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Chemical compositions and hypoglycemic activities of the protein and mucilage of *Casimiroa edulis* (Llave & Lex) seeds and fruits

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ABSTRACT

During the present study, chemical compositions of the protein and mucilage of seeds and fruits of *Casimiroa edulis* (Llave & Lex) as well as their hypoglycemic effects and other related biochemical parameters were studied for the first time. The protein content of *C. edulis* seeds was prepared with 4% (w/w) yield. The amino acid analyzer of protein hydrolysates led to the identification of 17 amino acids. Glutamic acid (10.25%) was considered the major amino acid. Moreover, the mucilage content of *C. edulis* fruits was prepared with 11% (w/w) yield. GLC analysis of mucilage hydrolysates revealed the identification of ten sugars represented (87.19%), with galacturonic acid (31.23%) as the main component. Significant hypoglycemic effects of protein and mucilage were recorded with respect to control and glibenclamide (standard drug). In addition, histopathological examination was performed, and the results showed that treatment with protein and mucilage remarkably improved Alanine Aminotransferase, Aspartate Aminotransferase, and Alkaline Phosphatase activities after streptozotocin induction with respect to control, also ameliorated the disturbances in Gamma Glutamyl Transferase, total protein & albumin, Glutathione levels, Catalase and lipid peroxidation activities. The prepared protein and mucilage exhibited properties that recommend them as natural supplements or in food industries for improving human being health.

INTRODUCTION

Casimiroa edulis belongs to family Rutaceae and it is widely distributed in Mexico and Central America (Landaverde *et al*., 2009). *C. edulis* is an evergreen tree that reaches up to 18 m tall and it is of economically high importance as it produces an edible fruit known as white Sapote (Ya-Ming *et al*., 2011), the plant under study previously reported to be used as sedative, hypnotic, anticonvulsant, and antihypertensive and also used for dermatological cases. Alkaloids, coumarins, limonoids, and flavonoids, i.e., zapotin and 3, 5-trimethoxy flavones, were previously reported (Awaad *et al*., 2011). Hyperglycemia is a chronic diabetic disease that led to the oxidative stress which was observed particularly in the liver that has a major and critical role in the regulation of carbohydrate metabolism (Uyar *et al*., 2017).

The present study aimed to investigate the hypoglycemic effect of seeds protein and fruits mucilage of *C. edulis* accompanied with other biochemical parameters including blood glucose level, total protein content, total albumin, liver functions [Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), and Gamma Glutamyl Transferase (GGT)], and the antioxidant activity, as well as studying the chemical composition of the prepared protein and mucilage hydrolysates responsible for this activity.

MATERIALS AND METHODS

Plant material

Casimiroa edulis (seeds and fresh fruits) were collected from El-Zohrya botanical garden, Giza, Egypt in April 2015 and the taxonomical identification was provided by Mrs. Threase Labib, plant taxonomy consultant at the Ministry of Agriculture and former director of El-Orman botanical garden (voucher No. 31-3-2015I).

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Investigation of protein contents of C. edulis seeds

The defatted dried powdered seeds (200 g) were stirred in 10% sodium chloride solution (Three times, 100 ml each) and then filtered. 10% trichloroacetic acid (TCA) equal volume solution was added to the filtrate to precipitate the protein as a white flocculent amorphous precipitate and the experiment was proceeded as reported by Aly *et al.* (2016). The previously prepared protein (10 mg) was hydrolyzed and the amino acid of the protein hydrolysate was investigated as previously mentioned conditions (Aly *et al*., 2016). The results were reported in Table 1.

Electrophoresis of protein

Polyacrylamide gel electrophoresis of the previously prepared protein was performed according to the methods described by Darwesh *et al*. (2015).

Preparation of staining solution

0.1 g coomassie brilliant blue powder, 10 ml of glacial acetic acid, 40 ml of ethanol then completed by distilled water to 100 ml and the solution was stirred for 1 hour then filtered through *Whatman No. 1 paper.* Relative mobility (R_f) and band percent (*B*%) of the electrophoretically separated proteins beside molecular weights (Mwts) were determined with respect to marker peptide of standard molecular weights ranging from 6.458 to 195.755 KDa then the polyacrylamide gel plates were photographed (Fig. 1) according to methodology reported previously by El-Sayed *et al*. (2018).

Investigation of polysaccharide contents in C. edulis fruits

Cold extraction of mucilage (C. E. M.)

