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EFFECT OF ETHANOLIC EXTRACTS OF ZIZYPHUS SPINA-CHRISTI ON HYPERLIPIDEMIA INDUCED IN RATS

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ABSTRACT **Background:** The objective of the study was to evaluate the ability of ethanolic extracts of Ziziphus Spina Christi (ZSC) seeds (S) fruits (F) and leaves (L) to ameliorate the adverse effects of hyperlipidemia induced in albino rats. **Methods:** This study used DPPH methods to evaluate the antioxidant activity of ZSC seeds, fruits, and leaf ethanolic extracts. Forty-two albino rats were divided into two groups: the normal group, which served as a negative control (NC), and the hyperlipidemic group, which was fed a hyperlipidemic diet for 2 months to induce hyperlipidemia. The hyperlipidemic group was divided into six subgroups: The positive control (PC) and the other subgroups were treated with ZSC seeds, fruits, and leaves ethanolic extracts separately and in combination and the statin group for 7 weeks. **Results:** The results showed that the Zizyphus spina-christi seeds, fruits and leaves ethanolic extracts were have antioxidant properties (87.38%, 74.45% and 62.49% respectively) when compared with standard antioxidant ascorbic acid 95.66%. additionally , the oral administration of ZSC (S,F&L) ethanolic extracts separately and in combination reduced the increase in body weight rate, serum AST ,ALT, total cholesterol, triglyceride (TG) and in low density lipoprotein (LDL) that induced by hyperlipidemic diet. Moreover, the extracts caused significant elevation in total protein, albumin high-density lipoprotein (HDL) levels of Hyperlipidemic rats Histological changes showed hemorrhage, fatty changes and amyloid, congested central vein and Inflammatory cells induced in liver by hyperlipidemic diet were ameliorated in the groups that treated with ZSC extracts. These findings led to the conclusion that the ethanolic extracts of ZSC seeds, fruit, and leaves, both separately and in combination, showed protective effects in hyperlipidemic rats by reducing fat and increasing antioxidants. ZSC extracts reduced the pathological alteration of blood parameters and liver histology in hyperlipidemic rats.

KEYWORDS : Ziziphus Spina Christi, antioxidants, hyperlipidemic diet, liver functions, lipid profile.

INTRODUCTION:

High-level fat, obesity, and metabolic syndrome may increase oxidative stress, and/or influence the levels of cellular homeostasis (Gao and Zhu. 2011). Thus, oxidative damage and its consequences may result in many chronic health problems. For example, atherosclerosis, cancer, hyperlipidemia, hyperglycemia, and arthritis have been correlated with oxidative damage (Brown and Bicknell. 2001)

Hyperlipidemia is a common disorder in developed countries. It results from abnormalities in lipid metabolism or plasma lipid transport or a disorder in the synthesis and degradation of plasma lipoproteins resulting in high levels of fat in the blood (Ducharme and Radhamma, 2008). Hyperlipidemia (including hypercholesterolemia and hypertriglyceridemia, or their combination) 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 (Yoon et al. 2011).

The extract of Z. spina-christi was shown to contain butic acid and ceanothic acid (a ring-A homologue of betulinic acid), cyclopeptides, as well as saponin glycoside and flavonoids, lipids, protein, free sugar and mucilage (Adzu et al. 2003). Cardiac glycosides and polyphenols (such as tannins) are also reported in the leaves (Abalaka et al. 2010). The aqueous extract of Z. spina-christi stem bark has shown significant antibacterial activity (El-Kamali and Mahjoub, 2009) while the methanol extract of Z. spina-christi roots showed antifungal activity (Adamu et al. 2006).

