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Study the Effect of "Sonchus Oleraceus" Leaf Extracts on Levels of Blood Glucose and Lipid on Rabbits Treated with Carbon Tetrachloride

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Abstract

In this study, we investigated the impact of "Sonchus oleraceus" leaf extracts on blood glucose levels and lipid in the serum of rabbits treated with carbon tetrachloride. Our results demonstrated qualitative phytochemical screening of aqueous and alcohol (Ethyle Alcohol) extract for the presence of plant flavonoids, alkaloids, tannins and phenolic, while saponins present only in an aqueous extract were not present in the ethanol extract and the quantitative analysis of the plant for alkaloids, flavonoids and saponnins were 17, 10 and 12% respectively. Also showed the effecting of the extracts on blood glucose (B.G) and lipid (LDL, HDL, total cholesterol and triglycerides) levels of non-infected control group of rabbits following 21 days of infused by the plant extract were 158mg/100 ml for B.G. and 13, 16, 168 and 62mg/dl respectively for lipid, whereas the control range of non-infected by CCl_4 (untreated) were 151mg/100ml for BG and for lipid were 12,15, 166 and 60mg/dl, whereas group treatment of infected by (75mg/1mg) of the aqueous extract were 120 mg/100 ml for BG and for lipid were 12, 24, 112 and 58mg/dl, whereas group treatment of infected by (75mg/1mg) of the alcoholic extract were 140mg/100 ml for BG and for lipid 12, 16, 151 and 56mg/dl whereas infected group and treated by (300mg/1mg) of the aqueous extract were 104mg/100ml for BG and for lipid 20, 33, 86 and 52mg/dl, whereas infected group and treated by (300mg/1mg) of the alcoholic extract were 108mg/100ml for BG and for lipid 19, 28, 100 and 57 mg/dl respectively ,whereas that values represent the average courses was 8.917mg/100ml and the values of LSD ($P < 0.05$) were NS 0.910, 3.010, 6.222 and 4.382. Finally, there were some differences between groups after we used ANOVA as statistical analysis.

Keywords: Sonchus oleraceus; rabbits; blood glucose; lipid; qualitative and quantitative; phytochemical screening.

1. Introduction

"Sonchus oleraceus", is a plant that had effectively used in traditional Libyan medicines for centuries. Abundant remedial plants were used as nutritional adjunct and in the treatment of many illnesses without appropriate acquaintance of their purpose. Several animal studies had showed that the ethanol leaf and flower extracts lowered the blood glucose levels [1]. Thorough of free radicals by antioxidants possibly will condense the fibrosis development in the tissues [2]. Non-natural medications charities in the treatment of liver diseases are insufficient and requirement serious contrary effects. Unconventional medicines for conduct of liver and kidney diseases had a requisite to auxiliary at present used treatments of unconvinced effectiveness and care. Consequently, nearby is a world-wide tendency to reoccurrence to traditional therapeutic plants to treat liver diseases [3]. Anti-fibrotic from natural prod-

ucts used in traditional remedy could condense the threat of toxicity and retain the therapeutic effectiveness when the drug is used clinically [4-6]. Carbon tetrachloride (CCl_4) is a common manufacturing solvent considered by its harmless properties on the liver [7]. The toxicity of CCl_4 affected from the bio-activation of CCl_4 into trichloromethyl free radical by cytochrome P450 system in the liver microsomes and subsequently this effects lipid peroxidation of membranes that leads to liver damage [8]. Many methodologies had revealed in what way CCl_4 might effect this damage. Individual of these methods showed that CCl_4 intoxication may major to hypo-methylation of cellular elements [9]. In addition, the cellular infiltration of stimulated neutrophils may increase the inflammatory response, which leads to the death of the cells owing to superoxide and other toxic mediators release [10].