The mucilage was prepared by using 500 g powdered plant of *C. edulis* fruits and the sugar of the mucilage hydrolysate was derivatized and analyzed by GLC using the conditions as previously reported (Ibrahim *et al*., 2016)**.**

Material for in-vivo hypoglycemic studies

Chemicals and biochemical kits

Kits used for the quantitative determination of different parameters were purchased from Bio-diagnostic. Streptozotocin (STZ) (Sigma Co.) for diabetes induction and glibenclamide (Daonil) ® (Sanofi Aventis) were used as a standard drug.

Table 1. Amino acid analysis of protein hydrolysate of *Casimiroa edulis* seeds.

Amino acid	Percentage	Amino acid	Percentage
1-Aspartic	5.86	10-Tyrosine	2.15
2-Therionine	1.97	11-Phenylalanine	2.94
3-Serine	2.21	12-Histidine	1.39
4-Glutamic *	10.25	13-Lysine	1.92
5-Glycine	2.57	14-Argnine	3.89
6-Alanine	2.29	15-Proline	2.01
7-Valine	3.98	16-Cystine	1.00
8-Isoleucine	2.28	17-Methionine	0.35
9-Leucine	4.05		

*Refers to the main component (Glutamic) in the protein hydrolysate of *C. edulis* seeds.

Animals and animal rights

Animal house of National Research Centre has provided male Wister Albino rats (150–170 g) for the experimental study, which were kept to accommodate on laboratory conditions for 2 weeks and were given food and water during the whole period of the experiment under constant environmental and nutritional conditions. Approvement of the anesthetic procedures complied with the legal ethical guidelines was approved by both the Ethical Committee of the Federal Legislation and National Institutes of Health Guidelines in USA and the ethical committee of the National Research Centre in Egypt with registration No. (15-028).

Doses and route of administration

Induction of diabetes

Intraperitoneal injection of the animals with STZ was done to induce diabetes (Uyar *et al*., 2017). Blood glucose level was measured after 72 hours by using glucometer after 1, 2, 3, and 4 week's intervals.

Experimental design

In the present study, 30 animals were divided into five groups and each group consists of six rats where:

- Group 1 is the normal healthy control group.
- Group 2 (+ control), induction of diabetes by (IP) injection (45 mg/kg body weight STZ dissolved in 0.01 M citrate buffer immediately before use). After injection, 5% glucose solution was orally administered. After 72 hours, fasting blood samples were collected and fasting blood glucose was measured (>600 mg/dl).
- Group 3 diabetic rats orally treated with the mucilage of *C. edulis* fruits (500 mg/kg).
- Groups 4 diabetic rats orally treated with the protein of *C. edulis* seeds (500 mg/kg).
- Group 5 diabetic animals treated with 10 mg/kg body weight glibenclamide as standard drug.

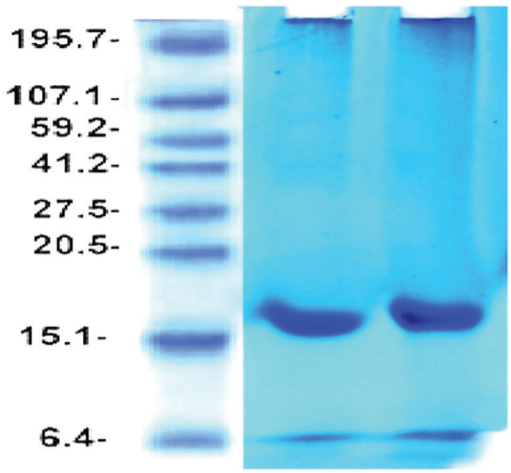

Figure 1. Electrophoretic pattern of the protein of *Casimiroa edulis* seeds. Lane 1: Marker & Lane 2: protein of *Casimiroa edulis* seeds.

Biochemical assays

Sample preparations

After 30 days of treatments, blood was collected from overnight fasted animals (12–14 hrs) from sublingual vein, left to clot then centrifuged at 3,000 r/minute at 4°C and the separated serum was stored at 80°C for further determinations of liver functions.

Parameters measured

Liver functions enzymes in serum: Total protein content, albumin level, ALT and AST and ALP as reported previously by Mohammed *et al*. (2018), (GGT) (Hyder *et al*., 2013). **Antioxidant parameters**: Lipid peroxide, glutathione concentration (GSH) and catalase as reported by Sadek *et al*. (2016) previously. Calculation percentages of change and improvement were calculated according to Ibrahim *et al*. (2016)**.** Analysis of variance (ANOVA) test was used in statistical analysis.

Histological study

Sections from liver and pancreas were fixed in 10% buffered neutral formalin and histopathological procedures were performed according to Ali *et al*. (2019).