El Rabey et al. (2014) concluded that Christ's thorn (Ziziphus spina-Christi) leaves powder has anti-oxidant activity and ameliorated hyperlipidemia, improved liver and kidney functions, and decreased lipid peroxide in hypercholesterolemic male rats. Previous studies recorded that consumption of 100 mg/kg Zizyphus spina-christi leaves extract greatly ameliorates Diabetes disorders in rats. In addition, the Zizyphus spina-christi leaves extract is effective to reduce hyperlipidemia, lipid peroxidation, and the activity of liver enzymes (Parsaeyan and Rezvani 2015). Therefore, this study aims to investigate the use of extracts of seeds, leaves, and fruits of Yemeni Ziziphus spina Christi in separately and as a combination of hypolipidemia and ameliorated liver functions and histological changes.

MATERIALS AND METHODS

1. Animals

This study was performed 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, Sana'a University for two weeks to adapt to the environment before experimentation. The study protocol was approved by the animal ethics committee of Biological Science, Sana'a University (ethical code: BAHSS101).

2. Plant material

The plant of Ziziphus spina-christi was collected from different areas (Sana'a, Al Mahwet, and Al Jwf governorates) in Yemen. The (seeds, Fruits, and leaves) were identified in comparison to the voucher specimen number (BHSS 720) in the Faculty of Science Herbarium, Sana'a University (Fig1).

Figure 1: General view of *Ziziphus spina-christi*

3. Preparation of plant extract

The leaves, Fruit, and seeds were washed under running tap water and rinsed with distilled water, and 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 a 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 (Parsaeyan and Rezvani, 2015; Al-Awar 2019).

4. Biological activity antioxidant assay (DPPH):

Antioxidant activity was carried out according to the method described by (Mensor et al 2001). The free radical scavenging activity of Ziziphus Spina Christi is based on its scavenging activity against the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The antioxidant activity of Ziziphus Spina Christi was analyzed in the National Center of Public Health Laboratories. The percentage of inhibition as stated by (Jha et al.2010) was calculated as the following:-

% inhibition = AControl - ASample/AControl × 100

5. Experimental design: Rats were divided into two groups:

1- Negative control (NC) group (n=6), was fed on standard diet for 130 days**.**

2- Hyperlipidemic group: were fed for 60 days with a hyperlipidemic diet (Matos et al.2005), then divided into 6 subgroups (n = 6 in each group) as follows:

- **First subgroup:** used as a positive control (PC)
- **Second subgroup:** was fed on a hyperlipidemic diet and administered orally seeds extract **800 mg/kg b.w/day** (Bhatia and Mishra 2010) for 70 days
- **Third subgroup:** was fed on a hyperlipidemic diet and administered orally fruits extract **500 mg/kg b.w/day** (Avizeh et al.2010) for 70 days**.**
- **Fourth subgroup:** was fed on a hyperlipidemic diet and administered orally leaves extract **100 mg/kg b.w/day** (Parsaeyan and Rezvani 2015) for 70 days
- **Fifth subgroup:** was fed on a hyperlipidemic diet and administered orally Ziziphus Spina Christi seeds, fruits, and leaves extracts as a combination for 70 days**.**
- **Sixth subgroup:** was fed on hyperlipidemic diet and administered orally Atrovastatin drug 10 mg/kg b.w/day for 70 days.

6. Parameters of study

6.1. Body weight: Body weight (b.w) is one of the most sensitive indicators for hyperlipidemia condition, so for that the rats were weighed once a week throughout the experimental period.

6.2. Liver Function Tests (LFT):

Glutamic Oxaloacetic Transaminase (SGOT) or Aspartate aminotransferase (AST) Glutamic Pyruvic Transaminase (SGPT) or Alanine aminotransferase (ALT), Albumin, and total protein were analyzed in General Military Hospital.

6.3. Lipid profile levels

- Total cholesterol.
- Triglycerides
- High-density lipoprotein-cholesterol (HDL-ch).
- Low-density lipoprotein-cholesterol (LDL-ch).
- Lipid profile levels and Liver Function Tests (LFT) were analyzed in the National Center of Public Health Laboratories.

