Online ISSN: 2413-6096 http://www.ljmr.com.ly/

Original Article

The anti-hyperglycemic and the anti-hyperlipidemia effects of Cynara Cornigera heads alcoholic extract on Alloxan-induced diabetes in Rat.

Shaban E. A. Saad[1], Abeer F. E. Aljabo[1], and Akram Abrahem[2]
1. Department of Pharmacology and Clinical Pharmacy, University of Tripoli.
2. Libyan Center of Medical research, Alzawia.
Corresponding email: abeeraljabo202@gmail.com

Abstract

Background: Cynara Cornigera (CC), family Asteraceae, is locally known as Gaamool. It is widely distributed in the northeastern part of Libya. CC heads and leaves are used in folk medicine for various illnesses such as diabetes, hepatic dysfunction, hyperlipidemia, and dyspeptic disorders. Aim: This study aims to investigate the hypoglycemic and hyperlipidemia effects of CC-heads alcoholic extract in normal and Alloxan-induced diabetes in rats.

Methods: The acute hypoglycemic effect of 30% W/V CC was tested against the glucose tolerance test (GTT). In addition, the sub-acute (once a day for a week) effect of 15% W/V CC extract was studied in normoglycemic rats. In the Alloxan model, hyperglycemia was induced by a single 120 mg/kg intraperitoneal injection (IP), and then the acute and sub-acute effects of CC were tested. Blood glucose levels have been determined by diabetes test strips (on-call plus glucometer). Glucose and ketones in urine were also measured by using deep sticks. Blood has been collected by cardiac puncture for further study to determine triglycerides, cholesterol, and liver enzymes (AST and ALT).

Result: The administration of 30% W/V CC showed a significant decrease in glucose levels in the GTT. Furthermore, in Alloxan-induced rats, CC extract resulted in a significant decrease (P 0.001) in glucose levels after a single dose (30% W/V); furthermore, it reduced blood glucose levels (P 0.05) after a week of daily administration of a single dose of (15% W/V). Moreover, the CC decreased the concentrations of glucose and ketones in urine that had been produced as a result of Alloxan hyperglycemia. Blood cholesterol, triglyceride, and liver enzyme (AST-ALT) levels that had been raised by Alloxan were reversed by CC extract.

Conclusion: The study revealed that the CC-head extract may contain compounds that possess hypoglycemic and hypolipidemic properties.

Keywords: *Cynara Cornigera*, hypoglycemia, Alloxan

Online ISSN: 2413-6096 http://www.ljmr.com.ly/

Citation. Saad Shaban E. A The anti-hyperglycemic and the anti-hyperlipidemia effects of Cynara Cornigera heads alcoholic extract on Alloxan-induced diabetes in Rat.2023;16(1):https://doi.org/10.54361/ljmr.162b09
Received: 12/06/22accepted: 14/07/22; published: 31/03/23

Introduction

According to the World Health Organization (WHO), more than 60% of the world's population and 80% of the populations of developing countries rely on herbal medicine for the treatment of their illnesses (1) (2). Artichoke was known and started being cultivated around the 1st century of the modern era (3). Moreover, many reports revealed the use of artichokes as food and medicine by ancient Greeks and Romans (4). Arabs had a positive role in the dispersion of artichokes. Hence, Al hardship was the original Arabic name of this plant, whereas it was called in Italian, Spanish, and Portuguese (Carciofo, Alcachofa, and Alcachofra, respectively), which were all derived from Arabic (5, 6).In Libya, artichokes include four species reported in" Flora of Libya," i.e., the endemic Cynara Cyrenaica, Cynara Cornigera, Cynara Carunculous, and Cynara Scolymus (6). Cynara Cornigera (CC) belongs to the family Asteraceae (locally known as Gaamool), which is widely distributed in the Al-Jabal Akhder area east of Libya. Its heads are used as fruit and contain vitamins such as C, K, -tecopherol, and -carotene. It is also rich in natural antioxidants, mainly polyunsaturated fatty acids and polyphenols, including caffeoylquinic acids and luteolin (7). Cynara cornigera has been used in folk medicine to treat various illnesses such as diabetes, hepatic dysfunction, hyperlipidemia, and dyspeptic disorders (8). In general, Asteraceae are an important source of various medicinally active substances with a remarkably potent hypoglycemic effect (Nazni et al., 2006).Diabetes is one of the most common diseases in the world. In addition, diabetes is an important cause of many serious complications, such as blindness, kidney failure, heart attacks, stroke, and lower limb amputation. Diabetes is characterized by elevated blood glucose levels and some other metabolic changes, such as raising blood lipids (10). Although the prevalence of diabetes is worldwide, there is no cure for this devastating disease. Therefore, folk medicine could be a successful guide in searching for anti-diabetic drug(s) with better pharmacological therapeutic profiles. Moreover, edible plants have the advantage of their low toxicity; therefore, they are easier to be pharmacologically screened and developed. To mimic diabetes in animals, many animal models have been designed and developed to be used in such research. One of the well-validated models is inducing hyperglycemia in rodents by using Alloxan; this model was selected to be used in this study.