RESULTS AND DISCUSSION

Protein and mucilage composition

In the present study, the protein of *C. edulis* seeds (4%). 17 Amino acids were identified; Glutamic 10.25% which represented the major amino acid, Aspartic acid (5.86%), Leucine 4.05%, Valine 3.98%, Arginine 3.89%, Phenylalanine 2.94%, Glycine 2.57%, Alanine 2.29%, Isoleucine 2.28%, Serine 2.21%, Tyrosine 2.15%, Proline 2.01%, Therionine 1.97%, Lysine 1.92 %, Histidine 1.39%, Cystine 1%, and Methionine (0.35%) as shown in Table 1. Protein was represented electrophoretically by two bands identified at relative mobility 0.683 and 0.937 (Molecular weights 16.009 and 6.480 KDa) in comparison with a marker. It was noticed that the first band (R_f 0.683; Mwt 16.009; B% 57.4%) considered the major band as shown in Figure 1.

Mucilage of *C. edulis* was prepared as mentioned above. The yield by the cold method gave (56 g) 11.2% (w/w) but no yield by the hot method. The isolated mucilage was pale gray in color and it gave positive Molische's test. Acid hydrolysis of the mucilage was done as mentioned above. GLC analysis of the mucilage hydrolysate, as shown in Figure 2 and Table 2, revealed the identification of ten sugar moieties which represented 87.19% of the total sugar hydrolysate, the main sugar identified is galacturonic acid (31.23%) arabinose (13.57%), galactose (10.17%), glucuronic acid (9.74%), glucose (6.82%), sorbitol (6.04%), mannitol (1.67%), and mannose & xylose (1.27% and 1.047%).

Hypoglycemic assay

The results demonstrated the use of protein and mucilage of *C. edulis* seeds and fruits (500 mg/kg) for the study of anti-diabetic activity against the standard drug glibenclamide (10 mg/kg). Protein remarkably decreases the blood glucose level which increased between 500 and 600 mg/dl after STZ induction. After the 1st week of protein seeds treatment, there was a reduction in blood glucose level to 403.47 mg/dl, and on 2nd week, the reduction reached 247.42 mg/dl, while highly significant results (141.56 mg/dl and 117.28 mg/dl) were recorded after 3 to 4 weeks of treatment in comparison to control (476.72 mg/dl and 273.80 mg/dl) and standard drug-treated groups (163.32 mg/dl and 107.15 mg/dl)as shown in Table 3.

Mucilage remarkably decreases the blood glucose level which increased between 500 and 600 mg/dl after STZ induction. Significant reduction of the blood glucose level in group treated with mucilage after 1 to 2 weeks, 414.94 mg/dl and 236.73 mg/dl while highly significant results recorded after 3 to 4 weeks of treatment, 125.41 mg/dl and 107.37 mg/dl in comparison to control (476.72 mg/dl and 273.80 mg/dl) and standard drug-treated groups (163.32 mg/dl and 107.15 mg/dl) as shown in Table 3.

Effect on hepatic liver enzymes

STZ induction led to the increase in the ALP, AST, and ALT by 146.79%, 63%, and 37%, respectively, with respect to

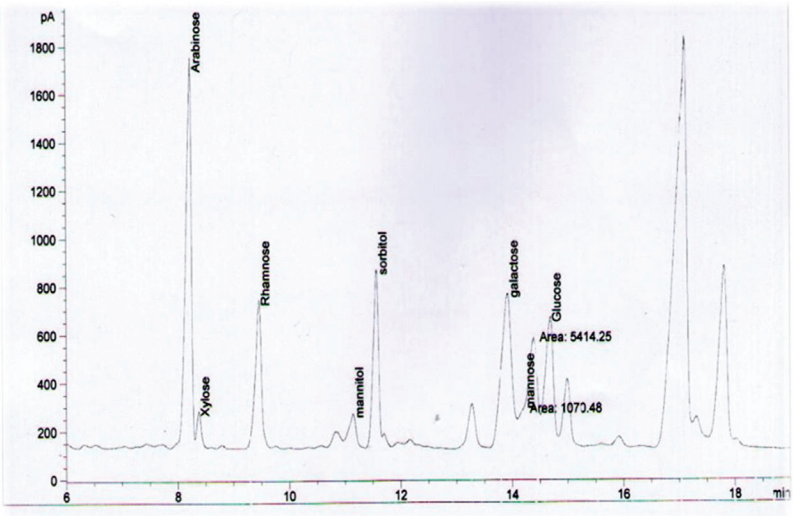

Figure 2. GLC chromatogram of mucilage hydrolysates of *Casimiroa edulis* fruits.

Table 2. GLC analysis of the mucilage hydrolysate of *Casimiroa edulis* (Llave & Lex) fruits.

