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Antidiabetic and renoprotective effect of *Fagonia cretica* L. methanolic extract and *Citrus paradise* Macfad. juice in alloxan induced diabetic rabbits

[Efecto antidiabético y renoprotector del extracto metanólico de *Fagonia cretica* L. y el jugo de *Citrus paradise* Macfad. en conejos diabéticos inducidos por aloxano]

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Abstract

Context: *Fagonia cretica* is a medicinal herb reported to have flavanoids of potential therapeutic value and *Citrus paradisi* is a fruit, whose juice is of great therapeutic value due to its anti-hyperglycemic effects.

Aims: To determine anti-hyperglycemic and renal protective effect of methanolic extract of *Fagonia cretica* and *Citrus paradisi* juice (grapefruit juice) in alloxan induced diabetic rabbits.

Methods: Diabetes was induced in rabbits by alloxan monohydrate (150 mg/kg, i.p.). The therapies including *Fagonia cretica* methanolic extract (500 mg/kg), *Citrus paradisi* juice (7 mL/kg) and sitagliptin (10 mg/kg) were administered (p.o.) to diabetic groups for 14 days. The biochemical parameters, glucose, creatinine, urea, bilirubin, albumin, total protein, globulins and albumin/globulin ratio were estimated.

Results: *Fagonia cretica* extract and grapefruit juice therapy significantly ($p<0.05$) reduced glucose levels in diabetic rats. *Fagonia cretica* extract was more effective anti-hyperglycemic agent than *Citrus paradisi* juice and sitagliptin. Significant ($p<0.05$) improvement in kidney function was observed in treated groups, the plant extract showing significant improvement as compared to the other two treatments. The histopathological results verified improvement in structural damage of kidney, liver and pancreas with these treatments.

Conclusions: *Fagonica cretica* and *Citrus paradisi* juice therapy markedly improved hyperglycemia and kidney functions in alloxan-induced diabetic rabbits.

Keywords: alloxan; *Citrus paradise*; diabetes; *Fagonia cretica*; kidney.

Resumen

Contexto: *Fagonia cretica* es una hierba medicinal que posee flavonoides de potencial valor terapéutico y *Citrus paradisi* es una fruta cuyo jugo es de gran valor terapéutico debido a sus efectos antihiperglicémicos.

Objetivos: Determinar el efecto antihiperglicémico y protector renal del extracto metanólico de *Fagonia cretica* y el jugo de *Citrus paradisi* (jugo de toronja) en conejos diabéticos inducidos por aloxano.

Métodos: La diabetes fue inducida en conejos por monohidrato de aloxano (150 mg/kg, i.p.). Se administraron (p.o.) los tratamientos incluyendo el extracto metanólico de *Fagonia cretica* (500 mg/kg), jugo de *Citrus paradisi* (7 mL/kg) y sitagliptina (10 mg/kg) a grupos diabéticos durante 14 días. Se estimaron los parámetros bioquímicos, glucosa, creatinina, urea, bilirrubina, albúmina, proteína total, globulinas y relación albúmina/globulina.

Resultados: El extracto de *Fagonia cretica* y la terapia con jugo de toronja ($p<0,05$) redujeron significativamente los niveles de glucosa en ratas diabéticas. El extracto de *Fagonia cretica* fue un agente anti-hiperglucémico más eficaz que el jugo de *Citrus paradisi* y sitagliptina. Se observó una mejoría significativa ($p<0,05$) en la función renal en los grupos tratados, el extracto de la planta mostró una mejoría significativa en comparación con los otros dos tratamientos. Los resultados histopatológicos verificaron mejoría en el daño estructural del riñón, hígado y páncreas con estos tratamientos.

Conclusiones: La terapia con extracto de *Fagónica cretica* y jugo de *Citrus paradis*i mejoró notablemente la hiperglucemia y las funciones renales en conejos diabéticos inducidos por aloxano.

Palabras Clave: aloxano; *Citrus paradise*; diabetes; *Fagonia cretica*; riñón.

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INTRODUCTION

Diabetes mellitus is a pandemic of 21st century and is growing faster day by day. In the present era natural and less toxic remedies are sought out for cure and management of diseases such as hypertension, cancer, liver diseases and diabetes mellitus (Ginter and Simko, 2009). With increasing incidence of diabetes, the prevalence of diabetic nephropathy is increasing in most countries and 40% of the patients diagnosed with diabetes are supposed to develop kidney disease (Mcknight et al., 2015). Medicinal plants have been used throughout human history for various diseases in humans. Plants synthesize wide variety of chemicals such as glycosides, alkaloids, flavonoids, and terpenoids, which have been identified with several pharmacological actions. It has been reported that 12 000 such compounds have been isolated and identified from plants possessing various pharmacological activities (Tapsell et al., 2006).

Fagonia cretica L. belongs to family Zygophyllaceae found in dry habitat all over Pakistan, commonly known as Dhamasa, Drahma or Dhamai botti. It contains flavonoids and triterpenoids, which have antioxidant activity and can be beneficial for disease where reactive oxygen species is involved. It also has anticancer, antioxidant (Iqbal et al., 2014) and antimicrobial activities (Gehlot and Bohra, 2000). *Fagonia cretica* has been reported to possess antidiabetic action when used in combination with stem of *Aloe vera* and fresh branches of *Tylophora hersuta* L (Ahmad et al., 2004). It showed *in vitro* activity of dipeptidyl peptidase-4 (DPP-4) inhibition, due to presence of quinovic acid, which can be helpful in diabetes management (Saleem et al., 2014). Further studies are required to evaluate the plant's pharmacological potential for it's safe utilization in humans (Qureshi et al., 2015).

Citrus paradisi Macfad. (grapefruit, family Rutaceae) is a fruit of subtropical areas, which is sour to semi-sweet in taste. Since ancient time grapefruit is associated with health benefits and is added in diet to reduce obesity. It reduces total cholesterol, triglycerides and low-density lipoproteins (Owira and Ojewole, 2010). Grapefruit consumption is associated with weight reduction and reduction in insulin resistance (Hayanga et al., 2016).

The aim of current study was to determine, estimate and compare antidiabetic activity of *Fagonia cretica* and *Citrus paradisi* with sitagliptin in rabbits. The renal profile was estimated to establish the renoprotective effects of the therapies in diabetic rabbits. Histopathological analysis of liver, pancreas and kidney showed the effectiveness of the therapies on cellular level.

MATERIAL AND METHODS

Chemicals

Alloxan monohydrate, methanol and formalin were purchased from Sigma Aldrich, Germany; sitagliptin phosphate from Sunji and company, China; dextrose monohydrate from Zheijang chemicals I/E corp. China; methanolic extract of *Fagonia cretica* from Common name Dhamasa or Dhamai botti, Kasur (Pakistan); and distilled water, normal saline solution from Medisole, Medipak pharma (Pakistan).

Collection of samples

Fagonia cretica (3 kg) was collected from East of Kasoor 2 km before Ganda Singh border 31°9'40" N 74°19'42" E in April 2014 Pakistan and fresh *Citrus paradisi* (5 kg) was purchased from the local market of Lahore, Pakistan. Both samples were identified by Dr. Uzma Hnaif, Assistant Professor Department of Botany, Government College University Lahore, and voucher numbers GC. Herb. Bot. 1929 and GC. Herb. Bot. 1930 were issued respectively.