Materials and methods:

2.1.Chemicals: glibenclamide 5mg (Remideca-Cyrpus), dextrose 25 g/50 ml (Amritsar, India), normal saline 0.9%, All the previous materials were provided by the local pharmacy. Alloxan (Biochemical, England), Gum Acacia (Sigma Aldrish, Germany), Elman reagent (Thermo Scientific Company, Sweden), EDTA, Trichloroacetic acid, Phosphate Buffer, Sodium Dodecyl Sulfate, Thiobarbetioric Acid, Butanol, Pyridine, and Acetic Acid (Chemical Laboratory of Zawia University),

2.2. Phytochemical Processes:

2.2.1. Plant Collection, Identification, and Preparation: The heads of Cynara cornigera were collected in March 2021 from Ghemines, a small town adjacent to the Gulf of Sidra in the Cyrenaica region of northeastern Libya. The identification of the plant was confirmed by the Department of Botany at the University of Tripoli under voucher number 685421. The heads were dried in the shade and ground into a powder by a mechanical grinder. The powder was kept in sealed amber glass bottles until the time of extraction.

2.2.2. Preparation of the Extract: The dried powder of 2000g was macerated in 6 L of 96.6% ethanol for 3 days with continuous agitation, then filtered and concentrated by a rotary evaporator at 45 C.The residue was weighed and stored in a tight, closed amber container at -20 C until use (11). 2.3. Pharmacological Screening:

2.3.1 Animals: Wister Albino rats weighing 180–200 g were obtained from the animal house of the Libyan Center for Medical Research, Alzawia. The rats were fed standard laboratory rat chow. Food and water were available ad libitum. They were kept in plastic cages at 20°C–25°C under a 12-hour light/dark cycle. All experiments involving animals were approved by the ethical committee of the Libyan Center for Medical Research. Different concentrations of treatment were given orally in a volume of 2 mL per 100 g of body weight.

2.5.2 Acute effect of CC on Glucose Tolerance Test (GTT) in normal rats: overnight-fasted 18 Albino Wister rats were divided into three groups (6 in each). Group I (control): received 5% W/V gum acacia in distilled water. Group II (positive control): received the standard drug, glibenclamide 1 mg/kg suspended in 5% W/V gum acacia, once. Group III (CC test) received CC 30% W/V CC extract suspended in 5% W/V gum acacia. All groups received glucose at 2 g/kg orally after 30 minutes of CC and gum acacia administration, and blood glucose levels were checked in 0, 30, 60, 90, 120, and 180 minutes (12) by using a glucometer (On call plus, LOT 3367778, Acon Laboratory, San Diego, USA).

2.5.3 Sub-acute effect of CC on normal Animals: 12 Albino Wister rats were divided into two groups (6 each); the control group was given vehicle control (5%w/v) gum acacia and the treatment group received 15% w/v CC extract. Each rat was separately housed in a metabolic cage, and the vehicle and treatment were given once daily for a week. Blood glucose level was measured on day 1 and day 7.Two hours after gavaging by using a glucometer (on call plus, USA).

2.5.4 Hyperglycemia induction by using Alloxan: Fasted male Wister Albino rats weighing 180–200g were selected and then marked for individual identification. The rats were injected with Alloxan monohydrate in freshly prepared saline (0.9% NaCl) at a dose of 120 mg/kg IP to induce diabetes in 12 h (13). After one hour of Alloxan administration, the animals were given water ad libitum. A 5% dextrose solution was given in a feeding bottle for a day after 6 hours of injections to overcome the early hypoglycemic phase. After 72 h, animals with blood glucose levels higher than 300 mg/dl were considered diabetic and were included in the study (14).

2.5.5 Experimental design for the effect of CC extract on alloxan-induced hyperglycemic rats: Rats were divided into three groups: two groups for diabetic animals and one group for normal animals.

Group I: normal animals received gum acacia (5% W/V) in distilled water once a day for a week.

Group II: Alloxan-induced hyperglycemic animals received gum acacia (5%) W/V in distilled water once a day for a week.

Group III: Alloxan-induced hyperglycemic animals received 30% w/v CC extract once on day one, and for the rest of the days (up to day 7), rats received one oral dose of 15% w/v CC extract. The tail blood glucose levels were determined by using Glucometer strips on day one (2 hours after treatment) and on day seven (2 hours after CC administration). Moreover, urine was collected on day seven to measure ketones and glucose levels.

At the end of the experiment, all animals (groups I, II, and III) were sacrificed under anesthesia, and the blood was collected by using cardiac puncture for other biochemical parameters, i.e., cholesterol, triglycerides, AST, ALT, and antioxidant enzyme activity (glutathione reductase and lipid peroxidase).

2.5.6 Measurement of glucose and ketone bodies in urine: Glucose and ketone bodies in urine were directly measured by insertion of the urine test strip in the collected urine, and the developed color was compared with the standard.

2.5.7 Measurement of Cholesterol and Serum Triglycerides levels: Enzymatic colorimetric (CHOD-PAP) testing (Bio Maghreb, Tunisia) was applied using the serum. The intensity of the color developed was measured spectrophotometrically against a blank at wavelengths ranging from 500 to 550nm (15) and 16).

2.5.8 Measurement of AST (GOT) and ALT (GPT): The IFCC Kinetic Method Test (Bio Maghreb, Tunisia) was applied by using serum (17) and (18). 2.5.9 Determination of an antioxidant enzyme: The pancreas of treated and control Rats were isolated and immediately washed with cold phosphate buffer (pH 7.4), and tissues were homogenized and prepared for antioxidant enzyme estimation (GSH, LP) (19).

