kidney	160.26±5.95a	103.02±15.30b	67.09±6.34c	42.95±3.09d
	Liver	141.57±12.23a	99.72±11.89b	56.75±4.53c	42.80±6.57c

Values are articulated as the means ± S.D of 8 rats, n=8. [a b c d] within rows represents the significant (P<0.05) difference among the groups when compared with DC (Diabetic Control). D1, D2 and D3 indicate 100, 200 and 300 mg/kg respectively, of MO leaf extract.

4. Discussions

A considerable decrease in blood glucose levels over period of time in treated samples was caused by MO extract. This decrease may be due increased secretion insulin (by the action of MO) from pancreatic β-cells of islets of Langerhans which may be damaged due to STZ induction [25]. Thus reduction in the blood glucose concentration declares the ability of extract to minimize hyperglycemia. Increase in the fall of blood glucose with the passage of time may be due to the components in extract which remains in the body for long time and prevents DM. In this study, DM induced with STZ significantly reduced body weight. STZ, a universal toxin not only induce DM but causes destruction of β-cells in the islets of Langerhans, which acts as a good enhancer of oxidative stress which is directly associated with reduction in weight in diabetic rats. Though in this study, the constituents of MO might recover islets of Langerhans which revealed insulin secretion over a period of time due to which reduction in weight recovered [26]. The ability of MO leaf extract to recover body weight loss may be due to its ability to reduce hyperglycemia. In the result of which the glucose digestion is totally disturbed, due to which the body weight of experimental animals fall down.

STZ induced DM destroys the β-cells of pancreas by which the body utilizes non-carbohydrate components such as protein for the production of glucose, results in sever loss in organ weight [27]. The present study suggested that MO leaf extract at dose of 300mg/kg b.w. is proficient of preventing organs weight gain. Our results were similar with the previous study [27] where the reduction of organs weight was observed with the administration of methanol extract of MO leaves. Thus it confirms that MO extract holds potential to recover the weight gain against diabetic rats.

The decrease in TP level was may be associated with enhanced catabolism of protein or may be due micro protein urea, which is clinical marker in diabetic nephropathy [12]. It has been reported that insulin motivates the incorporation of amino acids into protein and MO have potential to recover the insulin secretion from β-cells of STZ induced diabetic rats [28]. This study revealed that MO may be proficient for ameliorating the damaged diabetic organs function besides its hypoglycemic control.

Albumin is required for the supply of body fluids between intravascular compartments and body tissues, and works as a carrier by binding various hydrophobic hormones. Administration of STZ reduces albumin levels because of increased non-enzymatic glycosylation of protein. The presence of bioflavonoid in MO that may be responsible for the stimulation of glucose uptake in peripheral tissues and regulation of the rate-limiting enzymes activity that are associated in carbohydrate metabolism therefore MO treatment significantly elevated albumin concentration which is associated with decreased affinity of albumin towards glucose [28, 29].

Creatinine concentration decreased in the diabetic animals with the increase in MO extract. The renal function failure as a result of diabetic nephropathy was considered as a major complication of diabetes. The increased level of creatinine in STZ diabetic rats certifies a renal failure. Through acidification of urine and removal of these metabolites, kidney sustain optimum chemical composition of body fluids but during renal disease, these metabolites concentrations increase in blood [26]. This study suggested that the plant extract could reduce renal failure. MO has ability to restore the normal renal function and may be no pathological changes in histology of organs [5].

ALT and AST measurement is of clinical and toxicological importance because their activity indicates tissue damage by toxicants or in disease condition. Liver and kidney damage are prominent side effects of diabetes, though recovery of ALT and AST activity of diabetic rats towards normal reveals that the MO extract possess no adverse effect on liver functions [12].

In the present study decrease in LDH-C and ALP was observed in induced diabetic rats with increase in MO extract. DM induce hepatic malfunctioning i.e liver was necrotized in diabetic patients due to which percentage of LDH-C and ALP enzyme increases in plasma because of the spillage of these enzymes into the blood stream from liver cytosol, which indicates the hepatotoxic impact of STZ. However, administration of MO for 30 days might restore the action of LDH-C and ALP enzyme to their normal state and inhibits the liver damage induced by STZ [12].

The main 'anti-atherogenic' lipoprotein HDL is involved in the transportation of cholesterol from peripheral tissues into liver and thereby acts as a protective factor against coronary heart disease. STZ decreases HDL-Cholesterol level and are major factor for coronary heart disease which is one of complication of diabetes [30]. In this study, the increase in the HDL concentration might be due the MO leaf extract, which contains cardio protective potential against STZ induced diabetic rats [31].

