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Original Article

The Effect of Single Clove Garlic Extract (*Allium sativum)* Against Aspartame-Induced Hepatotoxicity in Diabetic Rats

Mohammed Ahmed Qasim[1], Fahmi S. Moqbel[1,*] and Nada M. H. Al Hamdani[2]

[1] Department of Biology, Faculty of Applied Science, Thamar University, Dhamar 87246, Yemen
[2] Department of Biology, Faculty of Sciences, Sana'a University, Sana'a, Yemen

*Corresponding author: Fahmi S. Moqbel
Department of Biology, Faculty of Applied Science, Thamar University, Dhamar 87246, Yemen
Email: fahmi.moqbel@tu.edu.ye

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Abstract:

Background: Aspartame is a widely used synthetic sweetener used for dietary control and by diabetics. **Objectives**: This study aims to investigate the effect of a single garlic clove extract against aspartame-induced hepatotoxicity in diabetic rats. **Methods**: Forty-eight experimental male albino rats were randomly divided into eight groups (6 rats per group) as follows: Group (G)1: served as normal control and received distilled water orally, G2: rats treated with single clove garlic (SCG) extract (0.5 g/kg body weight), G3: rats were treated with SCG extract (0.5 g/kg body weight) and alkaline phosphatase (ALP) (200 mg/kg body weight), G4: rats treated with ASP (200 mg/kg body weight), G5: was the diabetic control group (induced by alloxan (ALX) 120 mg/kg body weight), G6: diabetic rats were treated with SCG extract (0.5 g/kg body weight), G7: diabetic rats were treated with SCG extract (0.5 g/kg body weight) and ASP (200 mg/kg body weight) and G8: diabetic rats were treated with ASP 200 mg/kg body weight. **Results**: The results obtained showed that treatment with ASP caused a significant increase in serum levels of serum alanine transaminase (ALT), aspartate transaminase (AST), and ASP in SCG extract-treated rats in G3 and G4 and diabetic rats in G7 and G8, while treatment with SCG extract caused a significant decrease in serum levels of ALT, AST and ALP in SCG treated rats in G2 and G6 and diabetic rats in G7 compared to the control group (G1). After treatment with ASP, histologic changes in the liver were observed in G4, which were more pronounced in G8, indicating liver damage compared to the control group, while treatment with SCG extract showed further changes in liver tissue in the diabetic rats of G7 and G8. **Conclusion**: The present study suggests that administering ASP in normal rats leads to liver dysfunction. Moreover, our results proved that the application of ASP in diabetic rats may cause additional damage compared to ASP in normal rats. This study demonstrated that treatment with SCG extract significantly attenuated the biochemical and histopathologic changes induced by ASP and alloxan. From the data obtained, it appears that treatment with SCG extract has a protective effect against the toxicity of ASF and alloxan on the liver of normal and diabetic rats.

Keywords: Alloxan; Single Clove Garlic; Hepatotoxicity; Aspartame.

1. Introduction

Alkaline phosphatase (ALP) (E951) is one of the most widely used artificial sweeteners in many products worldwide and in various countries [1]. It is widely used (62%) as a non-nutritive sweetener in foods, beverages, and pharmaceuticals [2]. ASP is an artificial sweetener that has almost 180-200 times the sweetening power of sucrose as it has a low caloric value of 4 Kcal/g [3,4]. It is increasingly used by diabetic patients, is widely used in weight loss, and deceives almost 200 million people worldwide [5,6].