Peak number	Retention time	Area %	Name
1	8.229	13.57	Arabinose
2	8.378	1.047	Xylose
3	9.461	5.62	Rhamnose
4	10.480	0.91	unknown
5	11.145	1.68	Mannitol
6	11.572	6.04	Sorbitol
7	13.282	2.17	unknown
8	13.925	10.17	Galactose
9	14.211	1.27	Mannose
10	14.392	6.42	Unknown
11	14.693	6.82	Glucose
12	14.993	3.29	Unknown
13	17.115	31.23	*Galacturonic acid
14	17.819	9.74	Glucuronic acid

Total identified sugar represented : 87.19%

*Refers to the main component (Galacturonic acid) in the mucilage hydrolysate of *C. edulis* fruits.

control due to hepatic cellular damage (Ahmed *et al*., 2015). Group treated with protein of *C. edulis* seeds improved liver enzymes by 65%, −1.79% and 21.88%, respectively, and group treated with mucilage of *C. edulis* fruits enhanced the hepatic marker enzymes by 69.08%, 15.36%, and 17.49%, respectively, with respect to group treated with glibenclamide (40.04%, 10.59%, and 20.01%) in comparison with control untreated as supported by Table 4.

Antioxidative properties

The administration of STZ significantly increased the lipid peroxidation by 79.008% and also led to catalase activity reduction by −31.81% in the diabetic group comparing to control. Lipid peroxidation was remarkably decreased in the protein and mucilage treated groups by 26.45% and 4.87%, also the standard drug-treated group improved the lipid peroxidation by 9.91% comparing to the control group. Moreover, it was observed that both protein and mucilage have promising effects in the Catalase (CAT) activities of the diabetic rats by −19% and −13.13%, and on the other hand, glibenclamide can improve the catalase activity by −10.20% compared to control (Table 5).

Elevation of hepatic GSH level was shown in both the protein and mucilage treated groups by −6.58% and −15.91%, indicating that they can elevate the biosynthesis of GSH or decrease the oxidative stress resulting in reducing GSH degradation, or possess both effects, furthermore using glibenclamide as a standard drug for treatment may improve the GSH by 2.47% comparing to control as STZ-induced diabetic rats resulted in decreasing the hepatic GSH levels (−41.80%) with respect to normal rats as shown in Table 5. The previously mentioned agreed with Abd El-Fattah *et al*. (2018) and Ahmed *et al*. (2015). These results suggested that both mucilage and protein may have antioxidant activity, reducing free radicals and improving hepatic antioxidant enzymes.

Effect on total protein, GGT, and albumin

In case of STZ diabetic rats, reduction in albumin and total protein (−19.62% and -38.46%) were reported compared to control group due to hepatic metabolic disturbances in STZ-induced rats as stated previously by Ali *et al*. (2011), and increase in serum GGT by 91.34%. From Table 6, we concluded that treatments with the protein and mucilage of *C. edulis* in comparison with glibenclamide drug ameliorated these disturbances by different percentages as follows: slight elevations in groups treated with protein and mucilage in which albumin records −3.63% and −9.52%, while group treated with glibenclamide by −8.23% comparing to control. In case of total protein results, there was a significant amelioration with percentages −18.52% and −21.64%, in case of protein and mucilage treated groups while glibenclamide records −11.37% with respect to control group. According to GGT, slight reduction in protein, mucilage treated groups in comparison to glibenclamide with respect to control by 64.86%, 48.49%, and 68.31%, respectively, as shown in Table 6. Treatment with protein and mucilage of *C. edulis* seeds and fruits ameliorated the disturbances in GGT, total protein, and albumin as a result of STZ induction.

Effect on liver and body weights

Table 7 showed changes in liver and bodyweights and relative liver body weight in STZ-induced group, a slight decrease in liver weight by 90 g and body weight by 82 g while relative liver/body weights were 106 g compared to control was reported concurred with Ali *et al*. (2011) whom stated that the decrease in body weight and total hepatic protein related to liver metabolic disturbance was due to diabetes. Treatment with protein seeds and mucilage fruits improved liver weight by 112 g and 121 g and body weight by 93 g and 99 g comparing to glibenclamide (129 g); this led to relative liver/body weights of 117 g and 121 g for protein seeds and mucilage fruits comparing to glibenclamide (128 g).

Histopathological assay

The normal histopathological study of the liver was observed in the control group (Fig. 3A and B) Parenchyma and hydropic degeneration in hepatocytes and necrosis were observed in liver sections of the diabetic group. Severe fibrosis and inflammatory cell infiltration were detected in central and portal areas. Fig. 3D and C shows that in the protein and mucilage treated groups, the liver of diabetic rats was reduced as well as degeneration and necrosis was observed locally in hepatocytes comparing to the control. Figure 3E showed nearly normal histological structure in the group treated with glibenclamide.

