



The effect of *Zizyphus spina-christi* extracts on hyperglycemia induced by hyperlipidemic diet in Albino rats

Dawlat Ali A. Al-Ameri¹, Mohammed A. Al-Hegami¹, Elham A. S. Al-Shaibani^{2,*}.

¹Department of Science, Faculty of Education, Sana'a University, Sana'a, Yemen.

²Department of Biology, Faculty of Science, Sana'a University, Sana'a, Yemen.

*Corresponding author: el.alhaibani@su.edu.ye

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ABSTRACT

The study was carried out to evaluate the protective effects of *Zizyphus Spina Christi* (ZSC) leaves, fruits and seed extracts to ameliorate the adverse effects of hyperglycemia induced in Albino rats.

Zizyphus Spina Christi seeds, fruit and leaves extracts revealed positive results for carbohydrates, phenols, saponins, alkaloids, tannins, steroids, and flavonoids. High-fat diet intake has been associated with many pathological alterations in all studied parameters; one of these pathological alterations was hyperglycemia. Treatment of hyperglycemic rats with ZSC leaves, fruits and seeds extracts reduced these pathological alterations. Hyperglycemic rats were treated with ZSC seeds, fruit and leaves ethanolic extracts separately and in combination for 7 weeks. Results showed that the extracts succeeded in reducing the elevated glucose, insulin and HbA1C levels. On the other hand, the extracts improved histological alterations induced by a hyperlipidemic diet.

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1. Introduction:

Diabetes is a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys, and nerves. The most common is type 2 diabetes, usually in adults, which occurs when the body becomes resistant to insulin or doesn't make enough insulin. In the past three decades the prevalence of type 2 diabetes has raised dramatically in countries of all income levels. There is a globally agreed target to halt the rise in diabetes and obesity by 2025[1].

Hypercholesterolemia and hypertriglyceridemia, or their combination is called Hyperlipidemia and it is a prevalent health problem in modern societies. Due to their association with atherosclerosis, hyperlipidemia constitutes one of the major risk factors for vascular diseases such as coronary artery disease, a leading cause of death in developed countries, as well as hepatic steatosis [2]

The extract of *Z. spina-christi* was revealed to contain butic acid and ceanothic acid, cyclopeptides, as well as saponin glycoside and flavonoids, lipids, protein, free sugar and

mucilage [3]. Cardiac glycosides and polyphenols are also reported from the leaves [4]. Christinin-A is the major saponin of the leaves [5].

The aqueous extract of *Z. spina-christi* stem bark has shown significant antibacterial activity [6]. Methanol extract of *Z. spina-christi* roots showed antifungal activity [7]. *Z. spina-christi* leaf extract improved glucose utilization in diabetic rats [8]. Abdel-Zaher et al. [9] suggested that *Zizyphus spina-christi* leaves appears to be a safe alternative to lower blood glucose. The importance of this study lies in the use of extracts of seeds, leaves and fruits of *Zizyphus spina Christi* in Yemen separately and as combination as hypoglycemia

2. Materials and methods

2.1 Preparation of *Zizyphus spina Christi* extracts:

The parts of *Zizyphus spina-christi* (ZSC) were collected from Sana'a, Almahwet and Al jwf Governorates, in Yemen. The plant was identified in comparison to the voucher specimen number (BHSS 702) in the Faculty of Science Herbarium, Sana'a University. Th(seeds (ZSC.S), Fruits ((ZSC. F)) and leaves (ZSC.L) washed under running tap water and rinsed with distilled water, dried at room temperature, leaves, fruit and seeds every part was powdered alone. The powder of every part was carried out with up solute ethanol using shaker for 24 hours at room temperature after that was filtered by filter paper then the filtrate was kept in a rotary evaporator to obtain the crude extracts. Finally, the crude extracts were desiccated, collected, weighted and packed in dark glass containers and stored at -20°C until used [10].

2.2 Phytochemical analysis

Phytochemical screening of *Zizyphus Spina Christi* leaves, fruit and seeds extract was carried out according to the methods described by Trease and

Evans [11] for the detection of active components like carbohydrate, Tannine, saponine, glycosides, flavonoids, alkaloid, steroids.