Preparation of samples

The juice of the grapefruit was prepared daily using the pulp of the fruit in the juicer machine (Phillips). A quantity of 800 g of whole dried plant of *Fagonia cretica* was pulverized and extracted with methanol (1 L) by using mechanical shaker at 40 rpm (Memmert, Germany) for three days. After three days, the filtrate was separated and evaporated using rotary evaporator and placed in oven at 40°C until completely dried. Later the dried extract was stored in refrigerator (2-8°C) for further use. The percentage yield of *Fagonia cretica* plant ex-

tract was 5%. The powder was again soaked in methanol 1 L methanol for three days. The process was again repeated twice.

Animals

Forty adult rabbits of local breed with weight between 1- 1.5 kg were purchased from the market and acclimatized in the animal house of Punjab University College of Pharmacy, University of the Punjab for 7 days. The rabbits were placed in iron cages in the animal house with 12 hours light and dark period. They were fed on standard green fodder and water *ad libitum*. All protocols were approved by the Animal Ethical Committee of Punjab University College of Pharmacy, University of the Punjab, that are established under the guidelines of NIH guide for the care and use of laboratory animals, under the voucher no: AEC/UCP/1034/4313. The animals were kept under standard conditions throughout the experiment to reduce the error. Minimum number of animals were used to obtain reliable results.

Induction of diabetes

Food was withdrawn ten hours before the injecting alloxan monohydrate. A 10% solution of alloxan monohydrate (150 mg/kg) was injected intraperitoneally by dissolving in 0.9% saline solution (Ahmad et al., 2014). After 14 days, the animals having blood glucose levels greater than 200 mg/dL were included in the study.

Selection of dose of Citrus paradisi juice and Fagonia cretica extract

A preliminary group of animals was formed for the determination of dose for further studies. Fifteen rabbits were injected alloxan by the procedure as mentioned above and the animals with fasting blood glucose levels greater than 200 mg/dL were divided into three groups (n=4). The grapefruit juice was administered in doses of 5, 7 and 10 mL/kg separately to three groups (Table 1). While, the methanolic extract of *Fagonia cretica* was administered in doses of 25, 500, 750 and 1250 mg/kg orally separately to three groups (Table 2) and after the administration of the administration of treatments, blood glucose levels were determined at 2, 4, 6 and 24 hours.

The dose of grapefruit juice and *Fagonia cretica* extract that has maintained the glucose levels for up to 24 hours according to the criteria mentioned by International Diabetes Federation was selected for the further studies (Ceriello and Colagiuri, 2008). From the above study 7 mL/kg dose of grapefruit juice and 500 mg/kg of *Fagonia cretica* was selected for future research.

Table 1. Blood glucose levels in alloxan induced diabetic rabbits treated with different doses of grapefruit juice at different time intervals.

Doses (mL/kg)	FBG (mg/dL)				
	0 h	**2 h**	**4 h**	**6 h**	**24 h**
5	267 ± 24.6	277 ± 28.2	291 ± 39.1	278 ± 40.4	119 ± 3.6
7	251 ± 16.3	257 ± 24.5	225 ± 74.8	184 ± 48.3	107 ± 5.6
10	271 ± 9.7	284 ± 10.6	141 ± 28.4	120 ± 14.3	115 ± 3.5

FBG: Fasting blood glucose. Data represent mean ± SEM of four animals in each group.

Table 2. Blood glucose levels in alloxan induced diabetic rabbits treated with different doses of *Fagonia cretica* at different time intervals.

Dose (mg/kg)	FBG (mg/dL)				
	0 h	2 h	4 h	6 h	24 h
250	250 ± 7.2	219 ± 3.7	203 ± 2.3	195 ± 2.5	242 ± 5.4
500	242 ± 5.4	194 ± 3.9	108 ± 1.8	90 ± 2.9	115 ± 2.7
750	275 ± 26.1	177 ± 19.3	95 ± 9.4	85 ± 3.6	87 ± 5.3
1250	271 ± 31.2	146 ± 15.7	77 ± 1.9	76 ± 2.3	91 ± 5.2

FBG: Fasting blood glucose. Data represent mean ± SEM of four animals in each group.

Experimental design and treatments

A second group of thirty fresh rabbits were divided into 5 groups (n=6).

Negative Control (A): consisted of normal rabbits (n=6) and were administered distilled water at the interval when other groups were given respective treatments.

Other 4 groups (n=6) were treated with alloxan and animals having blood glucose levels greater than 200 mg/dL were included in the study.

Diabetic control group (B): The diabetic rabbits (n=6) were administered distilled water orally at the time of dosing of other groups throughout the study.

Plant extract treated group (C): The animals were given 500 mg/kg methanolic extract of *Fagonia cretica* in distilled water orally for 14 days. The extract was prepared daily by dissolving in distilled water prior to administration.

Grapefruit treated group (D): Grapefruit treated group was administered 7 mL/kg orally of fresh grapefruit juice was for 14 days.

Sitagliptin treated group (E): The sitagliptin treated group was administered standard drug (10 mg/kg) by filling in gelatin capsule of size 2 and administered orally.

All treatments were given in fasting state and food was provided after dosing the animals.

Sampling and testing

Blood sampling was done before starting the treatment (Day zero), on 7th and 14th days of treatment. The blood was collected from the marginal vein of the ear and plasma was separated. Plasma was stored at -20°C in refrigerator for further use. The glucose, albumin, globulin, total proteins, bilirubin, urea and creatinine levels were estimated by using Randox diagnostic kits (Washington, USA).

Histopathological analysis

On 14th day rabbits were sacrificed under anesthesia with chloroform. Liver, kidneys and pancreas were preserved in 26% formalin solution. The histopathological slides were prepared by using hematoxylin-eosin staining and observed under light microscope (Labomed) at 10x and 100x, photomicrographs were taken (Canon Powershot SX 220HS Japan).

Statistical analysis

Statistical analysis was done by GraphPad Prism version 6 using One-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test at 0.05 significant level. All data are presented as mean ± SEM.

RESULTS AND DISCUSSION

Medicinal plants have been used since ancient times with an increase in development of drugs from the plants and natural sources in recent years. Pharmacologist have keen interest in identifying remedies from the plants and natural sources (Tapsell et al., 2006). In our study, we selected a plant from Zygophyllaceae family, *Fagonia cretica*, and grapefruit juice to assess their antihyperglycemic and renoprotective activity.

Effect of different treatments on biochemical parameters

Diabetic control group showed significant ($p<0.05$) rise in glucose level after alloxan injection when compared to normal rabbits. Treatment with methanolic extract of *Fagonia cretica* showed significant decrease in plasma glucose levels on 7th and 14th day of treatment. Similarly, treatment with Grapefruit juice significantly reduced plasma glucose level. Results showed anti-hyperglycemic potential of *Fagonia cretica* and grapefruit as effective as sitagliptin (Table 3). It was observed that methanolic extract of *Fagonia cretica* and fresh grapefruit juice significantly ($p<0.05$) reduced hyperglycemia when compared with diabetic control group. Hypoglycemic effect of *Fagonia cretica* might be due to presence of anti-oxidants like flavonoids and triterpenoids, or quinovic acid, which is a DPP-4 inhibitor (Khaliq et al., 2000). The aqueous extract of *Fagonia cretica* as tea (500 mg/kg) decreased glucose levels in streptozotocin and nicotinamide treated diabetic rats (Nazir et al., 2017). Hypoglycemic effect of grapefruit juice might be due to presence of naringin, which is DPP-4 inhibitor (Parmar et al., 2012). Both *Fagonia cretica* and grapefruit contains flavonoids and flavonoids have the ability to regenerate pancreatic β-cells (Chakravarthy et al., 1981).