2. Material and Methods:

2.1. Plant collection and extract preparation

Fresh plant leafs of "Sonchus oleraceus" were collected in February 2016 from the wild around Sahel, Souk Alkhames Al-khums, Libya and Authenticated and identified the specie by Department of Botany, Education College, El- Mergeb University Alkhums Libya, The Fresh plant leafs washed by tap water, drayed in shadow, grinded to soft fine powdered and kept until used.

2.2. Extraction:

20g of the fine powdered leafs soaked in the 500ml appropriate solvent (Aqueous and Ethyl alcohol) for 72 hours, then separated the solvent by vacuum rotary evaporator and kept in refrigerator further use.

2.3. Phytochemical screening:

Phytochemical analysis were carried out for all the extracts as per the standard methods [11-15].

2.3.1. Qualitative Phytochemical screening:

Test for Alkaloids:

- **Hager's reagent:** To 10ml of crude extract few drops of Hager's reagent was added. Formation of yellow precipitate indicated the presence of alkaloids.
- **Wagner's reagent:** To 10ml of crude extract 2-3ml of wagner's reagent was added, Presence of reddish brown precipitate indicating presence of alkaloids.
- **Dragendroff's reagent:** To 10ml of crude extract few drops of dragendroff's reagent, formation of orange brown precipitate indicated the presence of alkaloids.

Test for cardiac glycosides:

- **Aqueous crude extract:** 10 ml of aqueous crude extract was boiled with 1mL of diluted H_2S0_4 in a test tube for 5 mints then filtered while hot. Cooled and added equal volume of C_6H_6(Benzene) and $CHCl_3$, shake well and separated the organic solvent then added the NH_3. The ammoniacal layer turned pink or red if cardiac glycosides were present.
- **Alcoholic crude extract:** Alcoholic extract was treated with 1 mL of pyridine and 1 mL of sodium nitroperside solution. Pink to red colour will appears. Extract (2 mL) was treated with 0.4 mL of glacial acetic acid containing a trace amount of $FeCl_3$ and 0.5 mL of concentrated H_2S0_4 was also carfully added by the side of the test tube. Persistent blue colour appeared in the acetic acid layer if cardiac glycosides were present.

Test for carbohydrates:

5 mL of crude extract treated with 3-5 drops of Mulish reagent (10% alcoholic solution of α- naphthol) was added and stirred for 5 mints. At that moment 5 mL of conc. H_2SO_4 was added. Violet ring was formed at the junction of two liquids, indicated the presence of carbohydrates.

Test for Tannins:

To 10 mL of crude extract 2 drops of 5% $FeCl_3$ was added. Presence of deep blue black colour indicated the presence of tannins.

Test for Flavonoids:

- **Lead acetate test:** 10 mL of crude extract was treated with 3-5 drops of 5% lead acetate solution, white precipitates appeared indicated the presence of flavonoids.
- **Shinoda Test:** To the crude extract a few fragments of magnesium ribbon and Conc. HCl was added. Appearance of magenta colour after few minutes indicates presence of flavonoids.

- **Alkaline Reagent Test:** To 10 mL of crude extract 3-5drops of NaOH solution was added. Formation of an intense yellow colour, which turns to colourless on addition of few drops of dil. H_2SO_4 indicated the presence of flavonoids.

Test for Protein:

- **Biuret Test:** The crude extracts were treated with 1 ml of 10% sodium hydroxide solution and heated. To this a drop of 0.7% copper sulphate solution was added. Formation of purplish violet colour indicates the presence of proteins.

- **Ninhydrin Test:** To the crude extract, 0.25% ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

- **Xanthoproteic Test:** The crude extracts were treated with few drops of concentrated Nitric acid solution. Formation of yellow colour indicates the presence of proteins.

Test for Saponins:

- **Foam Test:** 20ml of crude extract was shaken with small amount of water. If foam produced persists for ten minutes it indicates the presence of saponins.

