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RESEARCH ARTICLE

Effects of *psidium guajava* and *syzgium cumini* extracts on hepato-renal performance and lipid profile of induced diabetic male albino rats

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Diabetes mellitus is an endocrine disorder and traditional plants' treatment for diabetes has shown a lot of interest from last few decades, so the present study is designed to explore the effects of *Psidium guajava and Syzygium cumini* leaf extracts on hepato-renal performance and lipid profile of streptozotocin-induced diabetic male albino rats. The leaves of plants were washed, dried grinded to get fine powder. The leaves' powder was mixed with ethanol for 72 hours, filtered and evaporated by rotary evaporator to get extract as a soft pellet. Twenty-five (25) male Wistar albino rats, weighing 100-200g were randomly divided into five feeding groups. Diabetes was induced in rats of groups A, B, C & D by single intra-peritoneal injection of 55mg/kg BW of streptozotocin (STZ). The rats with glucose level over 200mg/dL were considered as diabetic. Rats of group A were fed basal diet supplemented with extract of *P. guajava* at a dose of 100 mg/kg BW. Group B were given leaf extracts of *S. cumini* (100 mg/kg BW), Group C rats were fed with combined leaf extracts of *P. guajava* and *S. cumini* (100 mg/kg BW of each plant extract). The rats of Group D & E were given only basal diet, hence considered as positive and negative control. All animals were provided free access to feed and water for 21 days. At the completion of the experiment, all the rats were anesthetized dissected and blood samples were collected by heart puncture method. The results revealed that, glucose level, organs' weight, TP, BIL, urea, creatinine, triglyceride, HDL, LDL and ALT concentration of experimental rats were different significantly, whereas, concentration of HbA_1C, ALP and GGT,AST and ALB differed non-significantly. It can be concluded that leaf extracts of *P. guajava* and *S. cumini* separately or combine presented a significant improvement in serum glucose level and other health promoting markers. It is therefore concluded that the extracts of both plants can be used synergistically for the management of diabetes.

Keywords: Syzgium cumini, Hepato renal, Diabetes,rat

INTRODUCTION

Diabetes mellitus is one of the metabolic disorders that happens when blood glucose level is elevated (De-Fronzo*et al.,* 2015) due to fault in insulin emission or insulin action (Pickup *et al.,* 2003). It is also measured as compact approval and filling of glucose as well as dense glucose use for energy purposes (Jacobsen *et al.,* 2009; Sangeetha *et al.,* 2010). Liver has a capacity to stock glucose in the form of glycogen and sustain concentration of blood glucose level in the body (Roden*et al.,* 2003). Dyslipidemia is tempted by the diabetes which consequences macro vascular and microvascular ailment. There are two main types of diabetes mellitus. The first type is Insulin dependent diabetes mellitus (IDDM), also called type-1 and the second type is non- insulin dependent diabetes mellitus (NIDDM) and also called as type-II.

Insulin dependent diabetes mellitus type-I is an autoimmune disease formed by auto caustic T-lymphocytes that enter the pancreatic cells and damage the β-cells that cause hyperglycemic situation. This develops different diseases with time period such as cardiomyopathy, retinopathy, nephropathy and neuropathy (Yadav *et al.,* 2004).

Diabetes mellitus is a universal syndrome due to which 382 million people were affected in 2013 all over the world. It is predictable that it will grasp 592 million through 2035 (Guariguata*et al.,* 2014).

Currently, the controlling of Diabetes mellitus depends upon several active ingredients present in plants and chemicals. A lot of numbers of Phyto-extraction with their yields can be verified to decrease oxidative stress automatically, that is distinctive properties of plant remedies confederated with cure a number of critical disorders containing diabetes (Ayodhya*et al.,* 2010).

Medicinal plants have been used in the treatment and improvement of human diseases and such plants with high antioxidant abilities can be used as natural medicines for preventing aging and chronic diseases. Herbs and vegetables have contributed significantly in progress of human health in terms of prevention and treatment of diseases. Herbal drugs are free of toxins and most effective (Rao *et al.,* 2003). Thousands of wild plant species grow in Africa and have both nutritional and therapeutic purposes. *P. guajava* and *S.cumini* plays an important role.

Psidiumguajava is also called guava, is ever green plant American native. First of all this study was designed to check the effects of ethanol and aqueous extracts of guava leaves on blood glucose and plasma insulin level in the severe and long-term feeding rats of type II diabetes. The effect of this extract will be observed in liver of usual and diabetic rats. Due to anti-diabetic properties type I or type II diabetes extracts of guava leaf shows positive effects (Mathur *et al.,* 2015).

The local name of*Syzygiumcumini* is Jamun, which belong to family *Myrtaceae*. Its fruit possess high quality medicinal functions against diabetes as it effects on pancreas (Modi *et al.,* 2010). Jamun have anti-hyper-cholesterolemic properties that help in regulation of lipid profile due to presence of bioactive components (Sinha *et al.,* 2014).

From above discussion we can conclude that diabetes is a lethal disease having dangerous effect on human health. Despite significant progress in management of diabetes mellitus by synthetic drug there is need of safe natural antidiabetic agents. Various plant extracts have been used for its remedies and plenty of data available. There is lot of medicinal plants available having antidiabetic effect, two of them is *P. guajava and S. cumini.* The leaf extract of both plants were reported to have antibacterial and antidiabetic effect.