2.5.10 Reduced Glutathione (GSH) estimation: The method was done according to (20). To precipitate tissue proteins, 1 ml of tissue homogenate (in 0.1 M phosphate buffer, pH 7.4) is taken, and 1 ml of 20% trichloroacetic acid (TCA) containing 1 mm EDTA is added.

The mixture is allowed to stand for 5 minutes before centrifugation for 10 minutes at 2000 rpm. The supernatant (0.2 ml) is then transferred to a new set of test tubes and added to 1.8 mL of Elman's reagent (DNTB 0.1 mm) prepared in 0.1 M phosphate buffer. The solutions are measured at 412 nm against a blank (21).

2.5.11 Lipid peroxidase (LPO) estimation: This method is illustrated in estimation: This method is illustrated in. LPO is determined by measuring the amounts of malondialdehyde (MDA) produced. A mixture of 0.2 ml of tissue homogenate (in 0.1 mM phosphate buffer, pH 7.4), 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid, and 1.5 ml of 8% TBA are added. The volume of the mixture is made up to 4 mL with distilled water and then heated at 95 °C in a water bath for 1 hour using glass balls as a condenser. After incubation, the tubes are cooled to room temperature, and the final volume was made at 5 mL in each tube. A mixture of butanol and pyridine (15:1) is added (5 ml) and then vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min, the upper organic layer is removed, and its OD is measured at 532 nm against an appropriate blank without the sample (21);

2.5.12 Statistical analysis: Descriptive statistical analysis was applied to the generated data from different samples using SPSS (software package, version 25) to find out whether the observed samples are normally distributed using the Kolmogorov-Samirnov maximum deviation test for goodness of fit. If the parameters were normally distributed, treatments were compared by applying a one-way ANOVA. Post-hoc tests (LSD and Duncan tests) were applied. The differences were considered to be significant at p 0.05. If the data was not normally distributed, the non-parametric Mann-Whitney test was applied.

3.6 Results:

3.6.1 Oral glucose tolerance test in normal rats: The blood glucose level in animals treated with CC 30% W/V extract was significantly lower than in the control group (Fig 1). In 30 minutes, the blood glucose level increased.

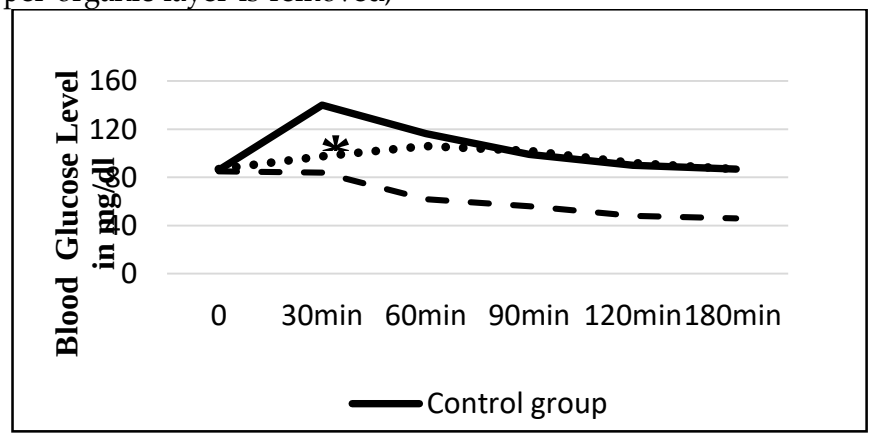

Figure 1: Effect of CC 30% W/V on the Oral Glucose Tolerance Test Values are expressed as mean S.D. * indicates that the cc extract group differed significantly from the control group at p0.05.

3.6.2 Sub-acute effects of CC on normal rats:
The result of the blood glucose level after one-week continuous treatment of CC 15% W/V was significantly lower than the Control group (Fig. 2).

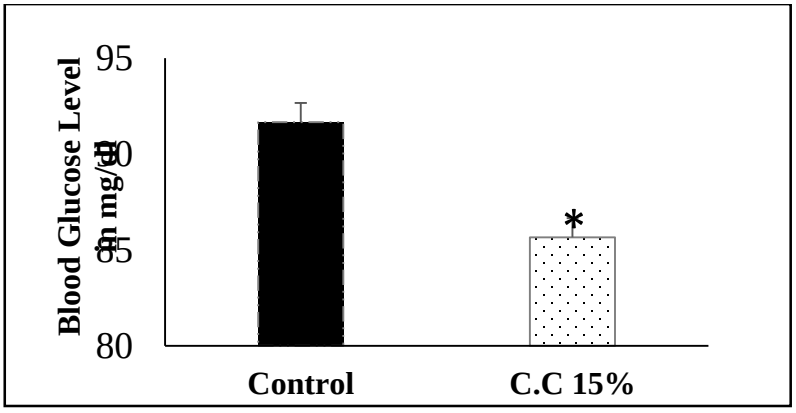

Figure 2: The effect of continuous CC 15% W/V treatment on blood glucose levels.

Values are expressed as mean standard deviation (mg/dl); * indicates a significant difference from the control (p 0.05).