7. Histological studies: The liver was removed after sacrificing the rats. Liver specimens were washed with normal saline and fixed with 10% formalin. Fixed tissues dehydrated with different grades of alcohol and then, infiltrated, and embedded with paraffin wax. Blocks were sectioned in rotary microtome (5 µm thick) and then stained with hematoxylin and eosin dyes (Velmurugan et al.2007). Tissue samples were coded and evaluated for any histological changes using a light microscope and photographed by a digital camera. The histological examination was done at the University of Science and Technology, Sana'a, Yemen.

8. Statistical analysis: The results were expressed as mean ± SE. The results were analyzed by one-way analysis of variance ANOVA using Graph Pad Prism 9.1.1. Differences with a P-value of 0.05 were considered statistically significant.

RESULTS

1. Radical scavenging activity of Zizphus spina-christi extracts:

The Zizyphus spina-christi seeds, fruit, and leaves ethanolic extracts have antioxidant properties compared with the standard antioxidant, ascorbic acid. The results are expressed as % inhibition as shown in (**Fig 2**) The results revealed, Zizyphus spina-christi seeds, fruit, and leaves ethanolic extracts exhibited potent total antioxidant activity, which was expressed as an equivalent of ascorbic acid.

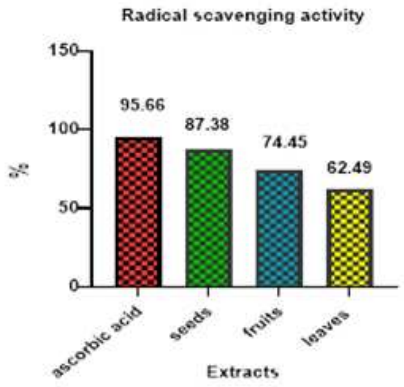


Figure 2: Comparative % inhibition of DPPH showed by ascorbic acid and ***ZSC*** ethanolic extracts.

2. Body weight:

The results recorded an increase in body weight in all groups at the end of the experiment. The negative control showed a consistent increase in body weight by 18.7% during the experiment, whereas the positive control group showed a high increase in its body weight by 27.08%, during eight weeks. The hyperlipidemic diet group that was treated with ZSC seeds, fruit, leaves ethanolic extracts separately and as a combination and statin showed 20.81%, 24.48%, 24.9%, 21.6, and 25.06% respectively gradual rise in their body weight, during the same period(Table 1).

Table (1): Effect of Zizyphus spina-christi ethanolic extracts on the body weight of the different groups.

Groups	N.C	P.C	Seeds(S) extract	Fruits(F) extract	Leaves (L) extract	S, F,& L extract	Statin
Weight							
Initial body weight	237.6 1.503	240.1 .594	2452.3 15	245.22. 083	245.2 3.54	245.0 ±1.38	240.2 3.277
Final body weight	282.2 4.55	3011. 76	2967.0 19	305.61. 86	3065. 481	298.0 ± 2.43	300.4 3.341
Weight increasing %	18.77 %	27.08 %	20.81 %	24.48 %	24.9 %	21.6 %	25.06 %

N.C = Negative control, P.C = Positive control
S = Seeds, F = Fruit, L = Leaves

3. Liver Functions Tests:

Serum ALT and AST levels in the Positive control group show a significant increase to 56.2 U/L and 176 U/L and a significant decrease in the value of total protein and albumin 6.46 g/dl and 3.76 g/dl when compared with those in the negative control group (30.8 U/L, 111.8 U/L, 7.34g/dl and 5.32g/dl respectively).

After administering ZSC seeds, fruits, and leaves ethanolic extracts separately and as a combination and statin these results of values of ALT, AST, total protein and were almost similar to that in the negative control group (Table 2).

4. Lipid Profile:

Data presented in Table 3 revealed that Hyperlipidemic-rats in the positive control group caused a significant increase in the level of T. cholesterol**,** Triglyceride. LDL and a significant decrease in the level of HDL when compared with the negative control group and the other treated groups.