MATERIALS AND METHODS

Collection of Plant Leaves

The leaves of *P. guajava* and *S. cumini* were collected from the local garden of Gujranwala after the permission of gardener in the month of July. The fresh leaves of *P. guajava* and *S. cumini* were washed with tab water. The leaves veins and unwanted particles were removed after washing. By using grinder the leaves were sliced into pieces and then air dried for one week and pulverized into fine powder. The subsequent powder was stored in air tight glass bottles and for further experimental procedure it was kept in laboratory at room temperature.

Extraction of leaves' extract

Two hundred and fifty gram (250g) of the prepared powder of each plant was mixed in 750 ml of ethanol in the ratio of 1:3 for 72 hours with continuous shaking in laboratory. After three days, the solution was filtered by Whatman no.1 filter paper. After filtration, the filtrate was evaporated using the rotary evaporator machine. A greenish paste was obtained for each extract. The extract was collected in petri dishes for further dry in open air, then the solid soft pallets extracts were stored in glass voiles and reserved in the refrigerator at 4°C for further usage.

Experimental conditions and animals

Twenty-five (25) healthy male albino rats (100-200 kg) were bought from the animal house of the department of Zoology, University of Lahore, Lahore. The rats were kept in standard animal cages. Before start of the experiment, rats were kept within the animal house for the period of one week for environmental adaptations. The standard pellet diet was balanced food, composed of protein 21.1%, fat 5.1%carbohydrates 60.0%, fiber 3.9%, vitamins 2.0%, and minerals 7.9%. Animals were housed and maintained at 22°C under a 12-h light/12-h dark cycle, with free access to food and water.

Induction of diabetes in animals

The rats were kept at overnight fasting and then streptozotocin (STZ) at the rate of 55mg/Kg body weight in 0.5ml physiological saline solution were injected in intra peritoneal cavity of 20 experimental rats. After 24hrs, this single dose induced the severe diabetes in rats by increasing the blood glucose level up to 250mg/dL. This diabetic condition was sustained throughout the experimental period. The rats with more than 200mg/dL blood glucose level were considered as diabetic and used in the experiment.

Experimental design

All rats were kept at the animal house in natural conditions for adaptation in the new environment for one week, following the guidelines of the OCDE (2008)**.** Prior permission was taken from the departmental animal welfare and ethical committees to use rats in present study.The rats were divided randomly in to five feeding groups to study the short term (21 days) comparative effects of *P. guajva* and *S. cumini* on blood glucose, hepato-renal performance, lipid profile and other parameters.

Animal treatment

All the rats were divided into five feeding groups randomly (A, B, C, D and E) having five rats in each group. All the experimental animals were fed either with basal diet or basal diet supplemented with plant extracts (100mg/Kg body weight).

Group A: Diabetic rats were administered orally with leaf extract of *P. guajava* (100mg/Kg body weight) during the experimental period.

Group B: Diabetic rats were administered orally with leaf extract of *S. cumini* (100mg/Kg body weight) during the experimental period.

Group C: Diabetic rats were administered orally with combined leaf extracts *P. guajava* and *S. cumini* (100mg/Kg body weight of each plant) during experimental period.

Group D: Rats of this group were diabetic control and were not treated with any plants extract

Group E: Rats of this group were normal control group and was not treated with any drug.

Table 1: Feeding diet for experimental and control groups

Groups	Dietary plan

A	Basal diet + *P. guajava* (100mg/Kg Body weight)
B	Basal diet + *S. cumini* (100mg/Kg Body weight)
C	Basal diet + *P. guajava* (100mg/Kg Body weight) + *S. cumini* (100mg/Kg Body weight)
D	Basal diet only (Diabetic control group)
E	Basal diet only (healthy control group)

Collection of blood samples:

On the completion of experiment all the experimental rats were weighted and anesthetized by using 0.5% chloroform. After measuring the glucose level with the Certeza glucometer (Certeza GL-110) the rats were fixed on the dissecting board and dissected. The blood sample of each rat was collected by heart puncturing and stored in the heparinized vacutainer tubes. The blood sample was then transfer to the laboratory and centrifuged for 15 minutes at 5000 x g for the collection of blood serum. The serum was collected and stored in the sterile tubes at -20^{O}C. A portion of the clear supernatant serum was used for biochemical analysis.

Specimen collection:

At the 22nd day of experiment after the collection of blood sample from the heart, theorgans like pancreas, liver and kidney were separated and measured by digital weighing machine and stored to check histopathology. The dissected tissues (liver, kidney, pancreases) were stored at −4°C for further experiments.

STATISTICAL ANALYSIS

One-way Analysis of Variance (ANOVA) was performed for all the recorded data and then interpreted by General Linear Model (GLM) procedures. The means of the values were compared by Duncan's Multiple Range test (DMR) with the help of software, statistical analysis system (SAS 9.1) for windows.

RESULTS

The present study was design to check the efficacy of ethanol plants extracts of *Psidium guajava* and *Syzygium cumimi* on hepato-renal performance and lipid profile of induced diabetic male albino rats. At the end of study following results were observed:

Body weight (g)

The current study revealed that the initial body weight of supplemented groups of experimental rats increased significantly as compare to control group with (P=0.0449). As the initial body weight of the experimental groups was 216.33±11.9758, 233.000±4.725, 188.50±16.50, 193.50±7.500, 186.500±4.500respectively. Whereas, the final body weight of the experimental rats decreased non-significantly as compare to control group (P=0.9675). The final body weight of the experimental rats was as following 178.00±41.01, 233.00±4.725, 197.50±50.50, 207.00±4.000, 215.00±6.000respectively.