3.6.3 Acute effect of CC 30% on alloxan-induced hyperglycemia in rats:
After 2 hours of 30%w/v CC extract treatment, blood glucose levels in hyperglycemic rats were significantly (P0.001) lower than in non-treated hyperglycemic rats (fig.3). Six normoglycemic rats comprise the control group.

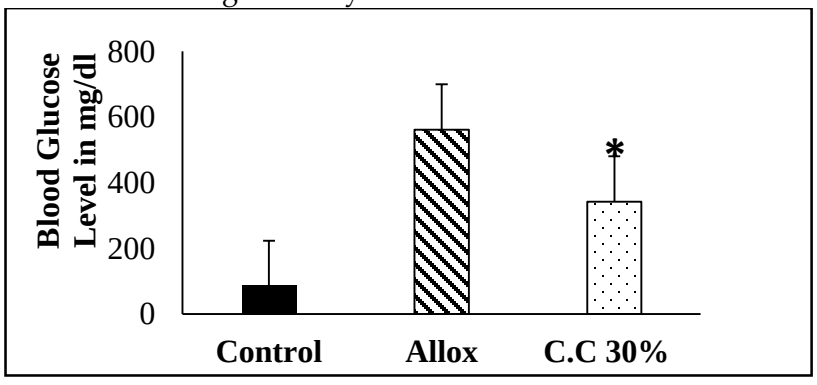

Figure 3: Acute effect of 30% W/V CC on Alloxan-induced rats' blood glucose levels.

Values are expressed as mean S.D. (mg/dl). * indicates a significant difference from the Alloxan group at p0.05.

3.6.4 Sub-acute effect of CC 15% on blood glucose in hyperglycemic rats:
The blood glucose level in alloxan-induced rats was significantly (P 0.05) decreased compared to the alloxan-induced group after continuous administration of CC 15% W/V for one week. (fig.4).

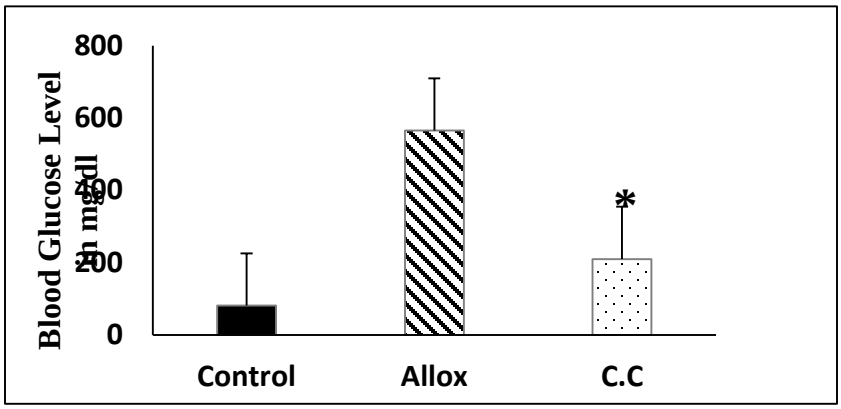

Figure 4: Sub-acute effect of 15% W/V of CC on Blood Glucose Levels of Alloxan-Induced Rats Values are expressed (mg/dl) as mean standard deviation; * means a significant difference from the Alloxan group as p 0.05

3.6.5 Detection of glucose and ketone bodies in urine:

Table.1. The presence of glucose and ketones in urine for vehicle control and the treated group.

The concentrations of glucose and ketones in urine were decreased in the group.
treated with 15% W/V CC compared to the Alloxan vehicle control group.

3.6.6 Effects of CC extract on cholesterol levels in alloxan-induced hyperglycemic rats:
The effect of 15% W/V of CC administration on serum cholesterol level was significantly (p 0.001) decreased compared to the Alloxan group (Fig. 5).

Groups	Glucose	Ketone
Control	No	No
Alloxan and vehicle	++++	++++
Alloxan and CC 15%w/v	++	++

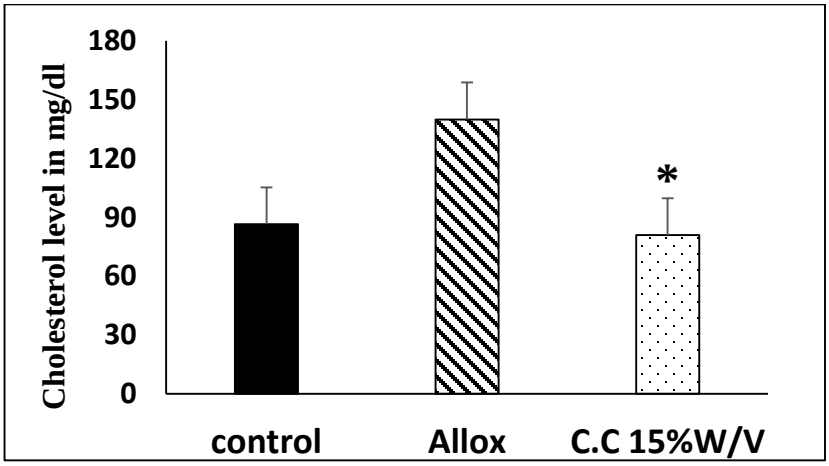

Figure 5: Effect of 15% W/V CC on Serum Cholesterol Levels of Alloxan-Induced Rats Values are expressed (mg/dl) as the mean standard deviation, which means a significant difference from the Alloxan group as *p 0.05*

3.6.7 Effects of CC extract on triglyceride levels in alloxan-induced hyperglycemic rats: The effect of 15% W/V of CC administration on serum Triglycerides level was significantly (p 0.001) decreased compared to the Alloxan group (Fig. 6).