Alloxan induces hyperglycemia by generation of ROS and inhibition of glucokinase activity (Ahmad et al., 2014). Hypoglycemic effect of *Fagonia cretica* and grapefruit juice might be due to inhibition of free radicals and activation of glucokinase, which is an insulin sensor of pancreatic β-cells. All the results were comparable with Sitagliptin, used as positive control. The anti-hyperglycemic activity of grapefruit has been found equivalent to that of metformin when administered to mice fed on high fat diet (Jung et al., 2004; Reimer *et al.*, 2014). Grapefruit juice should be taken with caution with metformin as it has shown to exacerbate lactic acidosis (Owira and Ojewole, 2009). Grapefruit juice has been reported to inhibit intestinal CYP3A4 and OATP1A2, therefor anti-diabetic drugs (for e.g. glibenclamide) metabolized by these enzymes should be taken with caution (Lilja et al., 2007). Beneficial effects of naringin, an active component of grapefruit, has been identified in lowering plasma glucose levels in diabetes (Mahmoud et al., 2012). Glucokinase in the liver is most sensitive in glycolytic pathway in diabetic condition. Naringin increases activity of glucokinase thus increases glucose use, storage and decrease gluconeogenesis by decreasing activity of glucose 6-phosphatase thus leads to its hypoglycemic effect. Supplementation with naringin leads to increase insulin production and decrease in insulin resistance by increasing insulin sensitivity (Jung et al., 2004; Chudnovskiy et al., 2014). Naringin showed *in vitro* DPP-4 inhibition activity. *In vivo* studies proved that naringin has hypoglycemic activity when administered to STZ induce diabetic rats. In the rats fed with high fat diet and made diabetic with STZ, naringin proved to reduce oxidative damage due to hyperglycemia and pro-inflammatory cytokine production (Mahmoud et al., 2012). Comparative effect of the treatments used during this study can be observed in Fig. 1A.

Table 3. Comparison of plasma glucose level (mg/dL) of different groups before and after treatment in alloxan induced diabetic rabbits.

Group	Dose	Day Zero	7th Day	14th Day
A- Negative Control	10 mL/kg	109.9 ± 4.6	97.6 ± 5.9	101 ± 6.6
B- Diabetic Control	10 mL/kg	253 ± 14.2^{a}	258 ± 7.7 ^{a}	237 ± 9.4^{a}
C- *Fagonia cretica*	500 mg/kg	266 ± 19.4^{b}	108 ± 8.4^{c}	89 ± 7.5^{c}
D- *Citrus paradisi* juice	7 mL/kg	248 ± 11.8^{b}	107 ± 4.8^{c}	98 ± 7.0^{c}
E- Sitagliptin	10 mg/kg	251 ± 20.0^{b}	114 ± 8.7^{c}	86 ± 6.1^{c}

Results are represented as mean ± SEM; n = 6. $^{a, b, c}$ and d superscript shows the mean significant difference at $p<0.05$ using one-way ANOVA post-hoc Bonferroni multi-comparison test. ^{a} represents comparison of group B with A. ^{b} represents comparison of groups C, D and E with A at day zero. ^{c} represents comparison of groups C, D and E with B. In negative and diabetic groups was used water as vehicle.

Kidney disease due to hyperglycemia is a common complication of diabetes, which leads to end stage renal disease if remain untreated. Urea and creatinine are two important markers of kidney disease (Okada et al., 2015). The total protein, albumin and globulin levels showed a significant decrease after induction of diabetes. Treatment with *Fagonia cretica* extract, grapefruit juice and sitagliptin significantly improved the levels of total protein and albumin, while globulin and albumin/globulin didn't improve significantly (Table 4). In diabetic condition hyperglycemia results in kidney damage, which in turn cause poor renal filtration of urea and creatinine. Poor filtration results in elevated urea and creatinine level in blood (Boland et al., 2014). In current study results showed that blood urea level significantly ($p<0.05$) reduced by methanolic extract of *Fagonia cretica* and grapefruit juice as compared with sitagliptin. While grapefruit juice showed highest decrease in blood urea level. When selected remedies was compared with sitagliptin, results showed that all selected remedies are as effective as sitagliptin in reducing blood urea level by improving kidney function, however grapefruit juice was found to reduce urea levels as presented in Fig. 1D more than sitagliptin. Grapefruit contains flavanoids that have shown to possess capability to trap the free radicals and improve the oxidative status of the body (Schubert et al., 1995). Improvement in kidney function with *Fagonia cretica* and grapefruit juice might be due to restoration of glucose levels and protective effects on renal tubular cells as observed in histopathological analysis. Serum creatinine was also reduced significantly by selected remedies. While *Fagonia cretica* significantly ($p<0.05$) decreased the creatinine levels, therefore it has potential to improve kidney function in diabetic condition. Comparison of urea and creatinine levels before and after treatments can be observe in Fig. 1C and D.

Albuminuria is another manifestation that detects the kidney damage due to diabetes. If hyperglycemia persists and remain untreated it leads toward the diabetic nephropathy and end-stage renal failure (Hull and Agarwal, 2014). In present study, it was clearly observed that hyperglycemia damages kidney, which was analyzed by estimating the reduction in serum total proteins, albumin and globulin. *Fagonia cretica* extract, grapefruit juice and significantly ($p<0.05$) improved total protein and albumin, which clearly depicted that selected remedies have great potential to reduce kidney damage. It could be suggested that these therapies can reduce the incidence of developing diabetic nephropathy and end-stage renal failure. This improvement in kidney function might be due to anti-hyperglycemic activity of *Fagonia cretica*, which was observed in our study. Antioxidant activity of plant might be responsible for amelioration of kidney damage. Grapefruit juice contains several flavonoids, such as naringin, quercetin, kaempferol and lycopene that act as anti-oxidant (Boland et al., 2014), that reduce hyperglycemia and thus kidney damage as seen in our results. However, serum globulin level didn't improve significantly by these treatments, which may require some higher dose. Comparative effect of *Fagonica cretica* and grapefruit juice on albumin, globulin and total protein can be observed in Fig. 1B.

Table 4. Effect of different treatments on total protein, albumin, globulin and albumin/globulin (A/G) ratio after 14th days in alloxan induced diabetic rabbits.

Group	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A/G ratio
A- Negative Control	14.3 ± 0.8	8.1 ± 0.4	6.2 ± 0.6	1.4 ± 0.2
B- Diabetic Control	4.6 ± 0.3[a]	2.9 ± 0.3[a]	1.6 ± 0.3[a]	2.3 ± 0.8[a]
C- *Fagonia cretica*	6.9 ± 0.3[b]	5.6 ± 0.4[b]	1.2 ± 0.3[c]	6.9 ± 2.6[c]
D- *Citrus paradisi* juice	7.6 ± 0.5[b]	5.5 ± 0.4[b]	2.4 ± 0.4[c]	2.5 ± 0.4[c]
E- Sitagliptin	7.4 ± 0.4[b]	5.4 ± 0.2[b]	1.9 ± 0.3[c]	3.2 ± 0.5[c]

Results are represented as mean ± SEM; n = 6. [a] and [b] superscript shows the mean difference is significant at the 0.05 level. [c] represents the mean difference is non-significant at the 0.05 level. [a] represents comparison of group B with A. [b] represents comparison of groups C, D and E with B. [c] represents comparison of groups C, D and E with B.