Higher level of TG reveals that there may lack of insulin in diabetic animals which is directly responsible for coronary heart diseases and dyslipidaemia [32]. This study revealed that administration of MO leaf extract during 30 days reduced TG. Thus MO aqueous extract might have a potential to protect cardiovascular disturbance in diabetic animals.

DM is associated with high levels of circulatory TC and severe coronary heart disease [33]. The serum lipids is often increased in diabetes mellitus and such an increase in lipids prompts coronary illness because of the uninhibited activities of lipolytic hormones on the fat stores primarily due to the low activity of insulin. Under typical conditions, insulin endorses the catalyst lipoprotein lipase, which is not initiated because of insulin inadequacy that is connected with hypercholesterolemia. Since insulin has an inhibitory activity on reductase, a key rate-constraining catalyst responsible for the metabolism of TC. Therefore the system of hypercholesterolemia is uncontrolled in diabetes patients [34]. However, MO increases insulin level by inhibiting the destruction of pancreatic β-cells [25].

Decrease in LDL-C was observed in diabetic animals with the administration of MO leaf extract. Insulin is responsible to increase receptor-mediated removal of LDL-C from the body and hence decreased activity of insulin during diabetes leads to increased level of serum LDL-C and consequently hypercholesterolemia. Therefore, significant control in the serum lipid level in MO treated diabetic rats might be due to the insulinotropic action upon MO administration [35].

5. Conclusions

Overall results reveal that STZ injection persuaded hyperglycemia, body and organs weight loss. This study

demonstrated that MO extracts at doses of 200 and 300mg/kg b.w. in most samples have non-significant effect therefore treatment of the diabetic rats with the dose of 200mg/kg b.w. has exerted a considerable hypoglycemic effect. Some components of MO leaf extract remain in the body over a period of time which implies anti-diabetic effect consequently. The organs effect in this study in response to MO leaf extract treatment of STZ induced diabetic rats implies that this plant may be beneficial against cardiovascular diseases, hepatotoxic activity and hepatocellular injury in STZ induced diabetic rats.