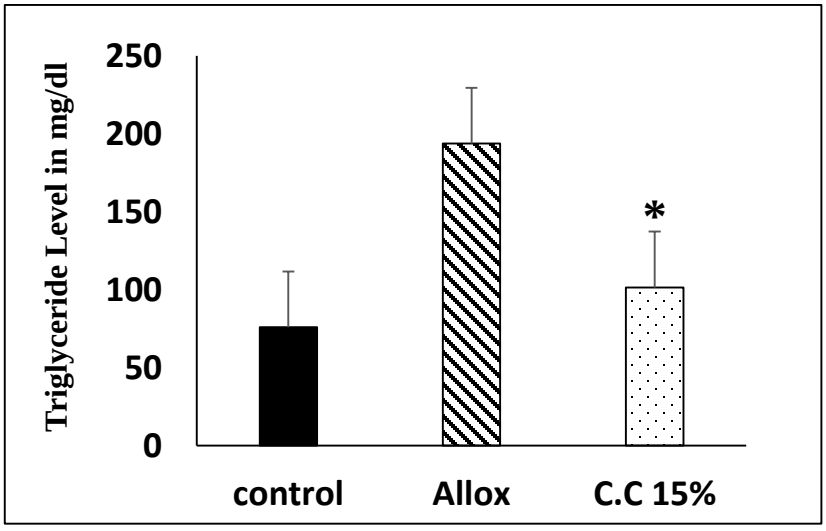

Figure 6: Effect of 15% W/V of CC on Serum Triglyceride Levels in Alloxan-Induced Rats Values are expressed (mg/dl) as mean standard deviation; * means a significant difference from the Alloxan group as *p 0.05*.

3.6.8 Levels of AST (GOT): The effect of 15% W/V of CC on liver enzyme (Aspartate aminotransferase, AST) level was

significantly (p 0.001) decreased compared to the Alloxan group (Fig. 7).

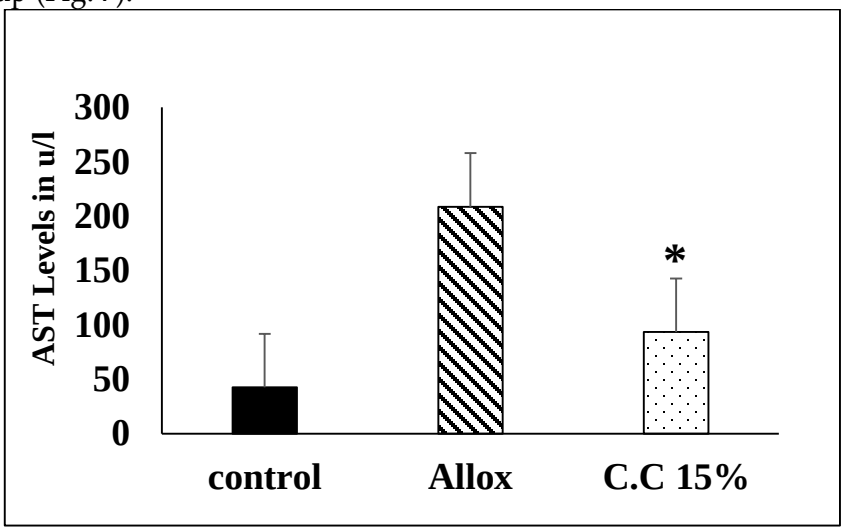

Figure 7: Effect of 15% W/V of CC on the AST level of alloxan-induced rats Values are expressed (u/l) as mean S.D.; * indicates a significant difference from the Alloxan group at p 0.05.

3.6.9 Measurement of ALT (GPT):

When compared to the Alloxan group, the effect of 15% W/V CC on liver enzyme (Alanine aminotransferase, ALT) levels was significantly (0.001) lower (Fig.8).

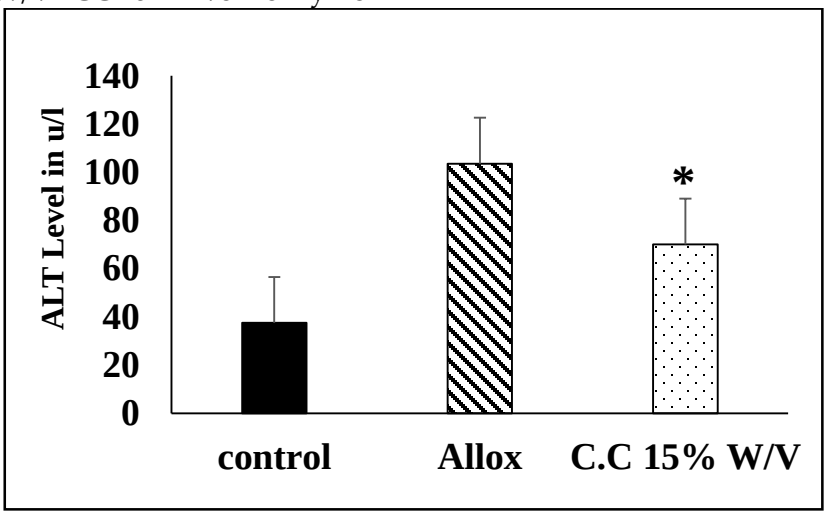

Figure 8: Effect of 15% W/V of CC on the ALT level of alloxan-induced rats Values are expressed (u/l) as mean SD. * indicate
significant difference from the Alloxan group as *p 0.05.*

3.6.10 Reduced Glutathione (GSH) estimation:

Glutathione reductase, the antioxidant enzyme, is significantly increased in

pancreatic tissue in the group that received 15% w/v CC compared with the alloxan-induced group (Fig. 9).