- **Froth Test:** About 1ml of crude extracts was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Test for Phenols:

10ml of crude extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

2.3.2. Quantitive Analysis:

The quantities of the phytochemicals present were determined using the methods of Harborne J.B., 1973 and Obadoni B.O., Ochuko B.O, 2001 and D. Krishnaiah 2009. [16-17] as shown below:
The extracts were weighed after separating the solvents by evaporated under reduced pressure and dried using a rotary evaporator at 55 °C then a percentage yield for each extract was calculated as Equation 2.1:

$$Yield = \frac{Final\ weight\ of\ extract}{Total\ weight\ of\ Ground\ plant} \times 100 \qquad (2.1)$$

Determination of Total Alkaloids:

5g of the sample was weighed into a 250ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed [18].

Determination of Total Saponins:

The samples were ground and 20g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were the purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponins content was calculated [19].

Determination of Flavonoid:

10g of fine powdered plant sample was extracted with 100 ml of 80% aqueous methanol repeatedly at room temperature. The whole solution was filtered through Whatman filter paper 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath, the weight of the material and percentage quantity was calculated [20].

2.4. Animal Treatment

15 male rabbits Collected from Khums region, and placed in Rabbit cages in the Animal House of The Bio-logy Department, Faculty of Education, Souk Alkhames Khums, Al-Mergib University, Al-Khums, Libya leaving four weeks adapt to the environment and the age ranges this topic rabbits of 5-7 months the weight was from 1100-1700kg. They were maintained under standard conditions (temperature of 24 to 28°C) with free access to food (Alfalfa and carrots diet) and water. All procedures were in accordance with the Guide for the Care and use of

Laboratory Animals remained castoffs in the present study. Previously experimentation, rabbits were adapted for 2wks., and the experiment initiated when the rabbits were 90 days old. Each day at 7am, body weight was recorded and animals were treated.

2.4.1. Experimental disposition

Fifteen matured male rabbits were subjectively assigned to three groups (4, 5 and 6 rabbits) as the following: first group consist of non-infected control group of rabbits were injected by Olive's oil only (1ml/1Kg) intraperitoneal, second group control range of non-infected by CCl_4 (untreated) were injected by a mixture of olives oil and CCl_4 (Olives oil 0.5: 0.5 CCl_4), the third group was injected by the plant extract within concentration (75 and 300mg/1Kg) per day sequins, were numbered this group with (1, 2, 5 and 6), Where injected (75 and 300mg of aqueous and ethanolic extract separately) and inoculated extracted after dissolving in water to the third set only for four days before being injected the carbon tetrachloride. Experiment continued for 21 days then after twenty-four hours from the last injection course, the animals were anesthetized with chloroform and then collected blood samples been separated by centrifuges (300rpm/min) for 15 minutes to get the blood serum and were kept frozen samples until examined [21].

2.5. Statistical Analysis:

Data were statistically analyzed using statistical analysis system (SAS, 2006). One way analysis of variance (ANOVA) was used to test the variations among groups. Were the values of LSD ($P<0.05$).

3. Results and Discussion:

As showed in Table 3.1 and Table 3.2 The qualitative and quantities revealing of the chemical constituents in the crude plant extracts (aqueous and ethanolic) for the presence of flavonoids, alkaloids, tannins, cardiac glycosides, protein and phenols, while saponins present only in an aqueous extract were not present in the ethanol extract and the quantitive analysis of the plant for alkaloids, flavonoids and saponnins were 17, 10 and 12 % respectively. Whereas the percentage yield for ethanolic and aqueous extracts 15 and 4% correspondingly, where existence of such components henceforward the inactivity of "Sonchus oleraceus" as antidiabetic treaty and for many other infections treatments.