During metabolism, ASP is broken down in the intestinal lumen into the three hydrolysis products phenylalanine (50%), aspartic acid (40%), and methanol (10%) [7,8,9,10]. The production of the essential amino acid phenylalanine is hazardous to the health of people born with a rare genetic disease called phenylketonuria (PKU). However, methanol production during ASP metabolism is not very high but still contributes to toxicity [11]. The effect Long-term consumption of ASP in rats leads to liver cell damage and changes in antioxidant status in the liver [12]. The long-term effect of aspartame (75 mg/kg) on the antioxidant status of the liver and brain with histopathologic changes in the liver and renal cortex in albino rats of the Wistar strain was investigated. Aspartame was reported to induce marked changes in the expression of key oncogenes. Harvey's sarcoma of rat viral homozygous h-Ras and viral homologous myeloma c-myc in rat livers [13]. Several clinical disorders, including hepatotoxicity, nephrotoxicity, neurotransmitter imbalance, and cognitive impairment [14]. Measurement of the activities of these marker enzymes in tissues and body fluids can be used to assess the degree of damage and toxicity of a chemical compound on organs/tissues [15,16].

Garlic (*Allium sativum* L): is a bulbous herb of the Alliaceae or Lilliaceae family. *Allium sativum*, commonly known as garlic, has more than 500 species in 30 genera and the average family is classified between Lilliaceae and Amaryllidaceae [17]. *Allium sativum* contains more than a hundred biologically useful secondary metabolites. Garlic also contains sulfur compounds such as aliin, allicin, allylpropyl, diallyl trisulfide, sallylcysteine, vinyldithiine, and S-allyl mercaptocysteine [18]. Other compounds such as enzymes, vitamins (A, B1, B2, B6, C, E), fibers (1.5%), water (65%), 17 amino acids (1.2%), carbohydrates, organic sulfur compounds (2.3%), proteins (mainly allinase; 2%), fatty acids, glycolipids, phospholipids, saponins, glycosides, and minerals such as calcium (Ca), iron (Fe), copper (Cu), magnesium (Mg), potassium (K), zinc (Zn), germanium (Ge) and selenium (Se) [19,20,21].

Garlic has many medicinal properties such as blood sugar-lowering, anti-inflammatory, anti-cancer, antioxidant, antiviral, antibacterial,

antifungal, cholesterol-lowering, and immunomodulating effects [22,23]. Due to cultivation practices and climatic conditions, the bulbs of garlic are sometimes not divided into cloves and produce a single clove of garlic known as single garlic clove, solo garlic, and pearl garlic. The amount of research on the bioactivity of single clove garlic is limited. Recently, a single clove of garlic was found to have a stronger hepatoprotective effect than regular garlic, known as multi-clove garlic, in the CCl4-poisoned rabbit model [24,25]. Garlic has been studied in various forms of extracts: aqueous, ethanol, and dried powder. Garlic extracts are effective in gentamicin-induced renal damage and oxidative stress in rats [26]. Adriamycin-induced toxicity was found for garlic powder [27].

Aspartame (ASP) is a dipeptide sweetener found as the main ingredient in most sugar-free products on the market today. The Food and Drug Administration (FDA) has approved the use of aspartame, but since then the safety of aspartame consumption has been questioned. In this study, the protective effect of a single clove of garlic on the biochemical and histologic changes against aspartame-induced nephrotoxicity in normal and diabetic rats was investigated.

2. Materials and Methods

2.1 Experimental animals

Forty-eight male albino rats with an average weight of 200 ± 50 g, obtained from the Biology Department, Faculty of Science, Sana'a University, participated in this study. The rats were housed in stainless steel cages and the entire experiment was conducted under ambient conditions at a room temperature of 25 ± 3 °C and humidity of 50 ± 3 % and under a 12-hour light and 12-hour dark schedule. The rats received food and water ad libitum 14 days prior to the experiment.

2.2 Chemicals

Alloxan (ALX) monohydrate: obtained from Dhafar Pharma Sana'a, Yemen (S.D Fine - Chem. Ltd., Mumbai, India). Aspartame (ASP): Aspartame obtained from Dhafar Pharma Sana'a, Yemen (Alexandria Company, Egypt). It is available in the form of tablets, each containing 20 mg of aspartame (aspartame 25 g/0.2 ml distilled water) to achieve a concentration of (200 mg/kg body weight) per rat [28].