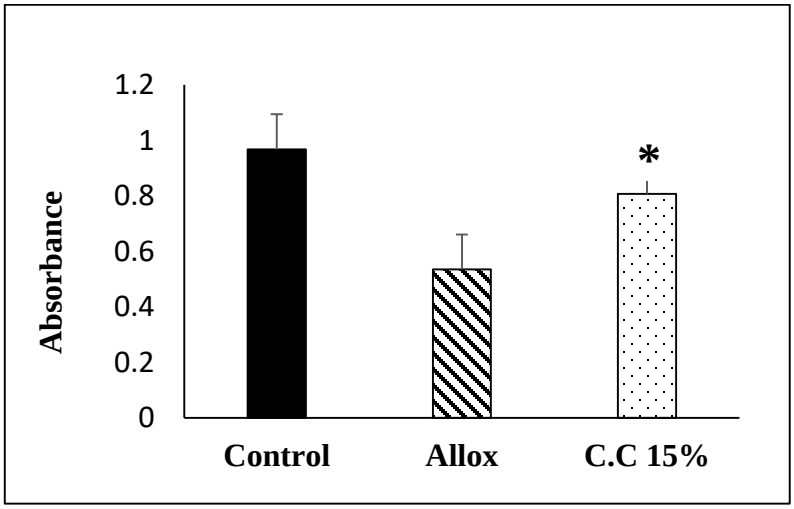

Figure 9 shows the effect of CC at 15% W/V on the GSH in normal and Alloxan-induced rats. Values are expressed (u/l) as mean SD, * indicating a significant difference from the Alloxan group at *p 0.05*.

3.6.11 Lipid peroxidase estimation:

Malondialdehyde (MDA), which is produced by lipid peroxidation in the pancreatic tissue of rats, is significantly lower in the group that received CC compared to the Alloxan group (Fig. 10).

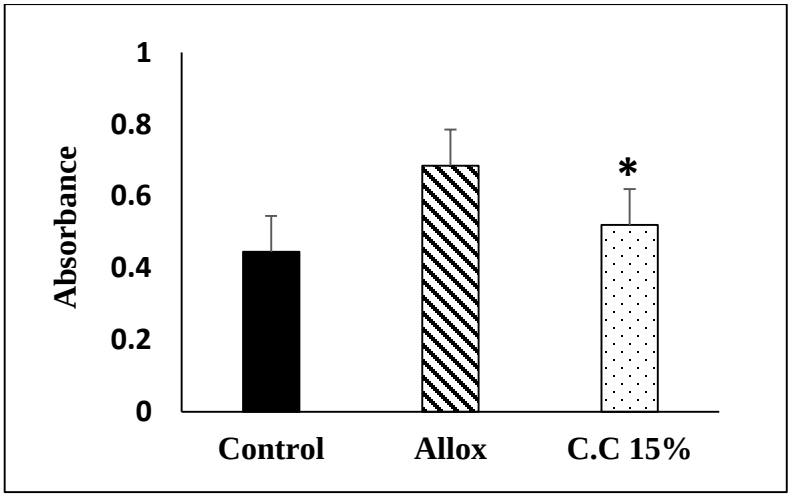

Figure 10 depicted the effect of CC 15% W/V on LP in both normal and Alloxan-induced rats. Values are expressed (u/l) as mean SD, * indicating a significant difference from the Alloxan group at p 0.05.

Discussion

The glucose tolerance test (GTT) is a widely used test to investigate glucose homeostasis in rodents. It is an easy and informative test. However, it does not tell much about the mechanism of action (12). Accordingly, the effect of the CC extract on the profile of GTT was undertaken. In the treated group, there was a significant improvement in the glucose profile as glucose levels shifted toward normal (Fig. 1). However, the decrease in glucose levels by CC extract was not as much as that produced in the Glibenclamide group that was used as a positive control at which glucose levels go beyond the normal levels. Thus, data generated from GTT point to the ability of CC extract to normalize high glucose levels. Nevertheless, using a reliable hyperglycemia model was needed to collect more data about the effectiveness and potency of this plant.