Glucose level (mg/dL)

According to present study it is evaluated that the initial glucose (mg/dL) level of supplemented group increased while final glucose (mg/dL) level decreased significantly as compare to diabetic control group.The initial glucose level of experimental group is as following 340.666±38.167, 300.00±58.968, 274.00±87.00, 219.00±8.000, 79.500±2.000 with P-value (P=0.0656) and the final glucose level of experimental group was 152.333±19.029, 153.333±7.838, 136.00±25.000, 212.50±6.500 and 78.500±2.500 with P-value (P=0.9675) respectively.

Organs' weight (g)

Current study evaluate that the organs' weight of experimental rats differ significantly among the treatment group. The weight of kidney decreased significantly in diabetic group as compare to control group (P=0.0679).The liver weight of experimental rats of group A, B, C, D and E with P-value (P=0.0243) is 6.200±0.500, 6.566±0.266, 6.750±0.550, 9.050±0.350 and 6.700±0.600 respectively. The kidney weight of experimental rats were 1.366±0.176, 1.600±0.0577, 1.750±0.050, 2.00±0.100 and 6.700±0.600 respectively. The experimental rats having the pancreas weight 0.366±0.088, 0.466±0.033, 0.400±0.100, and 0.800±0.100 and 0.450±0.500 respectively. The pancreas and liver weight of experimental rats differ significantly among treatment groups (P=0.0389).

Liver performance

The liver parameter was measured at the end of the experiment. The results of liver performance were as following.

Alanine aminotransferase (U/L)

According to the results it is observed that the concentration of ALT (U/L) of supplemented groups increased significantly as compare to control group (P=0.1323). The concentration of ALT of group A, B, C, D and E was 89.00±9.00, 44.00±4.00, 54.500±23.500, 50.00±1.000 and 39.500±0.500 respectively.

Aspartate transferase (U/L)

The present study evaluate that the concentration of AST (U/L) of supplemented groups decreased non-significantly as compare to the control group (P=0.7604). The concentration of experimental groups were as following 155.50±13.500, 142.50±0.500, 147.50±26.500, 143.00±3.000 and 164.00±3.00 respectively.

Alkaline phosphatase (U/L)

The results of present study show that the concentration of ALP(U/L) of supplemented groups increased non-significantly as compare to control group (P=0.2934).The concentration of ALP of group A, B, C ,D and E was as following 259.25±15.15, 210.25±62.65, 180.65±58.45 178.95±3.650 and 121.75±0.55 respectively.

Total proteins (g/dL)

The present results revealed that the concentration of TP (U/L) differ non-significantly as compare to control group (P=0.0008).The concentration of group A, B, C, D and E was 5.700±0, 5.900±0.300, 6.350±0.050, 7.850±0.050 and 5.750±0.100 respectively.

Albumin (g/L)

The concentration of ALB (U/L) decreased non-significantly in experimental rats (P=0.6447).The concentration of group A, B, C, D and E was 35.55±1.750, 35.75±1.050, 28.950±8.450, 33.75±2.100 and 37.75±1.950 respectively.

Gamma-glutamyle transferase (U/L)

The present study revealed that the concentration of GGT (U/L) differ non-significantly among the experimental groups (0.9178). The concentration of GGT among group A, B, C D, and E was 2.050±0.650, 2.800±0.400, 2.500±1.00, 2.550±0.350 and 2.550±0.050 respectively.

Bilirubin (mg/dL)

The present study revealed that the concentration of BIL (U/L) of supplemented groups increased significantly as compare to control group (P=0.0037). The concentration of group A, B, C, D and E was 0.055±0.005, 0.050±0, 0.045±0.005, 0.085±0.005 and 0.035±0.005 respectively.

Renal performance

According to the currents study the results shows that the

concentration of urea (mg/dL), and creatinine(mg/dL), of supplemented group decreased significantly as compare to diabetic control group (P=0.041) and (P=0.0040). Whereas, concentration of HbA_1C (percent) differ non-significantly among supplemented groups (P=0.3168). The concentration of urea of experimental group A, B, C, D and E was 38.500±2.500, 38.00±1.00, 36.00±8.000, 51.500±5.500 and 22.00±1.00. The concentration of creatinine was 0.350±0.050, 0.350±0.050, 0.300±0, 0.750±0.050 and 0.350±0.050.Whereas the concentration of HbA1C was 5.665±1.555, 3.700±0.070, 3.410±0.200, 3.765±0.445 and 3.710±0.160 respectively.

Lipid profile

The results of current study shows that after the experiment the concentration level of lipid profile differ significantly (P≤0.05). The concentration level of Cholesterol (mg/dL) differ significantly among experimental groups (P=0.0447). The concentration level of cholesterol (mg/dL) of experimental groups A, B, C, D and E were 59.00±6.00, 64.00±3.00, 43.500±12.50, 83.500±5.500 and 46.500±0.500 respectively. The concentration of triglyceride (mg/dL) and HDL (mg/dL) decreased significantly. Whereas the concentration of LDL (mg/dL) of supplemented group increased significantly as compare to control group (P=0.1536). The concentration of triglyceride of the supplemented groups A, B, C, D and E was 105.0±3.500, 112.50±1.500, 99.500±1.500, 160.00±3.00 and 123.50±2.500 respectively. The concentration of HDL of experimental groups was 38.08±3.780, 28.160±1.760, 32.720±11.38, 12.050±0.850, 15.300±1.00 whereas, the concentration LDL of experimental rats was 23.060±0.370, 15.515±5.275, 14.140±2.740, 27.100±5.800 and 13.385±0.1800 respective.