The treatment with ZSC seeds, fruit, and leaves ethanolic extracts separately and as a combination and statin exhibited a significant

decrease in serum T.Cholesterol, TG, and a significant increase in HDL values as compared with the positive control group. These results were almost similar to the results of the negative control. While the treatment with ZSC leaves ethanolic extract and statin caused a non-significant decrease in the level of the LDL, other treatment groups caused a significant decrease when compared with the negative and positive control groups.

Table (2): Effect of C. Zizyphus spina-christi ethanolic extract on liver function tests (AST, ALT, T.Protein and Albumin).

Groups	N.C	P.C	Seeds (s) extract	Fruits(F) extract	Leaves (L) extract	S, F,& L extract	Statin
Parameter							
ALT (U/L)	30.81.48	56.2 ± 2.3^{a****}	36 85.06^{b***}	30.44. 7^{b****}	301.87 ^{b****}	33±1.52 b****	33.84.54^{b*} ***
AST (U/L)	111.8 ± 3.06	176±9 .67^{a****}	126.4± 1.97^{a*b***} *	107 ± 1.58^{b***} *	120.4 ± 2.66^{b****}	126.8±1 .93^{a*b****}	132 ±4.18^{a**b} ****
Protein (g/dl)	7.340.403	6.46± 0.09 ^{a**}	540.258 ^{b***}	8.04±0 .24^{a*b***} *	8.060.646^{a**b****}	7.6 ±0.16^{b****}	7.900.46^{b****}
Albumin (g/dl)	5.320.486	3.76± 0.27^{a**} **	4.78 ±0.17^{b**}	4.9 ± 0.18^{b***}	5.12 ± 0.21^{b****}	4.96 ± 0.15^{b***}	5.02 ± 0.15^{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

Table (3): Effect of C. Zizyphus spina-christi ethanolic extract on lipid Profile levels (T. cholesterol, TG, HDL, and LDL).

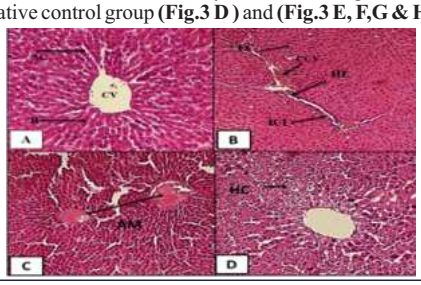
Groups	N.C	P.C	Seeds(s) extract	Fruits(F) extract	Leaves (L) extract	S, F, & L extract	Statin
Parameter							
T. cholesterol (mg/dl)	68.8± 7.63	157.80 ±3.56 ^{a****}	92.0±2 .59 ^{a**b****}	87.40± 4.18 ^{a*b****}	103.80 ±5.82 ^{a***b****}	88.20±1 .39^{a*b****}	66.80±0, 66 ^{b****}
TG (mg/dl)	215.2 ± 13.4	435.4± 12.25^{b*} ***	132.2 ± 2.82 ^{a***b****}	200.4 ±10.5^{b*} ***	160.6± 12.11 ^{a***b****}	129.6±3 .42 ^{a****b****}	120.8 ±3.17 ^{a****b****}
HDL (mg/dl)	63.00 ± 3.7	46.40± 3.55^{b***}	57.4 ± 2.42^{b*}	57 ± 2.51^{b*}	74 ±3.17 ^{a**b****}	56.60 ± 1.89^{b*}	56 ± 2.12^{b*}
LDL (mg/dl)	7.18± 0.41	10.40± 0.68^{b***} *	8.8±0. 30^{a*b*}	7.8 ± 0.59^{b***}	9.86 ±0.4^{a***}	8.360 ±0.37^{b**}	9.36±0.23^{a**}