The normal histopathological study of pancreas revealed normal pancreatic cells (Fig. 4A), while Figure 4B showed basic lesions in Langerhans islets of pancreas; reduction in islets of Langerhans numbers and their cellular content, also the necrosis and lymphocyte infiltration of some islets, were noticed in diabetic groups in agreement with Taie *et al*. (2015). In treated protein and mucilage groups, these findings were reduced (Fig. 4D and C) compared to control. Figure 4E showed normal histological structures of islets of Langerhans in the group treated with glibenclamide.

Hyperglycemia decreases the insulin level leading to initiating beta-oxidation of fatty acids through increasing the activity of fatty acyl Coenzyme-A (oxidase enzyme) resulting in peroxidation of cell membrane main components (lipid or protein), which impairs membrane functions by changing the activity of

Table 3. Effect of protein and mucilage of *Casimiroa edulis* seeds and fruits on blood glucose level in normal and diabetic rats in comparison with glibenclamide (standard).

Duration	Positive control	Mucilage (fruits)	Protein (seeds)	Standard
Control (mg/dl)	99.833 ±7.35	101.66±6.0	103.6±5.4	92.33±5.11
72 hrs (mg/dl) % of change	579.6 ± 29.6 +480.57	545.3.5 ± 15.9 +436.44	477.8 ± 12.3 +361.2	477.8 ± 12.3 +417.49
1 week (mg/dl) % of change	569.5 ± 19.5 +470.45	421.83 ± 13.52 +314.94	418.17 ± 18.8 +303.47	440.16 ± 20.3 +376.72
2 weeks (mg/dl) % of change	446.5 ± 13.9 +347.24	240.66 ± 13.6 +136.73	256.33 ± 13.3 +147.42	252.8 ± 15.3 +173.8
3 weeks (mg/dl) % of change	421.66 ± 18.11 +322.37	127.5 ± 6.4 +25.41	146.66 ± 8.3 +41.56	150.8 ± 9.3 +63.32
4 weeks (mg/dl) % of change	417.8 ± 12.3 +318.49	109.16 ± 7.1 +7.37	121.5 ± 5.3 +17.28	108.16 ± 6.3 +7.15

Data are means ± SD of six rats in each group, Blood glucose level expressed as mg/dl.

Table 4. Effect of protein and mucilage of *Casimiroa edulis* seeds and fruits on serum blood ALP, AST, and ALT in normal and diabetic rats in comparison with glibenclamide.

Parameters	Control G1	Diabetes G2	Mucilage (fruits) G3	Protein (seeds) G4	Standard G5	ANOVA
ALP (U/l) **% of change**	28.17 ± 1.66^{e}	69.52±2.02^{a} **+146.79**	47.77±3.38^{b} **+69.58**	46.48±1.05^{b} **+64.99**	39.45±2.70^{d} **+40.04**	**0.0001**
AST (U/l) **% of change**	36.92 ± 2.89^{c}	60.46 ± 1.67^{a} **+63.76**	42.59 ±2.75^{b} **+15.36**	36.26±4.52^{c} **-1.78**	40.83 ±2.94^{b} **+10.59**	**0.0001**
ALT (U/l) **% of change**	24.59 ± 2.65^{c}	33.71 ±2.12^{a} **+37.09**	**28.89 ± 3.40^{b}** **+17.49**	29.97±4.01^{b} **+21.88**	29.51 ± 2.07^{b} **+20**	**0.0001**

Data are expressed as means ± SD of six rats in each group.

Values of AST, ALT, and ALP are expressed as U/L.

p is the level of significance, where p<0.05 is significant. ANOVA p<.0001.

Analysis of data is carried out by one way (ANOVA) accompanied by post hoc (least significant difference [LSD]) (SPSS computer program).

Table 5. Effect of protein and mucilage of *Casimiroa edulis* seeds and fruits on oxidative parameters (Lipid peroxide, catalase, and glutathione) in normal and diabetic rats in comparison with glibenclamide.