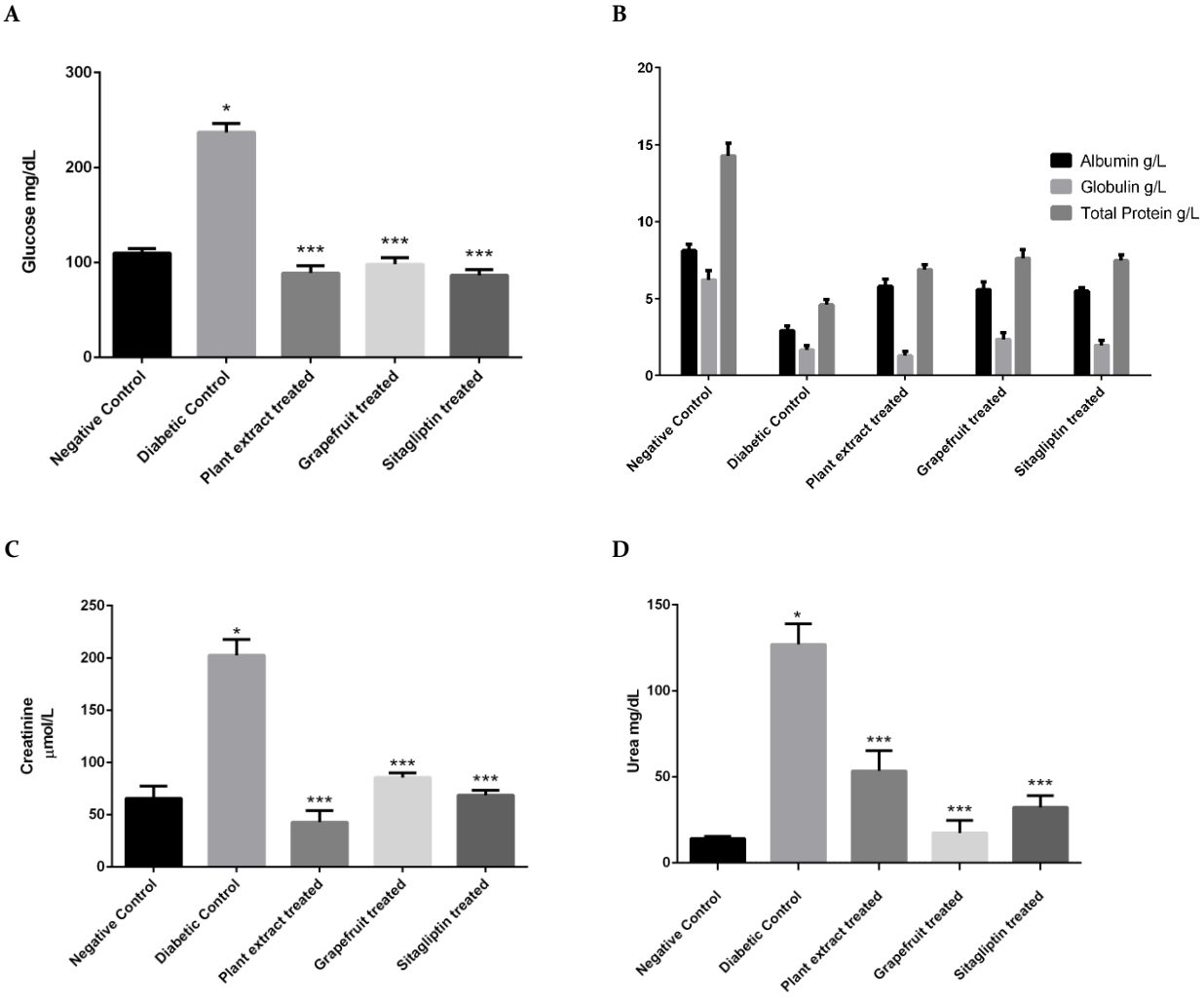

Figure 1. Comparison of different treatments on biochemical parameters in alloxan induced diabetic rabbits. A) glucose levels; B) albumin, globulin and total proteins; C) creatinine levels; D) urea levels and E) bilirubin levels.

Results are represented as mean ± SEM; n = 6. *p<0.05 difference statistically significant with respect to the control group. ***p<0.05 difference statistically significant with respect to the diabetic control.

Control group consisted of normal rabbits (n=6) and were administered with distilled water. Diabetic control consisted in animals (n=6) treated with alloxan having blood glucose levels greater than 200 mg/dL and administered with distilled water orally.

Urea and bilirubin levels significantly ($p<0.05$) increased in diabetic rabbits as compared to normal rabbits. Treatment with *Fagonia cretica* extract, grapefruit and sitagliptin significantly reduced urea and bilirubin concentration as compared to diabetic control group. The levels of creatinine increased in diabetic rabbits. Plant extract significantly ($p<0.05$) reduced creatinine level as compared to diabetic control. Grapefruit juice and sitagliptin also cause significant reduction in creatinine level by improving kidney function (Table 5). Bilirubin excreted during kidney damage causes accumulation of bilirubin in the blood and hepatocellular dysfunction also leads to increased bilirubin concentration in blood (Hull and Agarwal, 2014). In this study, it was observed that total bilirubin concentration significantly increased in diabetic control group, while treatment with *Fagonia cretica* extract and grapefruit juice decreased total bilirubin. Grape fruit juice was most active in decreasing the bilirubin levels as could be observed in Fig. 1E. There was significant decrease in total bilirubin with *Fagonia cretica* extract as well. Decrease in bilirubin level depicted that selected remedies have protective effect on kidney and liver as well.

Histopathological analysis

In Fig. 2A and D showed damage to the Bowman's capsules and distortion of distal and proximal tubules. In Fig. 2B and E the kidney, treated with *Fagonia cretica* extract, showed lesser damage to the Bowman's capsules and preservation of cellular structures of nephron were also clear. The sitagliptin treated kidney in Fig. 2C and F also showed lesser damage to the tubular structures and Bowman's capsule. The liver in Fig. 3A and D treated with alloxan showed damage to the hepatocytes and dilation and enlargement of sinusoids were visible. The improvement in cellular structures and sinusoids by *Fagonia cretica* and sitagliptin could be clearly depicted in Fig. 3B, C, E and F. The alloxan treated pancreas showed morphological damage and depleted islet cells and destroyed acinar cells in Fig. 4A and D. The B and E of the same figure treated *Fagonia cretica* extract showed less shrinkage of the acinar cells with less damage to the islet of Langerhan. The sitagliptin treated pancreas could be observed in Fig. 4C and F showing clusters of normal acinar cells.

The treatment with grapefruit juice has also shown improvement in cellular structures of kidney, liver and pancreas. The grapefruit juice treated kidney, liver and pancreas could be observed in Figs. 5B and E, 5B and E and 6B and E). The grapefruit treated kidney showed intact bowman's capsule with less damage to tubular structures (4B and E) when compared with alloxan treated kidney (4A and D). The grapefruit treated liver showed normal sinusoids with very little dilatation and intact hepatocytes could be clearly depicted in Fig. 6B and E. The Fig. 7B and E showed intact acinar cells with very less damage in certain areas when compared with the alloxan treated pancreas (Fig. 7A and D).

Table 5. Biochemical levels of urea, bilirubin and creatinine after 14th days of different treatments in alloxan induced diabetic rabbits.