Alloxan monohydrate is a toxic substance that is used to induce diabetes in experimental animals. It works by generating ROS, which result in serious damage and necrosis in the pancreatic -cells that are responsible for insulin production and secretion. Therefore, the resulting situation mimics a state of insulin-dependent diabetes (alloxan diabetes) (23). Hence, diabetes induced by alloxan is a commonly used model for investigating the glycemic control potential of plant extracts (13). Treated animals with CC extract either with a single dose or repeated doses for a week resulted in a significant reduction in the high glucose levels resulting from Alloxan on animals. As Alloxan has a direct effect on the insulin-producing cells of the pancreas, the anti-hyperglycemic effect produced by the CC extract may be directly related to improving the ability of -cells to produce insulin by reversing the action of Alloxan and/or enhancing the sensitivity of cells to insulin. Since alloxan-induced diabetes is considered a form of insulin-dependent diabetes mellitus (13), The Alloxan model has two pathological effects: inhibition of glucose-stimulated insulin secretion and generation of ROS that promotes selective necrosis of pancreatic insulin-producing cells (24). Accordingly, the levels of glutathione reductase in pancreatic tissue (the antioxidant enzyme) went up in response to CC treatment (Fig. 9) and the levels of malondialdehyde produced from peroxidation went down. Both indicate an anti-oxidant action resulting from CC administration in pancreatic tissue. Furthermore, Alloxan has been shown to have liver effects that are similar to the natural complications of diabetes (25).

In addition, Alloxan has a direct oxidative necrosis effect on hepatocytes, leading to the burst of their contents such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (26). Then, evaluating ALT and AST after sub-acute treatment of CC pointed to a protective and/or regenerative effect on liver cells as the values of these enzymes were reduced.

Diabetes is often accompanied by metabolic problems such as dyslipidemia as a result of the unusual metabolism of carbohydrates (27). It is well documented that alloxan-induced diabetes dyslipidemia includes an

elevation of cholesterol and triglyceride levels (28).

Consequently, the levels of cholesterol and triglycerides were increased in alloxan-diabetic animals before giving CC extract, but the levels were restored upon treatment for a week with CC extract. Hence, data from cholesterol and triglyceride tests go hand in hand with the reduction in hyperglycemia resulting in Alloxan-diabetic animals. Glucose and ketone bodies are usually detected in the urine of alloxan-diabetic rats. Treatment of diabetic rats with CC extract resulted in a depression in their levels in urine, which is in accord with the lowering effect of CC on blood glucose levels. Therefore, in general, the hyperglycemia and the related toxic and metabolic effects produced as a result of Alloxan administration have been reduced by CC extract. In addition, in normoglycemic animals, CC extract was also able to reduce blood glucose levels. Therefore, the hypoglycemic activity might be regulated by raising the levels of insulin or increasing the sensitivity of the cells to it. However, further investigations are needed to determine and isolate the compound(s) responsible for the above-discussed effects, and mechanistic studies are also required to elucidate the exact mechanism of action.

Conclusion:

This study revealed that the ethanolic extract of CC heads at 30% W/V and 15% W/V may contain ingredients able to lower blood sugar. Therefore, the generated data is in agreement with what has been practiced in Libyan folk medicine.

References:

1. Wang, X., Wu, F., Sun, H., Zhang, A., & Wei, W. (2017). Identification of the Absorbed Constituents of Schisandra Lignans by Serum Pharmacochemistry in TCM *Serum Pharmacochemistry of Traditional Chinese Medicine*, 337–350. https://doi.org/10.1016/B978-0-12-811147-5.00024-8
2. Farnsworth, N. R. (1994). Ethnopharmacology and drug development. *Ciba Foundation symposium*, *185*, 42–59. https://doi.org/10.1002/9780470514634.ch4
3. Foury, C. (1989). Ressources génétiques et diversification de l'artichaut (Cynara scolymus L.) *Acta Horticulturae*, (242), 155–166. https://pascal-francis.inist.getRecordDetail&idt=7371141
4. Sonnante, G., Pignone, D., & Hammer, K. (2007). The domestication of artichoke and cardoon: from Roman times to the genomic age *Annals of Botany*, *100*(5), 1095–1100 https://doi.org/10.1093/aob/mcm127
5. Lonitzer, A. (1551). Naturalis historiae opus novum *Frankfurt am Main* Online version at http://www.uni-mannheim.de.

6. Alavi S. A., Jafri S. M. H., and El-Gadi A. (1983). *Flora of Libya: vol. 107. Asteraceae* Al Faateh University Faculty of Science Department of Botany https://www.worldcat.org/title/flora-of-libya-vol-107-asteraceae/oclc/475212679
7. Vardavas, C., Majchrzak, D., Wagner, K., Elmadfa, I., & Kafatos, A. (2006). The antioxidant and phylloquinone content of wildly grown greens in Crete *Food Chemistry*, *99*(4), 813–821. https://doi.org/10.1016/j.foodchem.2005.08.057
8. Englisch, W., Beckers, C., Unkauf, M., Ruepp, M., & Zinserling, V. (2000). Efficacy of artichoke dry extract in patients with hyperlipoproteinemia *Arzneimittelforschung*, *50*(03), 260–265 https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-0031-1300196
9. Nazni, P., Vijayakumar, T. P., Alagianambi, P., & Amirthaveni, M. (2006). Hypoglycemic and hypolipidemic effects of Cynara scolymus among selected type 2 diabetic individuals *J. Nutr.*, *5*(2), 147–151. https://dx.doi.org/10.3923/pjn.2006.147.151
10. Preis, S. R., Pencina, M. J., Hwang, S. J., D'Agostino Sr., R. B., Savage, P. J., Levy, D., & Fox, C. S. (2009). Trends in cardiovascular disease risk factors in individuals with and without diabetes mellitus in the Framingham Heart Study, *Circulation*, *120*(3), 212-220. https://doi.org/10.1161/CIRCULATIONAHA.108.846519
11. Abubakar, A. R., & Haque, M. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes *Journal of Pharmacy & Bioallied Sciences*, *12*(1), 1–10. https://doi.org/10.4103/jpbs.JPBS_175_19
12. Bowe, J. E., Franklin, Z. J., Hauge-Evans, A. C., King, A. J., Persaud, S. J., & Jones, P. M. (2014). Metabolic phenotyping guidelines: assessing glucose homeostasis in rodent models *Journal of endocrinology*, *222*(3), G13–G25. https://doi.org/10.1530/JOE-14-0182
13. Ighodaro, O. M., Adeosun, A. M., Asejeje, F. O., Soetan, G. O., & Kassim, O. O. (2018). Time-course effects of 5, 5-dihydroxyl pyrimidine-2, 4, 6-trione (alloxan) as a diabetogenic agent in an animal model *Alexandria journal of medicine*, *54*(4), 705-710. https://doi.org/10.1016/j.ajme.2018.05.005
14. Shah, N. A., & Khan, M. R. (2014). Antidiabetic effect of Sida cordata in alloxan-induced diabetic rats *Biomedical Research International*, *2014*. https://doi.org/10.1155/2014/671294
15. Richmond, W. (1973). Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum *Clinical*