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

5. Histological Observation of Liver:

Light microscopic examination of the liver in the control rats showed normal hepatocytes rows arranged around the central vein and the hepatic sinusoids are present between the hepatocytes., and Kupffer cells were clear **(Fig.3A).**

While, the section of the liver of the hyperlipidemic diet group showed congested central vein, infiltration, hemorrhage, fatty changes, and amyloid **(Fig. 3 B&C).** Other groups treated with Zizyphus spina-christi seeds, fruit, and leaves ethanolic extracts separately and as a combination and statin show nearly normal histological structures as in the negative control group **(Fig.3 D)** and **(Fig.3 E, F,G & H).**



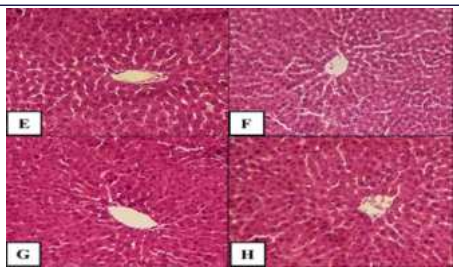


Fig.2: Photomicrograph of the liver sections in different groups (A): Negative Control liver shows normal hepatocytes HE architecture with normal central vein CV , Kupffer cells K and sinusoids S. (B &C): Positive control Liver without treatment shows congested central vein CCV, Inflammatory cells Infiltration ICI, hemorrhage H, fatty changes and amyloid . (D): liver treated with ZSC seeds extract shows slight hydropic changes HC.(E) liver treated with ZSC fruit extract. (F): liver treated with ZSC leaves. (G) liver treated ZSC S,F,L as combination ethanolic extracts. (H): Liver treated with statin show nearly normal histological structures as in the negative control group (H&E, 100X).

DISCUSSION

Radical scavenging activity of Zizyphus spina-christi extracts

This study was designed to determine the antioxidant properties of Zizyphus spina-christi seeds, fruit, and leaves ethanolic extracts by scavenging DPPH.

The results revealed, Zizyphus spina-christi seeds, fruit and leaves ethanolic extracts exhibited potent total antioxidant activity, which was expressed as an equivalent of ascorbic acid. Our finding indicated that ZSC ethanolic extracts had effective free radical scavenging similar to the findings of (Singh et al. 2012; Al Ghamdi and Shahat 2017)

Body weight:

Body weight was increased by the hyperlipidemic diet in the positive control, whereas it was decreased in other groups that were treated with Zizyphus spina-christi seeds, fruit, and leaves ethanolic extracts. These results agreed with (Al-Sieni et al. 2020)**,** and this may be due to the fact that the extracts of Zizyphus spina-christi contain antioxidants that work as antihyperlipidemic and reduce oxidative stress.

Liver Functions:

Liver transaminases (AST and ALT) are useful as biomarkers of liver injury in a patient with some degree of intact liver function (Mc Clatchey 2002). Moreover, an increase of AST is the most specific marker of liver cell damage (Chatterjee and Shinde, 2005)**.** The result of AST and ALT in the present study revealed that the serum levels of AST and ALT were increased in rats fed a hyperlipidemic diet for 60 days**,** our findings are complementary with (Al-Naqeeb, 2004; Al-Hammami, 2017 and Al-Sieni et al (2020)**.** While serum levels of total, protein, and albumin were decreased**.** Our results agreed with those of (Al-Hammami, 2017) who reported a decrease in total protein in the liver in high-fat diet-fed rabbits.

In the present study, we reported that after administering ZSC seed, fruit, and leaves separately and as combination extracts the result of AST, ALT, total protein, and albumin were almost similar to that of the negative control group**.** The current results are complementary to those results reported by **(**Al-Sieni et al. 2020) whose administration of the aqueous extract of Christ's thorn seed restored the liver enzymes to their normal levels**.** Moreover, our results agreed with (Yossef et al. 2011) who reported that the daily dietary containing powder of ZSC fruits improved serum levels of AST, ALT, total protein, and albumin against carbon tetrachloride (CCL4).