Group	Urea (mg/dL)	Bilirubin (µmol/L)	Creatinine (µmol/L)
A- Negative Control	14.0 ± 1.4	6.9 ± 0.92	65.5 ± 11.7
B- Diabetic Control	127.0 ± 12^{a}	39.7 ± 5.4^{a}	99.7 ± 8.8^{a}
C- *Fagonia cretica*	53.0 ± 11.7^{b}	17.3 ± 0.79^{b}	42.8 ± 10.9^{b}
D- *Citrus paradisi* juice	17.3 ± 7.2^{b}	9.8 ± 1.4^{b}	85.5 ± 4.3^{b}
E- Sitagliptin	32.2 ± 6.8^{b}	25.5 ± 2.2^{b}	68.6 ± 4.5^{b}

Results are represented as mean ± SEM; n = 6. ^{a} and ^{b} superscript show the mean difference is significant at the 0.05 level. ^{a} represents comparison of B with A. ^{b} represents comparison of C, D and E with B.

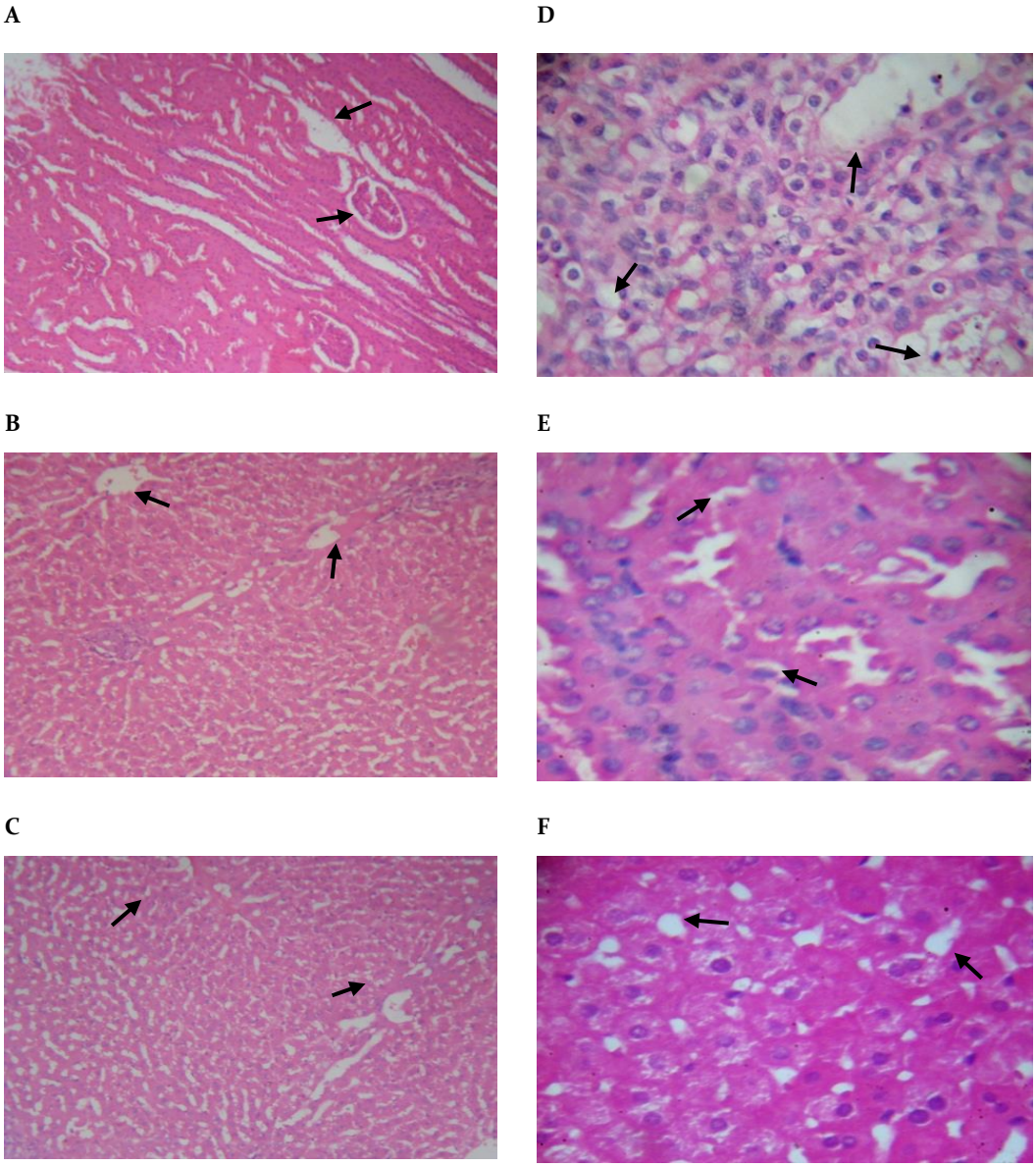

Figure 2. Comparison of *Fagonia cretica* extract treated kidney with diabetic control and sitagliptin treated rabbit kidney in alloxan induced diabetic rabbits.

A and D: Kidney of alloxan induced diabetic rabbit (H&E 10x and 40x, respectively); **B and E**: Kidney of alloxan induced diabetic rabbit treated with *Fagonia cretica* extract (H&E 10x and 40x, respectively); **C and F**: Kidney of alloxan induced diabetic rabbit treated with sitagliptin (H&E 10x and 40x, respectively).

The arrows in **A and D** show abnormal dilation of the tubular structures and distorted bowman's capsule whereas in **B and E** (*F. cretica* extract treated group) and **C and F** (sitagliptin treated group) the arrows show reduction in abnormal dilation with the restoration of nephron structure.