Table 2: Mean ± SEM value of body weight (g) and serum glucose (mg/dL) of albino rats fed with basal diet and basal diet supplemented with different plants' extracts (table 3).

Parameters	Feeding groups					
	P. guajava	***S. cumini***	***P. guajava + S. cumini***	**Diabetic control**	**Normal control**	**P. Value**
IBW	216.33± 11.9758^{ab}	233.000± 4.725^{a}	188.50± 16.50^{b}	193.50± 7.500^{b}	186.500± 4.500^{b}	0.0449
FBW	178.00± 41.01^{a}	233.00± 4.725^{a}	197.50± 50.50^{a}	207.00± 4.000^{a}	215.00± 6.000^{a}	0.9675
IG	340.666± 38.167^{a}	300.00± 58.968^{a}	274.00± 87.00^{a}	219.00± 8.000^{ab}	79.500± 2.000^{b}	0.0656
FG	152.333± 19.029^{d}	153.333± 7.838^{b}	136.00± 25.000^{b}	212.50± 6.500^{a}	78.500± 2.500^{c}	0.0086

Mean ± SEM values within rows with different alphabets are significantly (p≤0.05) different. **IBW:** Initial body weight, **FBW:** Final body weight, **IG**: initial glucose and **FG**represent`t the final glucose level of streptozotocin-induced diabetic albino rats.

Table 3: Mean ± SEM values of Kidney, Liver and Pancreas weight (g) of albino rats fed with basal diet and basal diet supplemented with different plants' extracts.

Parameters	Feeding groups					
	P. guajava	***S. cumini***	***P. guajava + S. cumini***	**Diabetic control**	**Normal control**	**P. Value**
Liver	6.200± 0.500^{b}	6.566± 0.266^{b}	6.750± 0.550^{b}	9.050± 0.350^{a}	6.700± 0.600^{b}	0.0243
Kidney	1.366± 0.176^{b}	1.600± 0.0577^{ab}	1.750± 0.050^{ab}	2.00± 0.100^{a}	6.700± 0.600^{ab}	0.0679
Pancreas	0.366± 0.088^{b}	0.466± 0.033^{b}	0.400± 0.100^{b}	0.800± 0.100^{a}	0.450± 0.500^{b}	0.0389

Mean ± SEM values within rows with different alphabets are significantly (p≤0.05) different.

Table 4: Mean ± SEM values of Liver function parameters (ALT, AST, ALP, TP, ALB, GGT and BIL) of albino rats fed with basal diet and basal diet supplemented with different plants' extracts.

Parameters	Feeding groups					
	P. guajava	***S. cumini***	***P. guajava + S. cumini***	**Diabetic control**	**Normal control**	**P. Value**
ALT	89.00± 9.00^{a}	44.00± 4.00^{b}	54.500± 23.500^{ab}	50.00± 1.000^{ab}	39.500± 0.500^{b}	0.1323
AST	155.50± 13.500^{a}	142.50± 0.500^{a}	147.50± 26.500^{a}	143.00± 3.000^{a}	164.00± 3.00^{a}	0.7604
ALP	259.25± 15.15^{a}	210.25± 62.65^{a}	180.65± 58.45^{a}	178.95± 3.650^{a}	121.75± 0.55^{a}	0.2934
TP	5.700±	5.900±	6.350±	7.850±	5.750±	0.0008

	0[c]	0.300[bc]	0.050[b]	0.050[a]	0.100[c]	
ALB	35.55± 1.750[a]	35.75± 1.050[a]	28.950± 8.450[a]	33.75± 2.100[a]	37.75± 1.950[a]	0.6447
GGT	2.050± 0.650[a]	2.800± 0.400[a]	2.500± 1.00[a]	2.550± 0.350[a]	2.550± 0.050[a]	0.9178
BIL	0.055± 0.005[b]	0.050± 0[bc]	0.045± 0.005[e]	0.085± 0.005[a]	0.035± 0.005[c]	0.0037

Mean ± SEM values within rows with different alphabets are significantly (p≤0.05) different. **ALT:** alanine aminotransferase, **AST:** aspertate phosphatase**, ALP:** alkaline phosphatase**, TP:** total proteins**, ALB:** albumins, **GGT:** gamma-glutamyletransferase and **BIL** represent the bilirubin of streptozotocin-induced diabetic albino rats.

Table 5: Mean ± SEM values of urea, creatinine and HbA1C of albino rats fed with basal diet and basal diet supplemented with different plants' extracts.

Parameters	Feeding groups					
	P. guajava	***S. cumini***	***P. guajava* + *S. cumini***	**Diabetic control**	**Normal control**	**P. Value**
Urea	38.500± 2.500[ab]	38.00± 1.00[ab]	36.00± 8.000[ab]	51.500± 5.500[a]	22.00± 1.00[b]	0.0471
Creatinine	0.350± 0.050[b]	0.350± 0.050[b]	0.300± 0[b]	0.750± 0.050[a]	0.350± 0.050[b]	0.0040
HbA_1C	5.665± 1.555[a]	3.700± 0.070[a]	3.410± 0.200[a]	3.765± 0.445[a]	3.710± 0.160[a]	0.3168

Mean ± SEM values within rows with different alphabets are significantly (p≤0.05) different.