2.3 Animals:

This study was executed on 42 albino rats, obtained from the Animal House of the Biological science Department, Faculty of Science, Sana'a University. They were housed in stainless steel cages in a well-ventilated room, under standard conditions of humidity at room temperature and a normal light-dark cycle in the Faculty of Education at Sana'a University for two weeks to adapt to the environment before experimentation. The animals in negative control were fed a control diet and water *ad libitum* during the entire period of the study, while the other groups were fed a hyperlipidemic diet [12]. The animals were weighed gravimetrically every week throughout the period of the experiment. The study protocol was approved by the animal ethics committee of Biological Science, Sana'a University (ethical code: BAHSS 101).

2.4 Experimental design:

Rats were divided into two groups:

- negative control (NC) group (n = 6) was fed on a standard diet for 130 days.
- hyperlipidemic groups (n = 36) were divided into six subgroups and fed on a hyperlipidemic diet for 60 days, and then:
- The first subgroup was sacrificed and used as a positive control (PC), while other groups were fed on a standard diet and administered orally (ZSC.S) extract 800 mg/kg b.w./day [13], orally (ZSC.F) extract 500 mg/kg b.w./day [14], (ZSC.L) extract 100 mg/kg b.w./day [15], (ZSC.S, F, and L) extracts in combination, and the Atrovastatin drug 10 mg/kg b.w./day, respectively, for 70 days

2.5 Parameters of study:

The body weights (b.w) of the rats were weighed once a week throughout the experimental period. Glucose levels, Insulin,

Insulin resistance and HbA1c were analyzed in National Center of Public Health Laboratories.

2.6 Histological studies:

The pancreas from each rat was removed after sacrificing it and fixed with 10% formalin, dehydrated in a descending series of ethanol, cleared in xylene, embedded in paraffin wax, cut at 5µm thick, and stained with haematoxylin and eosin (H&E) for histological examination under a light microscope at the University of Science and Technology, Sana'a, Yemen.

2.7 Statistical analysis

The statistical analysis was performed by Graph pad prism; continuous data are expressed as mean ±SE. Data was compared using one – way ANOVA P value <0.05 will be considered to be statistically significant

3. Results

3.1 Body weight:

The results recorded increasing gradual rise in body weight of the rat in all groups during the same period figure 1.

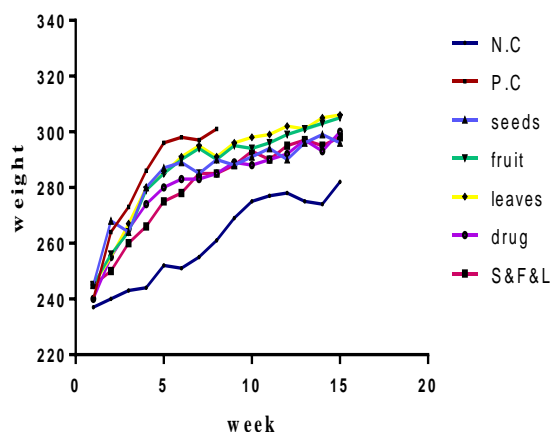


Figure. 1: Effect of *Zizyphus spina-christi* ethanolic extracts on the body weight of different groups induced by yperlipidemic diet.

3.2 Phytochemical analysis of ZSC seeds, fruit and leaves extracts

Examination of ZSC seeds, fruit, and leaves extracts revealed positive results for carbohydrate, phenols, saponins, flavonoids, alkaloids, tannins, Steroid, and flavonoids.

Table 1: Phytochemical analysis of ZSC seeds, fruit and leaves extracts

N	Examination	ZSCS extract	ZSCF extract	ZSCL extract
1	Carbohydrate	+	+	+
2	Saponins	+	+	+
3	Glycosides	-	-	-
4	Alkaloids	+	+	+
5	Steroid	+	+	+
6	Flavonoids	+	+	+
7	phenols	+	+	+
8	Tannins	+	+	+

3.3 Glucose, Insulin and HbA1C and Insulin Resistance levels:

As shown in Table 2, rats fed a hyperlipidemic diet showed a significant increase in glucose, insulin, HbA1C and insulin resistance compared to the NC group. There was a significant decrease in glucose levels and insulin and a non-significant decrease in levels of HbA1C in all treated groups as compared with the positive control group. Excepting the groups that were treated with ZSC leaves and statin, they had a significant increase in HbA1C compared to the NC group.