chemistry, *19*(12), 1350–1356. https://doi.org/10.1093/clinchem/19.12.1350

16. Young, D. S., Pestaner, L. C., and Gibberman, V. A. L. (1975). Effects of drugs on clinical laboratory tests. *Clinical Chemistry*, *21*(5), 1D-432D. https://pubmed.ncbi.nlm.nih.gov/1091375/
17. Bergmeyer, H. U., Bowers, G. N., Horder, M., & Moss, D. W. (1976). IFCC method for aspartate aminotransferase Appendix B. Conditions for the measurement of the catalytic concentrations of reagent enzymes and contaminants. *Clinica chimica acta, an international journal of clinical chemistry*, *70*(2), F41–F42. https://pubmed.ncbi.nlm.nih.gov/954208/
18. Bergmeyer, H. U., Scheibe, P., & Wahlefeld, A. W. (1978). Optimization of methods for aspartate aminotransferase and alanine aminotransferase *Clinical Chemistry*, *24*(1), 58–73 https://doi.org/10.1093/clinchem/24.1.58
19. Erejuwa, O. O., Sulaiman, S. A., Abdul Wahab, M. S., Nainamohammed Salam, S. K., Md Salleh, M. S., & Gurtu, S. (2009). Antioxidant Protective Effect of Glibenclamide and Metformin in Combination with Honey in Pancreas of Streptozotocin-Induced Diabetic Rats. *11(5), 2056-2066, International Journal of Molecular Sciences.*https://doi.org/10.3390/ijms11052056.
20. Ellman, G. E. (1959). Tissue sulphydryl groups, Arch Biochem Biophys 82: 70–77. https://doi.org/10.1016/0003-9861(59)90090-6
21. Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review of antioxidant activity evaluation methods in vivo and in vitro.*Saudi pharmaceutical journal: SPJ, the official publication of the Saudi Pharmaceutical Society*, *21*(2), 143–152. https://doi.org/10.1016/j.jsps.2012.05.002
22. Oshawa, H., Odisha, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*(2), 351-358. https://doi.org/10.1016/0003-2697(79)90738-3
23. Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes *Diabetologia*, *51*(2), 216-226. https://doi.org/10.1007/s00125-007-0886-7
24. Jörns, A., Munday, R., Tiedge, M., & Lenzen, S. (1997). Comparative toxicity of alloxan, N-alkylalloxans, and ninhydrin to isolated pancreatic islets in vitro *The Journal of Endocrinology*, *155*(2), 283–293. https://doi.org/10.1677/joe.0.1550283
25. Lucchesi, A. N., Cassettari, L. L., & Spadella, C. T. (2015). Alloxan-induced diabetes causes morphological and ultrastructural changes in the rat liver that resemble the natural history of chronic fatty

liver disease in humans. *Journal of diabetes research*, *2015*, 494578 https://doi.org/10.1155/2015/494578

26. Atawodi, S. E.; Yakubu, O. E.; Liman, M. L.; & Iliemene, D. U. (2014). Effect of a methanolic extract of Tetrapleura tetraptera (Schum and Thonn) Taub leaves on hyperglycemia and indices of diabetic complications in alloxan-induced diabetic rats *Asian Pacific Journal of Tropical Biomedicine*, *4*(4), 272–278 https://doi.org/10.12980/APJTB.4.2014C73
27. Babaei-Jadidi, R.; Karachalias, N.; Ahmed, N.; Battah, S.; & Thornalley, P. J. (2003). Prevention of incipient diabetic nephropathy by high-dose thiamine and benfotiamine *Diabetes*, *52*(8), 2110–2120. https://doi.org/10.2337/diabetes.52.8.2110
28. Yin, P., Wang, Y., Yang, L., Sui, J., & Liu, Y. (2018). Hypoglycemic Effects in Alloxan-Induced Diabetic Rats of the Phenolic Extract from
29. Mongolian Oak Cups Enriched in Ellagic Acid, Kaempferol and Their Derivatives. *Molecules (Basel, Switzerland)*, *23*(5

.