Table 6: Mean ± SEM value of lipid profile (CHOL, TRIG, HDL, LDL and VLDL) of albino rats fed with basal diet and basal diet supplemented with different plants' extracts.

Parameters	Feeding groups					
	P .guajava	***S. cumini***	***P. guajava* + *S. cumini***	**Diabetic control**	**Normal control**	**P. Value**
CHOL	59.00± 6.00ab	64.00± 3.00ab	43.500± 12.50b	83.500± 5.500a	46.500± 0.500b	0.0447
TRIG	105.0± 3.500cd	112.50± 1.500c	99.500± 1.500d	160.00± 3.00a	123.50± 2.500b	<.0001
HDL	38.08± 3.780a	28.160± 1.760ab	32.720± 11.38ab	12.050± 0.850b	15.300± 1.00b	0.0730
LDL	23.060± 0.370a	15.515± 5.275a	14.140± 2.740a	27.100± 5.800a	13.385± 0.1800a	0.1536

HDL: High Density lipo-proteins, **LDL:** low density lipo-proteins and **VLDL** represent the very low.

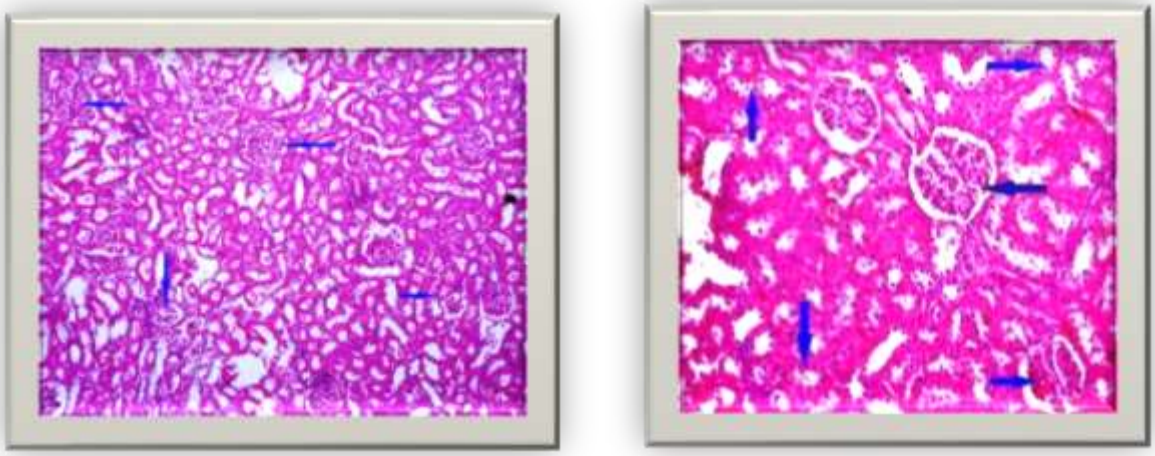


Fig A. Microscopic view of rats' kidney

Fig B. Microscopic view of rats' kidney tissue treated with *S. cumini* extract tissue treated with *P. guajava* extract

Microscopic histological examination of kidney tissues of rats treated with *P. guajava* extract (fig. A), showed that the kidney have mild to moderate renal tubular degeneration in its architecture. No severe inflammation was observed, no hydropic

degeneration seen, No necrosis, atypia, fibrosis, or malignancy seen in the selected tissue. When this slide was compared with the histological view of the diabetic control group, then it was observed severe inflammation and malignancy was observed. Microscopic histological examination of kidney tissues of rats treated with *S. cumini* extract (fig. B), showed that the kidney have normal glomeruli. The normal renal tubules, normal capsules and nephrons were seen. No atypia or malignancy seen. In comparison with other treatment groups and control groups, it was recorded that the supplementation of the plants extract has a positive influence over the architecture of the kidney tissue. When compared with the tissues architecture of fig D, it was much normal and that might be due to the positive influence of the *S. cumini* extract.