3.4 Histological altration of pancreas

The histological structure of the pancreas in control rats showed normal cells architecture: pancreatic cells (exocrine cells) and pancreatic islet of Langerhans (endocrine cells) (**Figure. 2A**). Sections of pancreas from the hyperlipidemic diet group revealed shrinkage in some islets and amyloid in the others (**Figures. 2 B and C**). Other groups treated with *Zizyphus spina-christi* seeds, fruit, and leaves and as combination ethanolic extracts and statine showed recovery on pancreatic pathological alterations (**Figure. 2 D**). (**Figures. 2 E, F, G, and H**).

Table (2) Effect of *C. Zizyphus spina-christi* ethanolic extracts on glucose, insulin, HbA1C and Insulin Resistance levels.

Groups Parameters	Negative control	Positive control	ZSCS extract (800 mg/kg b.w)	ZSCF extract (500 mg/kg b.w)	ZSCL extract (100mg/kg b.w)	ZSC- S,F,L extract	Statine (10mg/kg b.w)
Glucose (mg/dl)	71.4±3.36	110.4 ± 3.36 ^{a*****}	82.0±1.3 ^{a*b****}	103.4 ± 4.7 ^{a*****}	96.8 ± 3.46 ^{a*****b**}	86 ± 1.9 ^{a**b*****}	93.2±3.1 ^{a*****b****}
Insulin (µU/ml)	0.21 ±0.01	0.35±0.02 ^{a**}	0.19±0.03 ^{b**}	0.19±0.04 ^{b**}	0.18±0.05 ^{b**}	0.17 ± 0.04 ^{b***}	0.18±0.03 ^{b***}
HbA1C%	3.57±0.22	4.14±0.08 ^f	3.86±0.13	3.94±0.19	4.10±0.55 ^{a*}	3.72 ± 0.18	4.06±0.14 ^{e*}
Insulin Resistance	0.034±0.001	0.14 ± 0.010 ^{a*****}	0.04 ± 0.005 ^{b*****}	0.07 ± 0.006 ^{a***b*****}	0.05 ± 0.005 ^{a*b*****}	0.03 ± 0.004 ^{b*****}	0.04 ± 0.004 ^{b*****}

Values are expressed means ±S.E.M ^{a*} refer to significant difference compared to negative control group .^{b*} refer to significant difference compared to positive control group . ^{*}p<0.05, ^{**}p<0.005, ^{***}p<0.0005, ^{****}p<0.0001

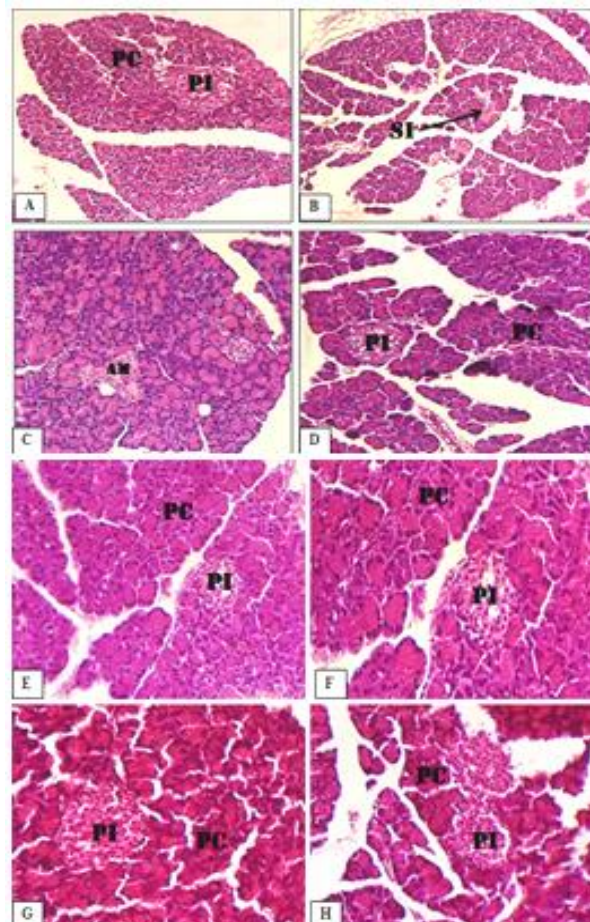


Figure. 2: Photomicrograph of the pancreas sections in different groups (A): Control pancreas shows normal islets of langerhans (PI) and normal pancreatic acini (PC). (B&C): Pancreas without treatment shows shrinkage in some islets and amyloid (D): Pancreas treated with ZSC seeds extract shows recovery islets of langerhans and pancreatic acini. (E): Pancreas treated with ZSC fruit extract; (F): Pancreas treated with ZSC leaves extract;(G): Pancreas treated with ZSC S,F,L as combination ethanolic extracts and (H): Pancreas treated with statin show recovery islets of langerhans and pancreatic acini (H&E, 100X).