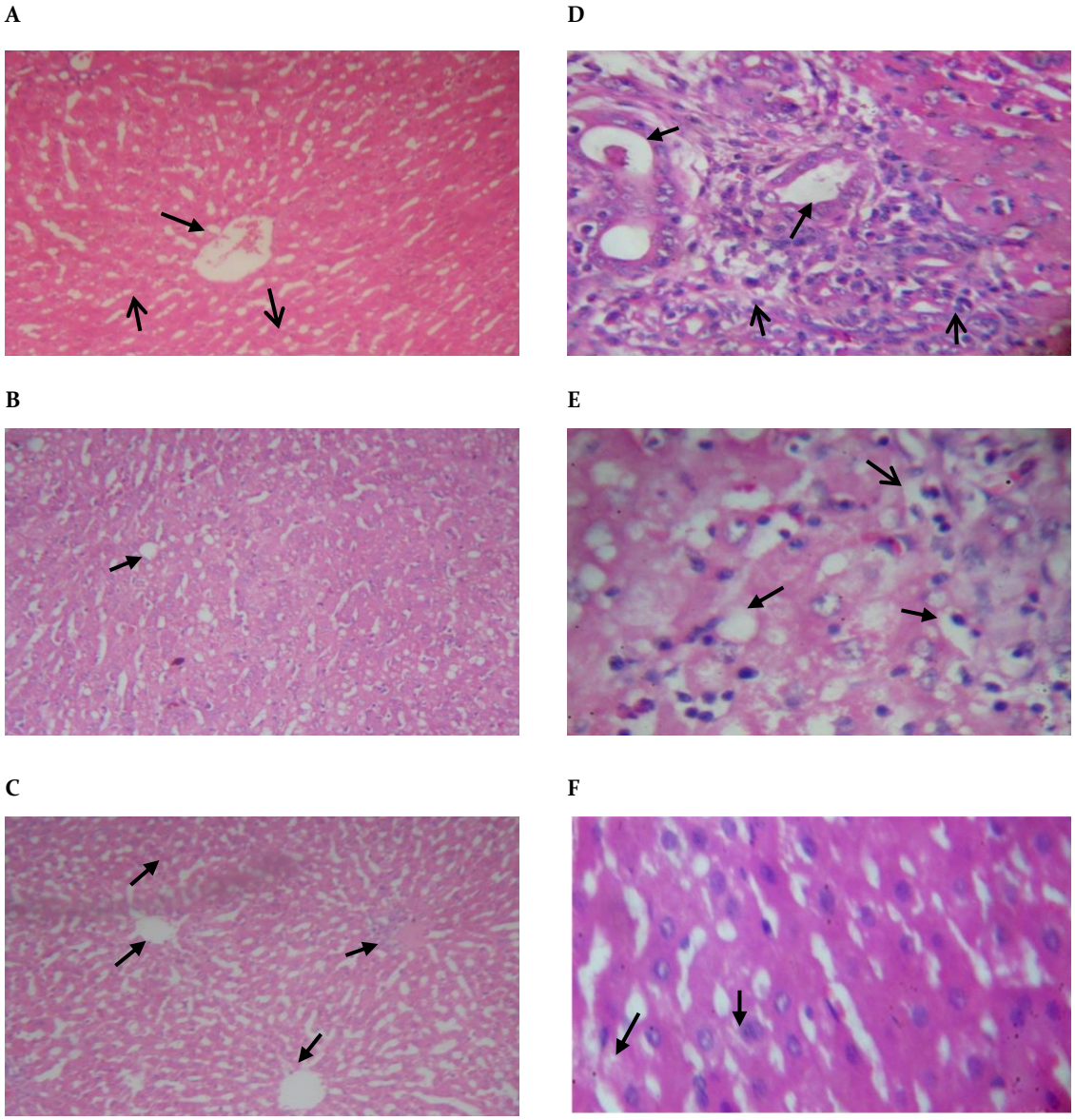

Figure 3. Comparison of *Fagonia cretica* extract treated liver with diabetic control and sitagliptin treated rabbit liver in alloxan induced diabetic rabbits.

A and D: Liver of alloxan induced diabetic rabbit (H&E 10x and 40x, respectively); **B and E**: Liver of alloxan induced diabetic rabbit treated with *Fagonia cretica* extract (H&E 10x and 40x, respectively); **C and F**: Liver of alloxan induced diabetic rabbit treated with sitagliptin (H&E 10x and 40x, respectively).

The arrows in **A and D** show damaged hepatocytes and macrophages. The spaces in the figure show distorted portal veins and bile ducts. In **B and E** (*F. cretica* extract treated group) and **C and F** (sitagliptin treated group) the arrows present normal portal vein, bile ducts and arteries. The restoration of hepatocytes is also visible.

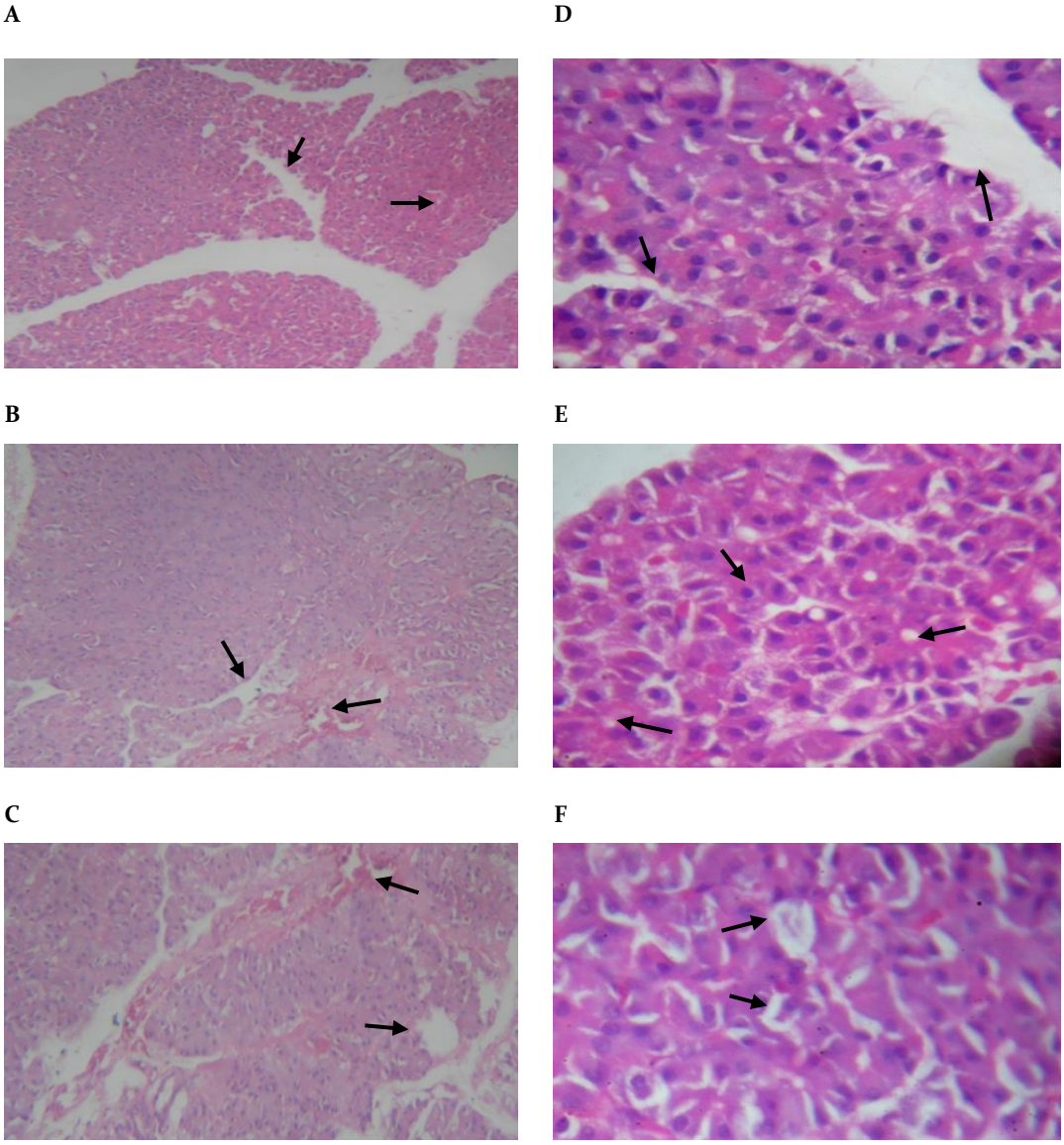

Figure 4. Comparison of *Fagonia cretica* extract treated pancreas with diabetic control and sitagliptin treated rabbit pancreas in alloxan induced diabetic rabbits.

A and D: Pancreas of alloxan induced diabetic rabbit (H&E10x and 40x, respectively); **B and E**: Pancreas of alloxan induced diabetic rabbit treated with *Fagonia cretica* extract (H&E 10x and 40x, respectively); **C and F**: Pancreas of alloxan induced diabetic rabbit treated with sitagliptin (H&E 10x and 40x, respectively).

The arrows in **A and D** present damage to acinar cells and shrinkage of islet of Langerhan. In **B and E** (*F. cretica* extract treated group) and **C and F** (sitagliptin treated group) the arrows present normal acinar cells.