Parameters	Control G1	Diabetes +ve G2	Mucilage (fruits) G3	Protein (seeds) G4	Standard G5	ANOVA
Lipid peroxide nmol/mg protein **% of change**	25.63 ± 1.83^{b}	45.88 ± 3.03^{a} +79	26.88 ±1.27^{b} +4.87	32.41 ± 2.03^{b} +26.45	28.17 ± 1.99^{b} +9.91	0.0001
Catalase U/mg protein **% of change**	211.19 ± 8.34^{a}	144.12 ±11.14^{d} −31.76	183.46 ±10.69^{bc} −13.13	171.07 ± 12.49^{c} −18.99	189.64 ± 6.26^{b} -10.20	0.0001
GST U/mg protein **% of change**	86.43 ± 7.00^{a}	50.30 ± 8.67^{c} −41.81	72.68 ± 8.47^{b} −15.9	80.74 ± 8.59^{ab} −6.58	88.56 ±6.19^{a} +2.46	0.0001

Data are means ± SD of six rats in each group.

Data are expressed as Ug/mg protein for glutathione and nmol/mg protein for lipid peroxides.

Unshared letters between groups are the significance values at $p < 0.05$.

Statistical analysis is carried out using one-way ANOVA using CoStat Computer Program.

membrane-bound enzymes and receptors cell membrane structure lose its continuity accelerating diabetes and other cellular damages like necrosis and inflammation which agreed with Ali *et al*. (2011), subsequently hypo-insulinemia led to an increase in the oxygen-free radicals, which could in-activate and reduce the catalase activity in the diabetic rats (Soon and Tan 2002). In addition to hepatic GSH reduction as result of increased ROS production and free radical scavenging activity inhibition agreed with Abd El-Fattah *et al*. (2018) and Ahmed *et al*. (2015), as well as other hepatic metabolic disturbances reduction in albumin and total protein, GGT serum elevation, AST and ALT, and ALP elevation after STZ induction was in accordance with histopathological findings and indicator of liver damage and thus otherwise alterations in the hepatic functions (Ahmed *et al*., 2015).

Wang *et al*. (2016) suggested that both oral anti-diabetic drugs and insulin may lead to insulin resistance besides many

Table 6. Effect of protein and mucilage of *Casimiroa edulis* seeds and fruits on blood serum albumin, GGT, and total protein in control and diabetic rats in comparison with glibenclamide.

Parameters	Control G1	Diabetes	Mucilage (fruits)	Protein (seeds)	Standard	ANOVA
		G2	G3	G4	G5	
Albumin (g/dl) **% of change**	10.19 ± 1.58^{a}	8.19 ±1.02^{b} -19.37	9.22 ± 0.72^{ab} -9.51	9.82±0.99^{ab} -3.63	9.30 ± 0.94^{ab} -8.23	0.0122
Total Protein (g/dl) **% of change**	3.51 ± 0.12^{a}	2.16 ±0.10^{d} -38.46	2.75±0.11^{c} −21.64	2.86±0.06^{c} -18.51	3.11± 0.16^{b} -11.39	0.0001
GGT (g/L) **% of change**	22.31± 3.16^{a}	42.69±4.35^{d} +91.35	33.13±3.17^{bc} +48.49	36.78±3.68^{b} +64.86	37.55± 5.36^{b} +68.31	0.0001

Data are expressed as means ± SD of six rats in each group.

Values of total protein are expressed as g/dl and albumin are expressed as g/l.

p is the level of significance, where $p < 0.05$ is significant. ANOVA $p < 0.0001$.

Statistical analysis is carried out using one-way ANOVA using CoStat Computer Program.

Table 7. Effect of protein and mucilage of *Casimiroa edulis* seeds and fruits on liver weight, body weight, and liver-body ratio in normal and diabetic rats in comparison with glibenclamide.

Parameters	Control G1	Diabetes	Mucilage (fruits)	Protein (seeds)	Standard	ANOVA
		G2	G3	G5	G7	
Liver Weight **% of change**	5.8 ± 1.07^{bc}	5.22 ± 1.01^{c} **−10**	7.02 ± 0.627^{ab} **+21.03**	6.53 ± 1.04^{abc} **+12.58**	7.53 ± 0.791^{a} **+29.82**	**0.0018 ****
Body Weight **% of change**	211 ± 9.07^{b}	173.17 ± 9.1^{C} **−17.93**	210 ± 8.39^{b} **-0.47**	197.33 ± 10.81^{b} **−6.48**	212 ± 10.93^{b} **+0.47**	**0.00001**
Liver/body/Weight. Ratio **% of change**	0.028 ±0.005^{b}	0.0299 ± 0.004^{ab} **+6.79**	0.034 ± 0.002^{ab} **+21.43**	0.0329 ± 0.004^{ab} **+17.5**	0.036 ± 0.0023^{a} **+28.57**	**0.0056 ****

Data are means ± SD of six rats in each group.

Data are expressed as weight in grams.

Unshared letters between groups are significance value at $p < 0.05$.

Statistical analysis is carried out using one-way ANOVA by Co Stat Computer Program.