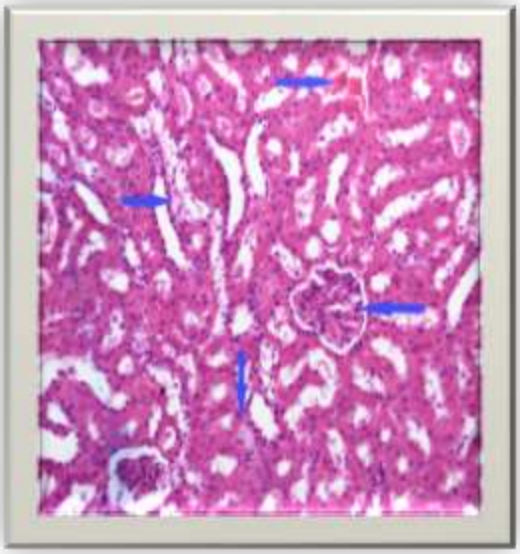


Fig C. Microscopic view of rats' kidney tissue basal diet

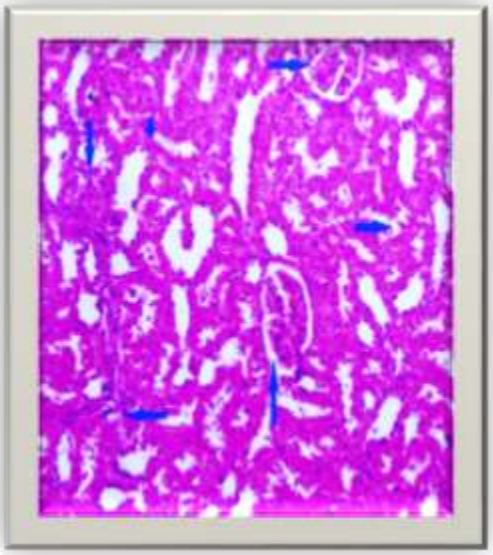
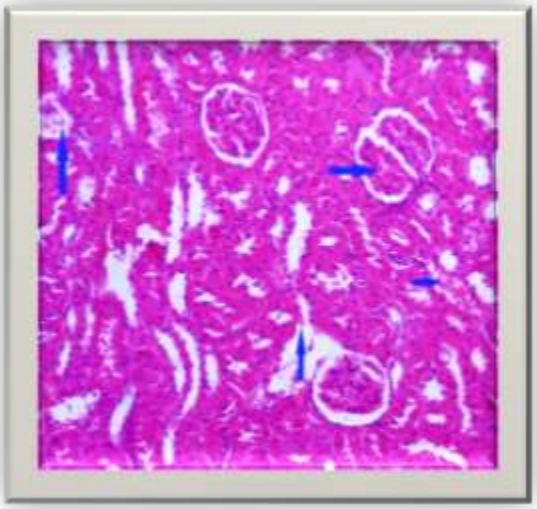


Fig D. Microscopic view of diabetic rats' (group D) kidney tissue fed with treated with *P. guajava* and *S. cumini* extract

Microscopic histological examination of rats' kidney tissues treated with *P. guajava* and *S. cumini* extract (fig. C) revealed normal renal capsule, nephrons and tubules. The kidney revealed normal renal nephrons, renal capsule and tubules. No inflammation has been recorded in the tissues, not necrosis nor fibrosis, atypia or malignant have been observed. This again have been confirmed that the normal architecture of the tissue might be due to the combine effects of the both plants extracts, i.e of *P. guajava* and *S. cumini* extract.

Microscopic histological examination of rats' kidney tissues treated with only basal diet (fig. D), showed that the kidney have mild to moderate nephron degeneration. The mild inflammatory cells aggregation (nephritis) is present at some areas of the tissues. This cell inflammation further cause malfunctioning of the nephron and kidney problems of the diabetic patients, so thirst issues have been very common among the diabetic patients, however, no atypia or malignancy seen in the section. When compared with the tissues of other treatment groups, it has been clearly noted that diabetic metabolism causes the inflammation, but treatments with plants extracts, it moves towards normality.



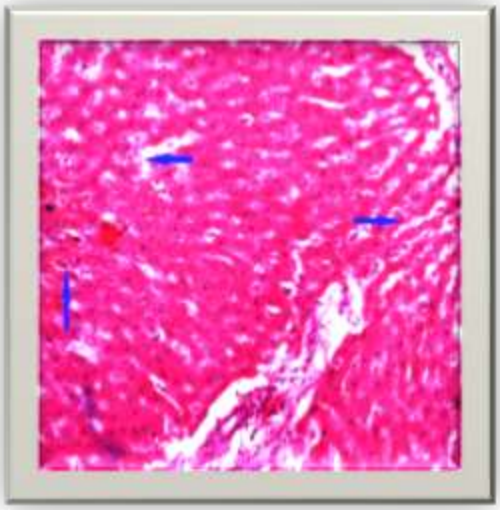
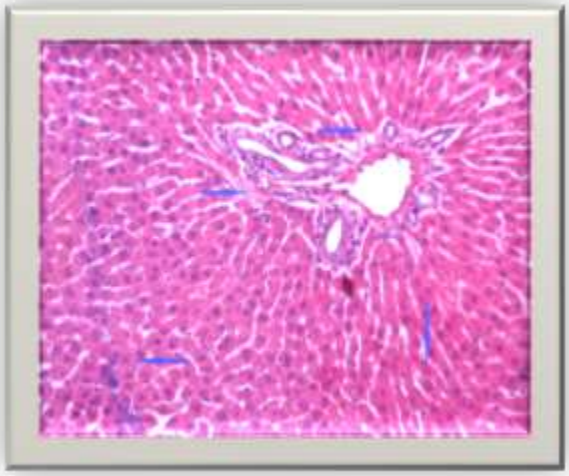


Figure E: Microscopic view of normal rats kidney (group E) tissue fed with basal diet

Figure F: Microscopic view of rats' liver tissue treated with *P. guajava* extract

Microscopic histological examination of rats' kidney tissues treated with basal diets only showed none of the abnormality in the architecture of the kidney tissues (fig. E). The kidney revealed normal renal nephrons, renal capsule and tubules. No inflammation has been recorded in the tissues, not necrosis nor fibrosis, atypia or malignant have been observed.

The histological examination of the liver tissues of rats treated with *P. guajava* extract (fig. F), showed that the liver have normal architecture. No inflammation was observed, no hydropic degeneration seen, No necrosis, atypia, fibrosis, or malignancy seen in the selected tissue. When this slide was compared with the histological view of the diabetic control group, then it was observed severe inflammation and malignancy was observed.