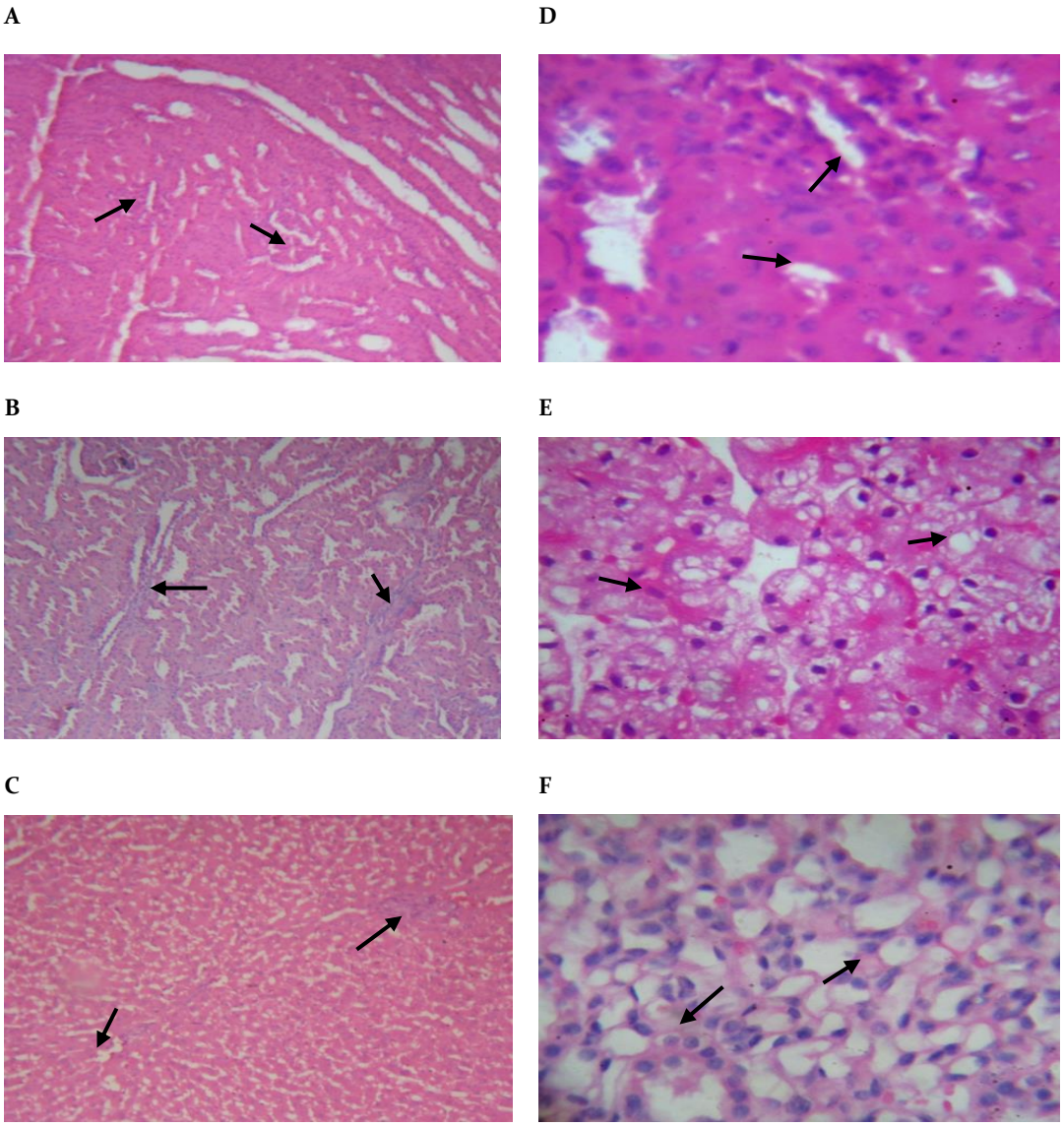

Figure 5. Comparison of *Citrus paradisi* juice treated kidney with diabetic control and sitagliptin treated rabbit kidney in alloxan induced diabetic rabbits.

A and D: Kidney of alloxan induced diabetic rabbit (H&E 10x and 40x, respectively); **B and E**: Kidney of alloxan induced diabetic rabbit treated with grapefruit juice (H&E 10x AND 40x, respectively); **C and F**: Kidney of alloxan induced diabetic rabbit treated with sitagliptin (H&E 10x and 40x, respectively).

The arrows in **A and D** show irregular spacing in bowman's capsule and distorted distal tubule whereas in **B and E** (*C. paradisi* juice treated group) and **C and F** (sitagliptin treated group) the arrows show reduction in abnormal dilation of the distal tubules and restoration of Bowman structure.