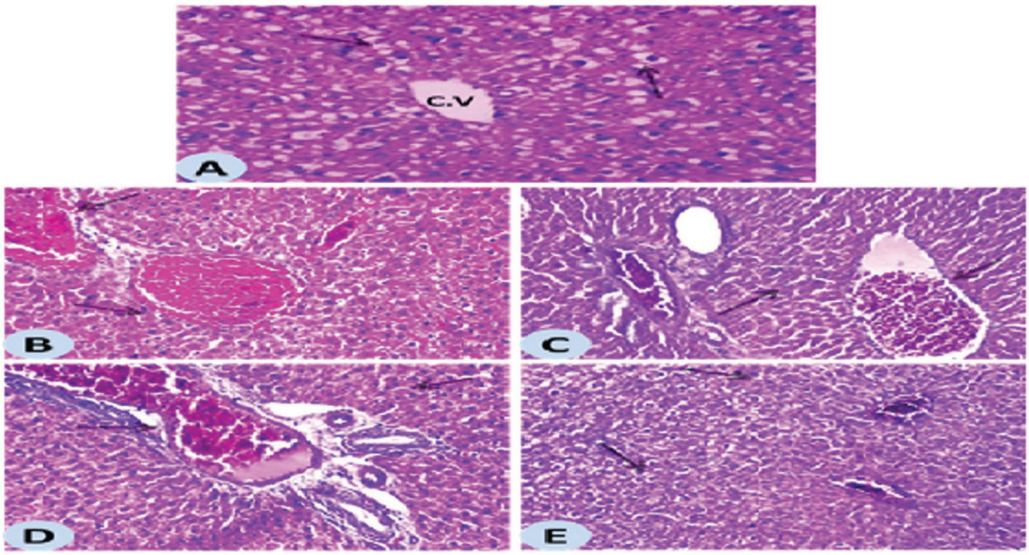

Figure 3. (A) A photomicrograph of rat hepatic tissue section showing normal histological structure of hepatic cells. (B) Severe dilatation congestion were detected in the central and portal veins as well as hepatic sinusoids in diabetic rats congestion in the central and portal veins were detected in group treated mucilage as in (C). (D) Some dilatation and congestion were observed in the central and portal veins in the portal veins in the portal area in group treated protein. [E] Showing nearly normal histological structure in group-treated glibenclamide.