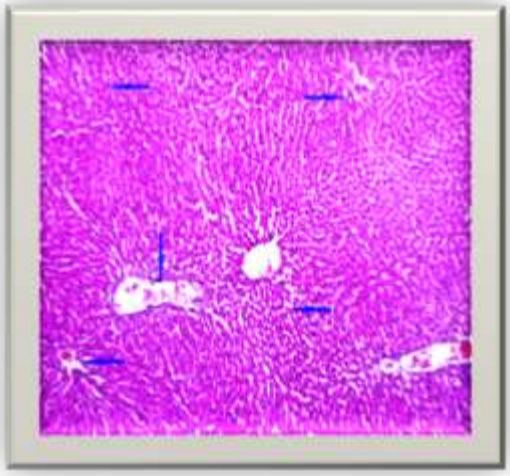
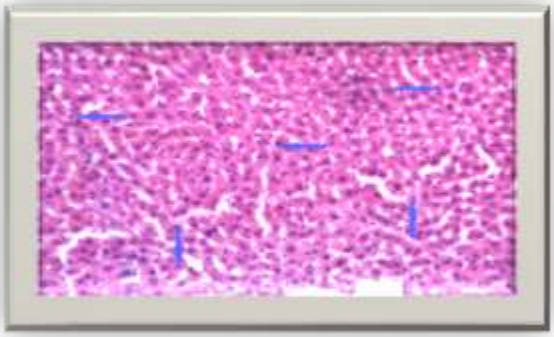


Figure G: Microscopic view of rats' Liver tissue tissue treated with with *P. guajava* and*S.cumini* extract

Figure H: Microscopic view of rats' Liver treated with *S. cumini* extract

Histological examination of the submitted liver of rats fed diet supplemented with *S. cumini* extract reveals normal hepatic architecture. No inflammation, hydropic degeneration, fibrosis, necrosis, atypia or malignancy seen, no atypia or malignancy seen in the section. When compared with the tissues of other treatment groups, it has been clearly noted that diabetic metabolism causes the inflammation, but treatments with plants extracts, it moves towards normality.

Microscopic histological examination of rats liver tissues treated with *P. guajava* and *S. cumini* extract (fig. H) revealed normal hepatocytic architechture, hepatocytes. No inflammatory cells aggregation seen around hepatic rim. No inflammation has been recorded in the tissues, not necrosis nor fibrosis, atypia or malignant have been observed. This again have been confirmed that the normal architecture of the tissue might be due to the combine effects of the both plants extracts, i.e of *P. guajava* and *S. cumini* extract.



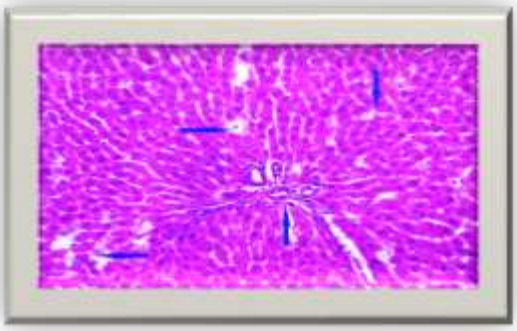


Figure i. Microscopic view of diabetic rats' liver only (group D) liver (group E) tissue fed with basal diet

Figure J. Microscopic view of normal rats' tissue treated with basal diet

Histological examination of the submitted liver tissues of rats fed diet with no extract (fig. I), showed moderate hepatocytic degeneration of the hepatocytes. The inflammatory cells of the hepatic tissue aggregations are present and are more prominent around hepatic rim. The hepatic array appears normal. No atypia or malignancy seen.

Microscopic histological examination of rats kidney tissues treated with basal diets only showed none of the abnormality in the architecture of the kidney tissues (fig. J). The kidney revealed normal renal nephrons, renal capsule and tubules. No inflammation has been recorded in the tissues, not necrosis nor fibrosis, atypia or malignant have been observed.

DISCUSSION

Diabetes mellitus is one of the metabolic disorder that happens when blood glucose level elevated (De-Fronzo *et al.,* 2015) due to fault in insulin emission or insulin action (Pickup *et al.,* 2003). Various method has been used for the treatment of diabetes mellitus including; allopathic homeopathic and herbal therapy. In different countries like Taiwan and Japan, people boil the guava leaves in water and drink this extract as a medicine for the treatment of diabetes. The *Syzygium cumini* fruit possess high quality medicinal functions against diabetes as it effects on pancreas (Modi *et al.,* 2010). A number of experiments and researches with different techniques have been performed since long to present day for the treatment of the diabetes but a successful treatment has not been discovered yet. Herbal products have shown a significant role in the prevention and treatment of the disease. For this study two important herbs have been used to define their importance and role in the treatment of the diabetes with hypothesizing that the *P. guajava* and *S. cumini* may have anti-hyperglycemic, hypolipedemic and health promoting effects in the diabetic organisms.

Body weight

In the present study, the leaf extracts of *P. guajava* and *S. cumini* were used to check their improving liver performance, lipid abnormalities and hypoglycemic potential in streptozotocin-induced diabetic male albino rats.It was observed that the initial body weight of supplemented groups of experimental rats increased significantly as compare to control group with (P=0.044). Whereas, the final body weight of the experimental rats decreased non-significantly as compare to control group (P=0.967). This difference in body weight was due to hunger, thirst or increased in diabetes level. These results are similar with the initial outcomes of several researchers. It was reported by Hong*et al.* (2019) that the compound grain of the plants improve the obesity and lipid profile of the diabetic rats, so lowers the body weight.