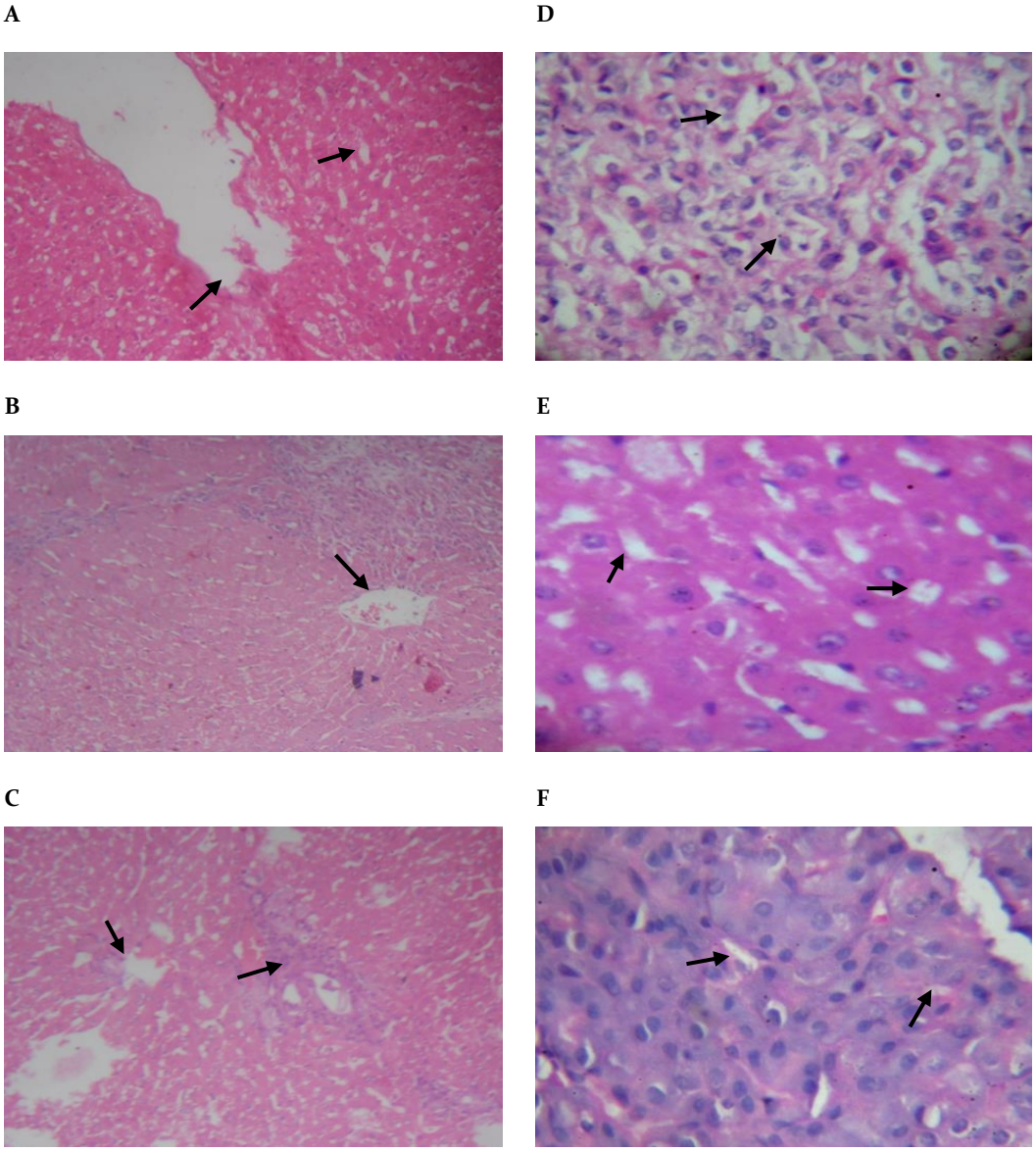

Figure 6. Comparison of *Citrus paradisi* juice treated liver with diabetic control and sitagliptin treated liver in alloxan induced diabetic rabbits.

A and D: Liver of alloxan induced diabetic rabbits (H&E 10x and 40x, respectively); **B and E**: Liver of alloxan induced diabetic rabbit treated with grapefruit juice (H&E 10x, respectively); **C and F**: Liver of alloxan induced diabetic rabbit treated with sitagliptin (H&E 10x and 40x, respectively).

The arrows in **A and D** show damaged hepatocytes and abnormal sinusoids. The spaces in the figure show distorted portal veins. In **B and E** (*C. paradisi* juice treated group) and **C and F** (sitagliptin treated group) cellular structures have been restored with treatments.

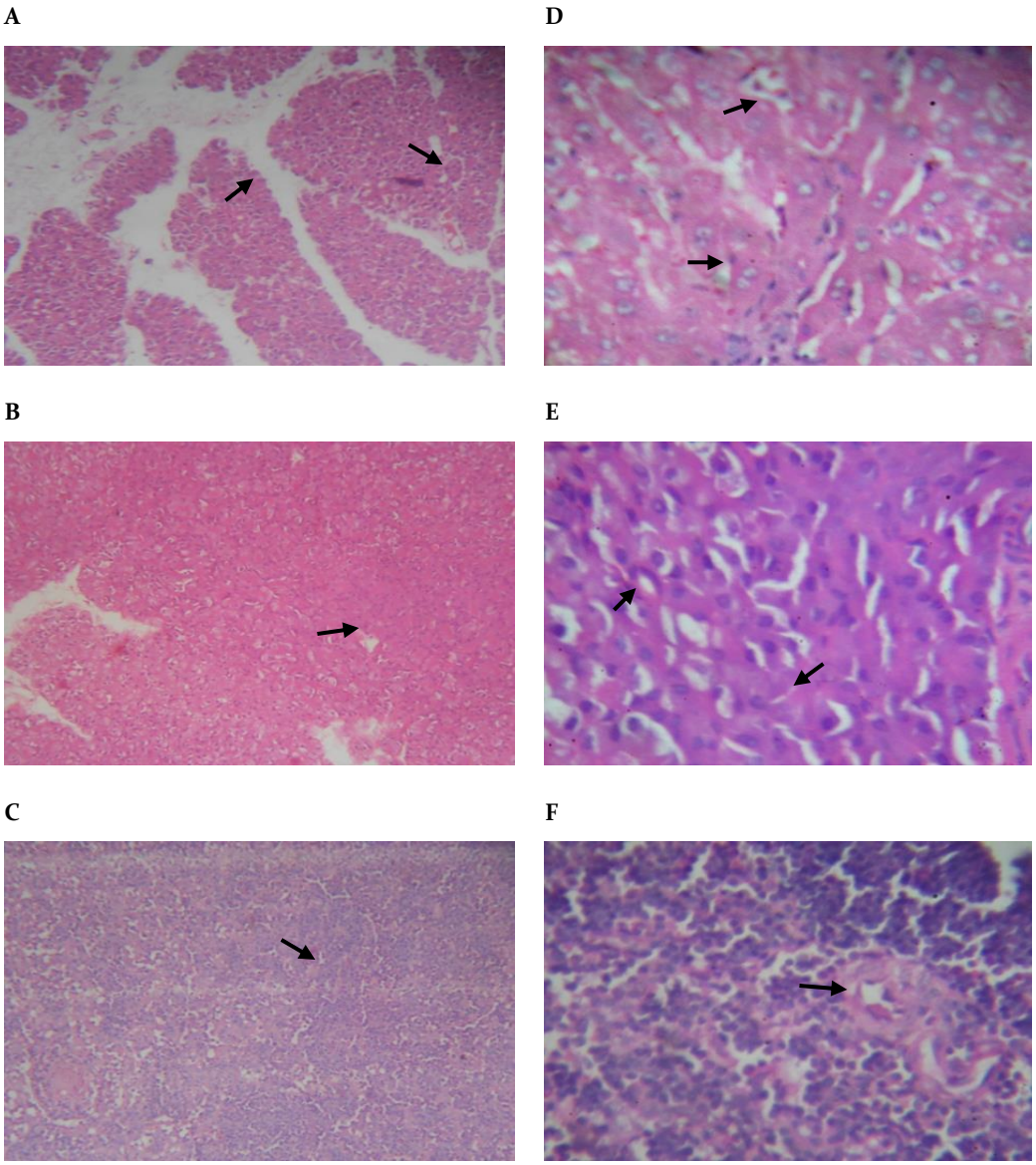

Figure 7. Comparison of *Citrus paradisi* juice treated pancreas with diabetic control and sitagliptin treated rabbit pancreas in alloxan induced diabetic rabbits.

A and D: Pancreas of alloxan induced diabetic rabbit (H&E 10x 40x, respectively); **B and E**: Pancreas of alloxan induced diabetic rabbit treated with grapefruit juice (H&E 10x and 40x, respectively); **C and F**: Pancreas of alloxan induced diabetic rabbit treated with sitagliptin (H&E 10x and 40x, respectively).

The arrows in **A and D** present damage to acinar cells. In **B and E** (*C. paradisi* juice treated group) and **C and F** (sitagliptin treated group) the arrows present normal acinar cells and islet of Langerhans.

CONCLUSIONS

From this study, it could be concluded that grapefruit juice and methanolic extract of *Fagonia cretica* possessed anti-diabetic activity when compared with sitagliptin. It could be clearly depicted that both selected remedies have great potential to reduce kidney damage, which would be helpful to reduce kidney damage in patients suffering from long term diabetes. It is suggested that active constituents of *Fagonia cretica i.e.* quinovic acid should be separated and evaluated further for its efficacy in incretin based therapy. Supplementary use of grapefruit juice could be suggested in diabetic pa-

tients and efficacy of grapefruit should further be explored clinically for risks and benefits in diabetic sufferers.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Contribution	Kamran SH	Shoaib RM	Ahmad M	Ishtiaq S	Anwar R
Concepts or ideas	X		X		
Design	X		X		
Definition of intellectual content	X	X	X		
Literature search	X	X			X
Experimental studies		X	X		
Data acquisition				X	X
Data analysis	X			X	X
Statistical analysis	X	X		X	
Manuscript preparation			X		X
Manuscript editing					
Manuscript review	X	X	X	X	X

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