2.3 Preparation of the extract from a single clove of garlic

Fresh garlic cloves were purchased from a local market in Sweida district near Dhamar city, Yemen, in October 2020. Dried and ground bulbs (approximately 100 g) were extracted with 300 ml ethanol (96%) in a Soxhlet apparatus for 72 hours. After extraction, the solvent was filtered and then evaporated with rotavapor. The alcoholic garlic extract obtained was stored at 4 °C until use.

2.4 Preparation of the diabetic rat

The animals were injected with alloxan (120 mg/kg body weight). Five days after injection, the rats with fasting blood glucose greater than 250 mg/dl were used for the experiments. Each animal was used only once in all experiments. Food and water were removed from the cages 12 hours before the experiment.

2.5 Experimental design

Forty-eight rats (24 diabetic, 24 normal rats) were used. The rats were divided into eight groups (six rats each) as follows:

Group 1: Normal control rats were administered 1 ml of distilled water.

Group 2: Normal rats were administered an extract of a single clove of garlic (SCG) (0.5 g/kg body weight).

Group 3: Normal rats were administered an extract of a single clove of garlic (SCG) 0.5 g/kg body weight and aspartame (ASP) 200 mg/kg body weight.

Group 4: Normal rats received aspartame (ASP) 200 mg/kg body weight dissolved in 25 g/0.2 ml distilled water.

Group 5: Diabetic control rats were administered 1 ml of normal saline.

Group 6: Diabetic rats were administered an extract of a single clove of garlic (SCG) (0.5 g/kg body weight).

Group 7: Diabetic rats were treated with an extract of a single clove of garlic (SCG), 0.5 g/kg body weight, and aspartame (ASP) 200 mg/kg body weight.

Group 8: Diabetic rats were treated with aspartame (ASP) 200 m /kg body weight.

All animals in the experimental groups were administered orally by gavage once daily for 30 days.

2.6 Body weight assessment

Rats in all experimental groups were weighed using electronic scales before the start of the experiment (day 0) and on day 3, day 10, day 20 and at the end of the experimental period (day 30). The changes in body weight were calculated using the following formula:

$$\textbf{Body weight gain (g)} = \frac{\textbf{Final body weight} - \textbf{Initial body weight}}{\textbf{Final body weight(g)}} \textbf{x 100} \qquad \textbf{(1)}$$

2.7 Relative liver weights

All rats were fasted overnight on day 29 of the experiment and live body weight (g) was determined on day 30 prior to euthanasia. At autopsy, the liver of each animal was removed, dried with tissue paper, and weighed on an electronic scale prior to fixation to determine and statistically analyze the change in organ weight relative to body weight. The relative liver weight of each animal was calculated using the following formula:

$$\textbf{Relative liver weight (g)} = \frac{\textbf{(Absolute liver weight (g)}}{\textbf{Body weight of rat on sacrifice day (g)}} \textbf{x 100} \qquad \textbf{(2)}$$

2.8 Collection of Blood Samples

On the 30th day of the experimental period, 5 ml of blood was collected from the ophthalmic vein (canthus) of each animal using capillary tubes in unsalted heparin tubes for biochemical analysis.

3. Biochemical analysis

3.1 Liver Function Tests

Serum aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) tests were measured. The blood samples were centrifuged at 3000 rpm for 10 minutes and then the blood serum was collected at 4 ºC for various physiological and biochemical assays. The blood samples were analyzed in the Automated Clinical Chemistry Analyzer, Dimension Type RXL Max (Dade Behring Delaware, DE 19714, U.S.A.) at Al-Asr Alhadith Specialized Medical Laboratory, Dhamar, Yemen.

3.2 Histologic Examination

At autopsy, the liver tissue of each group was washed in saline and fixed with 10% formalin, dehydrated in ethyl alcohol of various ascending grades, cleared in xylene and embedded in kerosene wax, cut into 4 µm thick sections, stained with hematoxylin and eosin (H & E) and examined under light microscope for histologic examination [29].