The present study agreed with (El Rabey et al. 2014) who reported that the extract of ZSC leaves succeeded to reduce the elevated serum of ALT in rats treated with a hyperlipidemic diet. This may be attributed to the presence of saponins in herbs that have hepatoprotective effects (Zhang et al. 2004).

Lipid profile:

The present study demonstrated that TC, TG, and LDL levels were increased in rats fed **a** hyperlipidemic diet for 60 days, and HDL levels were decreased as compared with rats fed a normal diet, whereas The treatment with ZSC seed, fruit, and leaves separately and as

combination extracts improved these levels and became almost similar to that in the negative control group. These results indicated that the ameliorative effect of ZSC S, F, and L extracts separately and as a combination helped to reduce the damage induced by a hyperlipidemic diet.

Our findings are complementary with (Al-Sieni et al. 2020) who reported that Phenolic constituents of Christ's thorn seed aqueous extract reduced the hypercholesterolemia in fat-rich diet-induced hypercholesterolemia in rats. These results were in agreement with (Yossef et al. 2011; Singh et al. 2012) who suggested that the serum lipid profile in hepatotoxic groups was improved by the addition of Ziziphus spina-christi fruit extract. On the other hand, our results coincided with the result obtained by (El Rabey et al. 2014; Al-Ghamdi and Shamhat 2017) who recorded that Ziziphus spina-christi leaf extract has beneficial effects on lipid profile.

This may be due to functional ingredients, saponins in Zizyphus spp which have hypolipidemic effects by decreasing total cholesterol, triglycerides, and LDL-C in hyperlipidemic rats (Zhang et al. 2004; Ejelonu et al. 2021). Moreover, this may be due to the presence of flavonoids. On the other hand (Weggemans and Trautwein 2003) reported that flavonoids intake decreased LDL-C and increased HDL-C and the effect of Zizyphus spina-christi leaves extract could be its phenol constituent which inhibits oxidative stress (Parsaeyan and Rezvani 2015) and may be due to the presence of saponin and glycosides (Othman et al. 2006; El-Beshbishy et al. 2006).

Histopathology:

Histological changes induced in the liver by a hyperlipidemic diet were ameliorated in the groups that were treated with Zizyphus spina-christi seeds, fruits, and leaves extract separately and as a combination. This refers to the antioxidant properties of ZSC extracts in addition to that the best findings were shown in the group that was treated with Zizyphus spina-christi seeds extract. This indicates that ZSC extracts improved liver and pancreas damage. These results were in conformity with (El Rabey et al. 2014) showed that the hepatic tissues of hypercholesterolemic rats that were treated with Christ's thorn leaves for 8 weeks showed a restored normal appearance and agreed with (Al-Sieni et al. 2020) who observed that tissues of the liver treated by Christ's thorn seed aqueous extract against hypercholesterolemia-induced by fat rich die. These findings were in agreement with other studies, which suggested that Ziziphus spina christi leaf extract alleviates diethylnitrosamine-induced hepatocarcinoma in rats (El-Din et al. 2019). The pretreatment of Ziziphus spina christi leaf extract inhibits liver injury in a mouse with sepsis through antioxidant and anti-inflammatory effects (Dkhil et al. 2018).

CONCLUSION:

This study reveals that ZSC seeds, fruit, and leaves ethanolic extracts separately and as a combination indicated amelioration in hyperlipidemic rats by Reducing fat and increasing antioxidants. High-fat diet intake has been associated with many pathological alterations in all studied parameters. Treatment of hyperlipidemic rats with ZSC extracts reduced these pathological alterations. This may be attributed to the antioxidative properties contained by ZSC extracts, which were revealed by scavenging DPPH (2, 2- diphenyl-1-picrylhdrazyl).

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