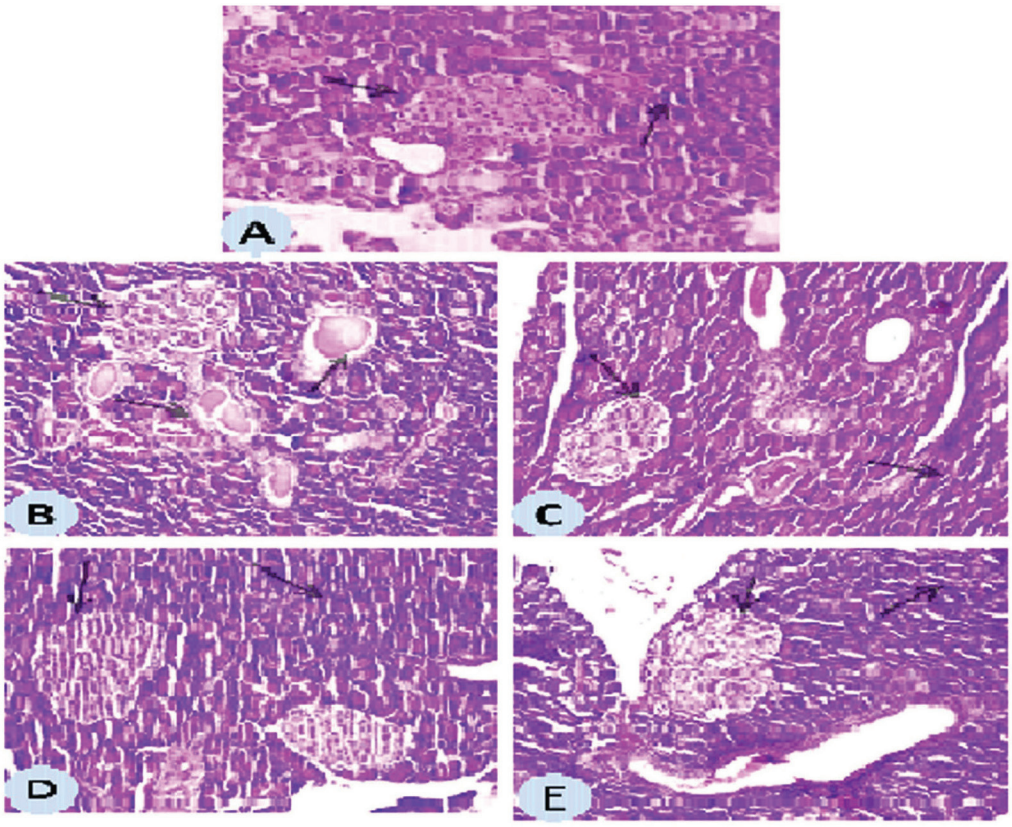

Figure 4. (A) A photomicrograph of rat pancreatic tissue section showing normal histological structure of islets of Langerhans cells as recorded in control group (H&E ×200). (B) The islets of Langerhans cells showed degeneration and atrophy as well as esoinophilic casts in the lumen of the pancreatic ducts in diabetic group. (C) Nearly normal histological structure in group treated mucilage. (D) Normal architecture of the pancreas as recorded in group treated protein. (E) Normal histological structure of islets of Langerhans cells in group treated with glibenclamide.

side effects, so we are looking for effective, non-toxic, and affordable drugs for diabetes. Protein and mucilage of *C. edulis* (Llave & Lex) seeds and fruits have been prepared to be used as anti-hyperglycemic agents for the first time during this study, improving blood glucose level successfully after 3 to 4 weeks of treatment as well as improving other liver functions (ALP, AST, and ALT). Moreover, the antioxidant activity was studied, in which lipid peroxidation was remarkably decreased in protein and mucilage treated groups. A significant increase in the CAT activity of the diabetic rats treated with protein and mucilage was proved. Noticeable elevation of hepatic GSH level was observed in both the protein and mucilage treated diabetic rats agreed with previously mentioned by Ahmed *et al*. (2015). According to albumin content slight elevation was observed in groups treated with protein and mucilage. In the case of total protein results, there was significant amelioration in case of protein and mucilage, while GGT slight reduction in protein, mucilage treated groups with respect to control.

Hypoglycemic and antioxidant activities, as well as the recovery from other diabetic complications, may be attributed to presence of amino acids considered as the principal protein components, especially glutamic acid, the most prominent amino acid in *C. edulis* protein, in which it was previously proved by Reimann *et al*. (2004) that glutamine activate and potentiate GLP-1 release, the principle metabolic gut fuel. The findings suggested that nutritional amino acid-like glutamine might have an effective role in obesity and diabetes**.**

Moreover Mansour *et al*. (2018) mentioned that glutamine as "essential" amino acid is the most human blood relevant element, playing a role as regulator of acid-base homeostasis and in gluconeogenesis and also a "nitrogen shuttle" between various organs, involved in as oxidizing agent for rapidly proliferating cells including enterocytes, reticulocytes, and immune cells. Also, glutamine has a signaling role in many processes including cell proliferation. Dietary intake of glutamine increased insulin circulation as explained above due to GLP-1 release.

Moreover, previous pharmacological studies proved that polysaccharides lowering blood glucose level by restoring pancreatic tissues roles and improving insulin output by beta cells (β-cell), as well as increasing the sensitivity of insulin peripheral cells (Hu *et al*., 2013), also it was demonstrated previously that polysaccharides possess prominent efficacies on diabetes (Wang *et al*., 2016). Thus, the hypoglycemic activity may be attributed to the presence of polysaccharides and amino acids as major components of the fruits and seeds.

More investigations should be undertaken to identify more bioactive compounds of both hypoglycemic and antioxidant properties in *Casimiroa edulis* besides studying their mode of action.

CONCLUSION

According to previous reported reviews, no study has up to the present date investigated the chemical compositions of protein and mucilage responsible for the hypoglycemic properties of *C. edulis* seeds and fruits, so this study for the first time focused on identifying the major amino acids and polysaccharides of the protein and mucilage accounted for the hypoglycemic activity of *C. edulis* as well as improving oxidative stress, liver functions, albumin, GGT, and total protein which is documented by the histopathological studies. Finally, further studies should be held to assure the beneficial effects of this plant for human being health and also its nutritional properties.

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ABBREVIATIONS

ALT: Alanine Aminotransferase
AST: Aspartate Aminotransferase
ALP: Alkaline Phosphatase
GGT: Gamma Glutamyl Transferase
CAT: Catalase
GSH: Glutathione

CONFLICT OF INTEREST

Authors declare that there are no conflicts of interest.

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ETHICAL APPROVAL

Approvement of the anesthetic procedures complied with the legal ethical guidelines approved by both the Ethical Committee of the Federal Legislation and National Institutes of Health Guidelines in USA and the ethical committee of the National Research Centre in Egypt with registration No. (15-028).

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