As showed in Table 3.3 the rabbits after 21 days from injecting them by two concentrations highest value of blood glucose were 158mg/100ml for the Non-infected control group of rabbits, and lowest value 104mg/100ml with the Infected group and treated by (300mg\1mg) of the aqueous extract in comparing between them. And this is indicating the extract of "Sonchus oleraceus" decrease the blood glucose level within the rabbit's serum. Where other groups (Group treatment of infected by (75mg\mg) of the aqueous extract, Group treatment of infected by (75mg\1mg) of the alcoholic extract 140, Infected group and treated by (300mg\1mg) of the aqueous extract and Infected group and treated by (300mg\1mg) of the alcoholic extract) 120, 140, 104 and 108. ,rising rate of diabetes with high oxidative stress induced by free radical generated in a short time from oxidation, Where the effective oxygen works to damage the enzyme insulin product of beta cells in the pancreas and created this free radical when injected rabbits with carbon tetrachloride [22]. And that the cause of low blood sugar when the dosages rabbits extract "Sonchus oleraceus" leaves back to consist of natural antioxidants (flavonoids), which is one of the natural compounds that have the activities of multiple and rich with physiological activity of this plant leaves, and also it contains a good percentage of vitamin C, which play an active role to encourage and strengthen the immune system [23], as showed on Table 3.3 the effect of the plant at the level of the magnesium element in the blood serum of rabbits and which led to increase it any meaning that this plant contains a small percentage of this element which is one of the major mineral elements are involved in more than 300 function in the human body, including the deficiency of this element increases diabetes by resisting the hormone insulin by cells and is considered one of the main components in the constituent of insulin [24].

CCl_4 – intoxicated rabbits showed a significant increase in the levels of serum cholesterol, triglycerides, HDL and LDL, As showed in Table 3.4 Effective of extract of "Sonchus oleraceus" on lipids in rabbit's serum were heights values 62mg/100 ml for cholesterol of Non-infected control group of rabbits followed by control range of non-infected by CCl_4 (untreated) 60mg/100 ml then Groups treatment of infected by (75mg\1mg) of the aqueous extract 58mg/100ml, Group treatment of infected by (75 mg\1mg) of the alcoholic extract 140 (56mg/100 ml), Infected group and treated by (300mg\1mg) of

Table 3.1: Phytochemical screening of aqueous and Ethanolic extracts of "Sonchus oleraceus"

Chemical Component		Observations	
		Aqueous Extract	**Ethanolic Extract**
Alkaloids	Hager's reagent	+	+
	Wagner's reagent	+	+
	Dragendroff's reagent	+	+
Cardiac glycosides		+	+
Carbohydrates		+	+
Tannins		+	+
Flavonoids	Lead acetate test	+	+
	Shinoda Test	+	+
	Alkaline Reagent Test	+	+
Protein	Biuret Test	+	+
	Ninhydrin test	+	+
	Xanthoproteic Test	+	+
Saponins	Foam test	+	-
	Froth Test	+	+
Phenols		+	+

Key: + = present; - = absent

Table 3.2: Percentage Yield of aqueous and Ethanolic extracts of "Sonchus oleraceus"

Plant Name	Percentage yield (%)				
	Ethanolic extract	Aqueous extract	Flavonoids %	Saponins %	Alkaloids %
"Sonchus oleraceus"	15	4	10	12	17

Table 3.3: Effective of extract of "Sonchus oleraceus" on Blood Glucose level in rabbit's serum

Rabbits treatment groups	Blood Glucose Levels (mg/100ml)	Magnesium (mg/100ml)
Non-infected control group of rabbits	158	4.1
control range of non-infected by CCl_4 (untreated)	151	3.0
Group treatment of infected by (75mg\1mg) of the aqueous extract	120	5.2
Group treatment of infected by (75mg\1mg) of the alcoholic extract140	140	5.4
Infected group and treated by (300mg\1mg) of the aqueous extract	104	5.3
Infected group and treated by (300mg\1mg) of the alcoholic extract	108	5
Average courses was 8.917mg/100ml	values of LSD ($P<0.05$) were NS0.910, 3.010, 6.222 and 4.382	8.092