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7. Literature Cited

1. Mbikay M. Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: a review. *Front Ethnopharmacol.* 2012; 3:1-12.
2. Adedapo AA, Mogbojuri OM, Emikpe BO. Safety evaluations of the aqueous extract of the leaves of Moringa oleifera in rats. J. Med. Plants Res., 2009; 3(8): 586-591.
3. Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera:* A food plant with multiple medicinal uses. Phytother. Res. 2007; 21:17-25.
4. Morton J F. The horseradish tree, *Moringa pterigosperma (Moringaceae)*. A boon to arid lands. *Econ. Bot.* 1991; 45(3):318-333.
5. Kasolo JN, Bimenya JS, Okwi AL, Othieno EM, Ogwal-Okeng JN. Acute toxicity evaluation of *Moringa oleifera* leaves extracts of ethanol and water in mice. Afr. J. Anim. Biomed. Sci. 2011; 7(1):40-44.
6. Bharali R, Tabassum J, Azad MRH. Chemomodulatory *effect of Moringa oleifera*, lam, on hepatic carcinogen metabolising enzymes, antioxidant parameters and skin papillomagenesis in mice, Asian Pac. J. Cancer Prev. 2003; 4(2):131-139.
7. Al-Malki AL, El Rabey HA. The antidiabetic effect of low doses of *Moringa oleifera* Lam. seeds on streptozotocin induced diabetes and diabetic Nephropathy in Male Rats. *Biomed. Res. Int.* 2014; 1-13.
8. Lidia ANB, Louis CM, Ali BA, John TA, Émile L, Pierre SH. Inhibition of intestinal glucose absorption by anti-diabetic medicinal plants derived from the James Bay Cree traditional pharmacopeia. *J. Ethnopharmaco.* 2010; 132: 473-482.
9. King H, Aubert RE, Herman WH. Global burden of diabetes 1995–2025: prevalence, numerical estimates and projections. *Diabetes Care.* 1998; 21:1414-1431.
10. Ndidi EA, Nneka OC, Ebere OO. Cytological and biochemical studies during the progression of alloxan-induced diabetes and possible protection of an aqueous leaf extract of Costus afer. Chin. J. Nat. Med. 2014; 12(10):745-752.
11. Liu X, Hering BJ, Brendel MD, Bretzel RG. The effect of streptozotocin on the function of fetal porcine and rat pancreatic (pro) islets. *Exp. Clin. Endocrinol.* 1994; 102:374-9.
12. Mansour HA, Newairy AA, Yousef MI, Sheweita SA. Biochemical study on the effects of some Egyptian herbs in alloxan-induced diabetic rats. *Toxicology.* 2002; 170:221-228.
13. Jemai H, El-Feki E, Sayadi S. Antidiabetic and antioxidant effects of hydroxytyrosol and oleuropein from olive leaves in alloxan-diabetic rats. J. agric. Food Chem. 2009; 57(19):8798-8804.
14. Thomas L. *Clinical Laboratory Diagnostics*. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft: 1998; 664-7.
15. Johnson A, Rohlfs EM, *Silverman LM. Protein.* In: Burtis CA, Ashwood ER, editors.
16. Tietz NW. Textbook of Clinical Chemistry, W. B. Saunders Co. Philadelphia, 1986; 588.
17. Tietz NW. Fundamentals of Clinical Chemistry, W. B. Saunders Co. 1982; 603.
18. Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 1957; 28:56-63.
19. McGowan MW. Clin. Chem. 1983; 29:538.
20. Deeg R, Zlegenhorn J. Kinetic Enzymic Method for Automated Determination of Total Cholesterol in Serum. Clin. Chem. 1983; 29(10):1798-1802.
21. Young DS. Effects of drugs on clinical laboratory tests. *AACC press*, Washington D. C. 1990.
22. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Amer. J. Med. 1977; 62(5):707-14.
23. Ahmadi SA, Boroumand MA, Gohari-Moghaddam K, Tajik P, Dibaj SM. The Impact of Low Serum Triglyceride on LDL-Cholesterol Estimation. Arch. Iranian Med. 2008; 11(3):318-321.
24. Steel RGD, Torrie JH. Principles and Procedures of Statistics, Second Edition. New York: McGraw-Hill, 1980.
25. Rafiu AA, Luka CD. Anti-diabetic activity of aqueous seed extract of (*Moringa oleifera*) in normal and alloxan induced diabetic rats. *J. Biol. Sci. Biocon.* 2015; 7: 24-37.
26. Une HD, Pradip S, Tarannum PR. A study on the effects of *Moringa oleifera lam*. Pod extract on alloxan induced diabetic rats. *Asian J. Plant Sci. Res.* 2014; 4(1):36-41.
27. Bais S, Singh GS, Sharma R. Antiobesity and Hypolipidemic Activity of *Moringa oleifera* Leaves against High Fat Diet-Induced Obesity in Rats. *Adv. Biol.* 2014; 1-9.
28. Gupta R, Mathur M, Bujaj VK *et al*. Evaluation of antidiabetic and antioxidant activity of *Moringa oleifera* in experimental diabetes. *J. Diabetes.* 2012; 4:164-171.
29. Zunszain PA, Jamie GJ, Komatsu T, Tsuchida E, Curry S. Crystal structural analysis of human serum albumin complexed with hemin and fatty acid. BMC Struct. *Biol.* 2003; 3:6.
30. Ananthan R, Latha M, Ramkumar K, Pari L, Baskar C, Bai V. *et al.* Effect of *Gymnema montanum* leaves on serum and tissue lipids in alloxan diabetic rats. Experimental Diabetes Research. 2003; 4:183-189.
31. Nandave M, Ojha SK, Joshi S, Kumari S, Arya DS. "*Moringa oleifera* leaf extract prevents isoproterenol-induced myocardial damage in rats: Evidence for an

antioxidant, antiperoxidative, and cardioprotective intervention," J. Med. Food. 2009; 12(1):47-55.
32. Florence NT, Benoit MZ, Jonas K *et al*. Antidiabetic and antioxidant effects of *Annona muricata* (Annonaceae), aqueous extract on streptozotocin-induced diabetic rats. *J. Ethnopharmacol.* 2014; 151:784-790.
33. Akah PA, Alemji JA, Salawu OA, Okoye TC, Offiah NV. Effects of *Vernonia amygdalina* on Biochemical and Hematological Parameters in Diabetic Rats. Asian J. Med. *Sci.* 2009; 1(3):108-113.
34. Shah NA, Khan MR. Antidiabetic effect of *Sida cordata* in alloxan induced diabetic rats. Biomed. Res. Int. 2014; 1-15.
35. Kim MJ, Kim HK. Insulinotrophic and hypolipidemic effects of *Ecklonia cava* in streptozotocin-induced diabetic mice. Asian Pac. J. of Trop. Med. 2012; 374-379.