4. Discussion

4.1 Phytochemical analysis of seeds, fruit and leaves extracts

The data of Phytochemical screening of ZSC seeds, fruit and leaves extracts revealed the presence of carbohydrate, phenols, glycosides, saponins, flavonoids, alkaloids, tannins, Steroid,

and flavonoids. These results conformity with [16], [17], [18] who revealed that the genus *Zizyphus* have revealed that peptide and cyclopeptide alkaloids, flavonoids, sterols, tannins, betulinic acid, and triterpenoidal saponin glycosides have been isolated and chemically identified. And agreement with the results obtained by Al-war [10] who suggested that the *Zizyphus spina-christi* seeds embryo extract revealed the presence of carbohydrates, glycosides, saponins, alkaloids, flavonoids, sterols, triterpenes, proteins, amino acids, polyphenols, tannins and flavonoids [19] reported that the preliminary Phytochemical analysis of ZSC seeds and fruit extracts gave positive test for tannins, alkaloids, flavonoids, cardiac glycosides. The extract of *Zizyphus spina-christi* leaves was containing bioactive saponins, including the major saponin christinin-A [20]. The methanol extract of the extract *Zizyphus spina-christi* showed the presence of carbohydrates, saponins and tannins [21].

4.2 Glucose, Insulin, HbA1C and Insulin Resistance

Although blood glucose levels, insulin, HbA1C and insulin resistance were in the normal range, they were significantly higher in the positive control group when compared with groups that were treated with ZSC seed, fruit, and leaves extracts. A significant increase in blood glucose levels, insulin, HbA1C and insulin resistance in the positive control group was improved in hyperlipidemic diet-fed rats that were treated with ZSC seed, fruit, and leaves extracts separately and in combination. The results of our study agreed with those of Abdel-Zaher et al. [9] and Michel et al. [8] noted that ZSC leaves extract lower blood glucose. And our results are concurrent with those of Avizeh et al. [14], who showed that treatment with ZSC fruit extract reduced blood glucose in rats and dogs, respectively. Also, our results conform to those of Bhatia and Mishra [13], who showed the aqueous-ethanol seed extract of *Zizyphus mauritiana* had hypoglycemic activity by

reducing blood glucose levels in alloxan-induced diabetic mice, and they agreed with Al-Awar [10], who demonstrated A significant reduction in blood glucose, insulin, pyruvate and lactate in diabetic rats treated with ZSC seeds embryos extract. In addition, the results accord with Ejelonu et al. [22], who revealed that antioxidants like saponin improved hyperglycemia as insulin resistance was induced by a high-fat diet in mice. This may be due to the presence of antioxidants such as saponin, polyphenols glycosides, flavonoids, and terpenoids which may inhibit oxidative stress that is induced by a hyperlipidemic diet. and reduce the accumulation of lipids around cells, thereby facilitating insulin binding to cellular receptors and glucose entry into cells.

4.3 Histological of pancreas

Pancreatic tissues of hyperlipidemic rats were treated with *Zizyphus spina-christi* seeds, fruits, and leaves ethanol extracts separately and in combination. These findings may be due to functional ingredients such as flavonoids, glycosides, saponins, and polyphenols that inhibit oxidative stress induced by a hyperlipidemic diet. These results were in conformity with Al-Sieni et al. [23], who showed that histopathological changes of the kidney, liver, heart, and testes that were induced by hypercholesterolemia were altered by Christ's thorn seed aqueous extract. Moreover, our results agreed with Al-Awar [10], who noted that *Zizyphus spina-christi* seed embryos extract improved remnant -cells of the pancreas.

5. Conclusion

This study reveals that ZSC seeds, fruit and leaves ethanolic extracts, separately and in combination, indicated amelioration in hypoglycemic rats by increasing antioxidants. This may be attributed to the antioxidative properties contained in ZSC extracts, which were revealed by phytochemical screening.

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