Table 3.4: Effective of extract of "Sonchus oleraceus" on lipids in rabbit's serum

Rabbits treatment groups	Cholesterol mg/dl	Triglycerides mg/dl	HDL mg/dl	LDL mg/dl
Non-infected control group of rabbits	62	168	16	13
control range of non-infected by CCl_4 (untreated)	60	166	15	12
Group treatment of infected by (75 mg\1mg) of the aqueous extract	58	112	24	12
Group treatment of infected by (75mg\1mg) of the alcoholic extract140	56	151	16	12
Infected group and treated by (300mg\1mg) of the aqueous extract	52	86	33	20
Infected group and treated by (300mg\1mg) of the alcoholic extract	57	100	28	19
Average courses was 8.917mg/100ml	4.382	6.222	3.01	NS0.91

the aqueous extract (52mg/100ml), Infected group and treated by (300 mg \ 1 mg) of the alcoholic extract (57mg/100 ml). Where the triglycerides was the highest value of the Non-infected control group of rabbits 168mg/100ml followed by control range of non-infected by CCl_4 (untreated) 166mg/100ml were the other groups as showed in table 3 had these values 112, 151, 86 and 100mg/100ml, respectively. And thus the highest values of the level of HDL to infected group and treated by (300mg\1mg) of the aqueous extract 33mg/100 ml and was at least the value of 15mg/10ml of group treatment of infected by (75mg\1mg) of the aqueous extract. Accordingly LDL values group of six rabbits were 13.12, 12.12, 20 and 19mg/100ml, respectively, largely due to lower fat of rabbits which was injected extract of leaves of the plant to reduce fat produced level in the liver tissue and reduce the level of liver enzymes in the produced fatty acids. this has approved what was found Kocsis et al. (2003), which is a decrease of the level of cholesterol and triglycerides and also a decrease in the effectiveness of pancreatic enzymes (alpha Omalaaz and Allabez) [25]. Regarding lipid profile as revealed on Table 3.4, the diabetic control rabbits exhibited marked progressions of serum total lipid, total cholesterol, triglyceride, low density lipoprotein (LDL)-cholesterol. The serum high density lipoprotein (HDL)-cholesterol concentration, conversely, was significantly ($P<0.05$) reduced in the diabetic control animals as compared with the normal ones.

4. Conclusion:

We conclude and recommend that, the studied plants "Sonchus oleraceus" reveal capable diabetes mellitus that require further studies to make them useful for treatment of many diseases. Hereafter, from the present study the plant leave crude extracts "Sonchus oleraceus" revealed a plentiful production of Phytochemicals as secondary metabolites and it can be used in the pharmaceutical industries for producing a potent drug against diabetes. The studies result of the above this plant gives a basis of its use in traditional medicine to manage illnesses and complaints. In addition, and according to these results of these investigation the daily consumption of "Sonchus oleraceus" can be used to protect hepatotoxicity and nephrotoxicity.

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References

[1] Ghosh RK, Gupta I.; Effect of Vincarosea and Ficusracemososus on hyperglycemia in rats. Indian J. Anim. Health.; 1980. 19: 145-148

[2] Thresiamma K.C. and Kuttan R. (1996). Inhibition of liver fibrosis by ellagic acid. Indian J. Physiol. Pharm.,40:363–366

[3] Ali S.A. and Hamed M.A.; Effect of Ailanthus altissima and Zizyphus spina Christi on bilharzial infestation in mice: Studies. J Applied Sci.; 2006; 6:1437-1446

[4] Ali S.A., El-Rigal N.and Rizk M.Z.; Nutritional supplementation with Ailanthus altissimia and Ziziphus spina Christi to compensate for some metabolic disorders in Schistosoma mansoni infected mice. Pak J Biol Sc.; 2006; 9: 1700-1706

[5] Ali S.A. and Hamed M.A.; Effect of Ailanthus altissima and Zizyphus spina Christi on bilharzial infestation in mice: Studies. J Applied Sci.; 2006; 6:1437-1446