Glucose level (mg/dL)

According to the present study the final glucose level of diabetic rats decreased significantly as compare to diabetic control group. The results are in accordance to the Batol *et al.* (2016) who conducted a study on therapeutic effects of *C. colocynthis* fruit in patients with type-II diabetes and the results showed the lowering blood glucose level. They observed that diabetes mellitus is a chronic metabolic disorder, which is caused by the deficiency of insulin. The results are also in accordance withAhmad *et al.* (2017) who reported that *P. cumini* extract was given to male rats to examine hyperglycemic properties for sixty days. The insulin level and serum glucose of rats was monthly evaluated. According to the results it is observed that both the seed and fruit extracts reduce the blood glucose level and regulate the insulin level in hyperglycemic rats. Similarly, Shadia *et al*. (2018) also presented a study on effect of *P. guajava* leaf extract, glibenclamide and their combination on rat model of diabetes induced by streptozotocin and reported that the serum insulin level was decreased significantly and glucose level was increased significantly and the aqueous extract of guava leaf improved the reduced insulin level and high glucose level in diabetic rats. The finding of study how that the aqueous extract of guava leaf has effective anti-hyperglycemic effects on diabetic rats.

Organs' weight

Toluwani *et al*. (2019) conducted a study on the effect of *P. guajava* aqueous leaf extract on liver glycogen enzymes, hormone sensitive lipase and serum lipid profile in diabetic rats. After the treatment period, blood, liver and adipose tissue samples were collected from the euthanized animals. According to the results it was observed that *P. guajava* extract decreased the Hormone Sensitive-Lipase (HSL) activity in adipose tissue and liver of diabetic animal and thus increases the glycogen level. Current study evaluate that the organs' weight of experimental rats differ significantly among the treatment group. The weight of liver and pancreas differ significantly among the treatment groups and the weight of kidney decreased significantly as compare to control groups (P=0.067). Accordingto the present findings it was observed that the weight of organs' (liver and pancreas) was improved whereas, the weight of kidney decreased by the plants extracts supplementation. This increase and decreased in organs' weight might be due to some vascular and muscular infections caused by accumulation of glucose in the tissues and abnormal metabolism.

Liver Performance

According to the results it is observed that the concentration of ALT (U/L) increased significantly as compare to normal control group. The concentration of AST (U/L) and ALB (U/L) decreased non-significantly as compare to control group. ALP (U/L) and GGT (U/L) differ non-significantly and the concentration of TP (U/L) and BIL (U/L) differ significantly among experimental group. The present studies are similar to previous researches. Shadia *et al*. (2018) reported that the activities of AST, ALT, ALP, and the level of bilirubin, urea, creatinine and uric acid increased significantly in diabetic rats. The present study show that the aqueous extract of guava leaf improved the reduced insulin level and high glucose level in diabetic rats. The finding of study how that the aqueous extract of guava leaf has effective anti-hyperglycemic effects on diabetic rats.

Renal performance

The present study elaborates that the concentration level of urea (mg/dL), and creatinine(mg/dL), of supplemented group decreased significantly as compare to diabetic control group (P=0.041) and (P=0.0040). Whereas, concentration of HbA_1C (percent) differ non-significantly among supplemented groups (P=0.3168). The results are in accordance to Shadia*et al*. (2018), who described that when streptozotocin-induced diabetes rats are treated with the extracts of *P. guajava* leaves, the concentration level of urea, creatinine and uric acid decreased significantly. The present findings showed that the reduction in the kidney performance markers might be due to the fact that the supplementation of the plants extract can improve the metabolic pathways by normalizing the liver enzyme concentration. As urea cycle occurs in the liver and the improved health condition of the liver might be co-related with the kidney performance.

Lipid profile

The results of current study shows that after the experiment the concentration level of lipid profile differ significantly (P≤0.05). The concentration level of Cholesterol (mg/dL) differ significantly among experimental groups (P=0.0447). The concentration of triglyceride (mg/dL) and HDL (mg/dL) decreased significantly. Whereas the concentration of LDL (mg/dL) of supplemented group increased significantly as compare to control group

(P=0.1536). The lipid profiles were assessed in both plasma and liver tissue of diseased and control rats that results the levels of total cholesterol, triglycerides, free fatty acids, phospholipids, and low-density lipoprotein cholesterol were increased, while, the level of high-density lipoprotein cholesterol was decreased in CCl4-induced hepatotoxic rats. The present study accomplished that the *P. guajava* leaf extracts can notably regulate lipid metabolism in CCl4-induced hepatotoxic rats and reduce the disease rate.

CONCLUSION

The leaf extracts of *P. guajava* and *S. cumini* presented a significant improvement in final body weight and serum glucose level in streptozotocin-induced diabetic albino rats. Likewise, other health promoting markers of body was significantly improved with the treatment of mixing of both plant extracts. It is therefore concluded that the extracts of both plantscan be used synergistically for the management of diabetes.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

AM designed the study,RA write the whole manuscript,MS arrange the data according to the journal's requirement,MAI and MS revised the manuscript,JK and SA draw the figures,SK,SS and UI help in writing whole summary and ABS make amendments in paper after revision.

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