[6] El-Ansary A.K., Ahmed S.A. and Aly S.A; Antischistosomal and liver protective effects of Curcuma longa extract in Schistosoma mansoni infected mice. Indian J Exp Biol.; 2007; 45: 791-801

[7] Seifert WF, Bosma A, Brouwer A, Hendriks HF, Roholl PJ, van Leeuwen RE, van Thiel-de Ruiter GC, Seifert- Bock I, Knook DL.; Vitamin A deficiency potentiates carbon tetrachloride- induced liver fibrosis in rats; Hepatology; 1994; 19: 193-201

[8] Gadgoli CH, Mishra SH.; Antihepatotoxic activity of Pmethoxy benzoic acid from Capparies spinosa; J Ethenopharmacol; 1999; 66: 187-192

[9] Weber LW, Boll M.; Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model; Crit Rev Toxicol; 2003; 33: 105-136

[10] Fanton JC, Ward PA.; Role of oxygen-derived free radicals and metabolites in leukocyte- dependent inflammatory reactions; Am J Pathol; 1982; 107: 395-418

[11] Kokate CK.; Practical Pharmacognosy; Vallabh prakashan; Delhi; 2005; 107-111

[12] Khandelwal KR.; Practical Pharmacognosy Techniques and Experiments; Edn 9, Nirali Prakashan, Pune; 2003

[13] Ansari SH.; Essentials of Pharnacognosy; 1st edition, Birla publications, New Delhi; 2006; pp. 357-359, 588-590

[14] Brain KR and Turner TD.; The practical evaluation of phytopharmaceuticals; 2nd Edn.; Bristol: Wright Sciencetechnica; 1975; 81-82

[15] Evans WC, Trease and Evan's Pharmacognosy; 14th Edn London; England: W.B.Sounders company limited; 1996; 545- 46

[16] Roopashree TS, Dang R, Rani RHS, Narendra C.; Antibacterial activity of antipsoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis; International J App Res Nat l Prod; 2008; 1:20-28

[17] Obadoni BO. Ochuko PO.; Global J Pure and Applied Sciences; 2001; 8b:203-208

[18] Harborne J.; Phytochemical methods. Chapman and Hall; Ltd London; 1973.p.49-88

[19] Obdoni B, Ochuko P.; Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria; Global J. Pure Appl. Sci; 2001; 8: 203–208

[20] Bohm BA, Kocipai- Abyazan R.; Flavonoid and condensed tannins from the leaves of Vaccinum raticulation and Vaccinum calcyimium; Pacific Sci.; 1994; 48: 458-463

[21] J. Akram, K. Javad, Z. Dehaghani, H. Ninahad; Hepatoprotective Activity of Cichroium Itybus L. Leaves Extract against Carbon Tetrachloride Induced Toxicity; Iranian Journal of Pharmacological Rsearch; 2006; 1: 41-46

[22] Ahmed, Nayeemunnisa; Alloxan Diabetes-induced Oxidative Stress and Impairment of Oxidative Defense System in Rat brain:Neuroprotective Effects of Cichorium Intybus; Bangalore; India; Int J Diabetes and Metabolism; 2009;17:105-109

[23] Belal, Nehal M.; Hepatoprotective Effect of Feeding Celery Leaves Mixed with Chicory Leaves and Barley to Hypercholesterolemia

Rats; Asian Journal of clinical Nutrition; 2011; 3(1).14- 24

[24] Cathy, Aunt.; Guide to Nutrition: Magnesium; UND School of Medicin; Dept. of Pediatrics Fargo; ND.; 2009

[25] Kocsis I., Krisztina K., Szoke and B. Anna.; Effective of Chicory on Pancreas Statues of Rats in Experimental Dislipidemia; Acta Biologica Szegediensis; 2003; 47(1-4): 143-146