3.3 Statistical Analysis

All data were expressed as mean ± standard deviation (SD) and statistically analyzed using one-way ANOVA to examine the differences between the studied groups. Subsequently, Dunnett's test was performed and compared with the respective control group of animals. The test for multiple comparisons between the groups was performed using SPSS software (SPSS version 21 IBM Chicago, IL, USA). All tests were considered statistically significant at a P-value of < 0.05.

4. Result

4.1 Effect of single clove garlic (SCG) extract against aspartame-induced on body weight (g) in normal and diabetic rats

Table 1 shows that the final body weight of 193.80 ± 7.01 g in the normal control group increased significantly compared to the initial body weight of 152.60 ± 3.78 g. Rats administered ASP showed a lower body weight gain (-5 g) compared to the normal control rats (+41.2 g). The diabetic control rats showed a lower body weight gain (-7.4 g) compared to the body weight gain in the normal control rats (+41.2 g). While the body weight gain in diabetic rats administered ASP showed less body weight gain (-15 g) compared to the normal control rats (+41.2 g), induction of diabetes in diabetic control rats showed less body weight gain (-7.4 g) compared to the body weight gain in the normal control rats (+41.2 g).

As shown in Table 1, the SCG extract treated rats showed a significant increase in body weight (+59.87 g) compared to the body weight gain of the normal control group (+41.2 g), while the SCG extract +

ASP treated rats showed no significant changes in body weight (+40.8 g) compared to the normal control rats (+41.2 g).

Accordingly, the diabetic rats administered SCG extract showed no significant change in their body weight gain (+40 g) compared to that of the normal control rats (+41.2 g) but showed an increase in their body weight gain (+40 g) compared to that of the diabetic control rats (-7.4% g). Similarly, the diabetic rats administered SCG extract + ASP showed no change in their body weight gain (+40.4 g) compared to the body weight gain of the normal control rats (+41.2 g), moreover, a remarkable increase in their body weight gain (+40.4 g) was observed compared to that of the diabetic control rats (-7.4% g). As shown in Table 1.

4.2 Relative Liver Weight

The results in Table 2 show that the ASP treated rat group had a significant increase in relative liver weight of 4.47 ± 0.539 g compared to that of the normal control rats of 3.92 ± 0.247 g. In addition, a significant increase in relative liver weight of 4.66 ± 0.287 g was observed in the diabetic ASP treated rats compared to the normal control rats 3.92 ± 0.247 g. There was also an increase in relative liver weight of 4.03±0.296g in the diabetic control rats compared to the normal control rats 3.92 ± 0.247 g.

In Table 2, the rats administered SCG extract showed no remarkable increase in relative liver weight 3.59 ± 0.256 g compared to that of normal control rats 3.92 ± 0.247 g. In the rats administered the SCG extract with ASP, there was no significant increase in relative liver weight 3.60±0.258g compared to that of control rats 3.92±0.247g, while it decreased significantly compared to that of normal ASP rats 4.47±0.539g. Similarly, diabetic rats treated with SCG extract showed no significant increase in relative liver weight of 3.56 ± 0.043 g compared to control rats 3.92 ± 0.247 g and a significant decrease compared to diabetic control rats 4.03 ± 0.296 g.

The result presented in Table 2 shows that the rats treated with the ethanolic extract of SCG and ASP group showed no remarkable increase in relative liver weight (3.50 ± 0.273 g) compared to the normal control rats (3.92 ± 0.247 g) and a remarkable decrease in relative liver weight compared to the diabetic ASP rats (4.66 ± 0.287 g).

4.3 Serum Biochemical Markers

4.3.1 Liver Function Tests

A deeper scientific look at the results in Tables 3, 4 and 5 shows that the orally administered normal ASP-treated rats exhibited a remarkable increase in ALT, AST and ALP serum levels compared to the normal control group: 74.40 ± 11.92 U/L, 165.60 ± 21.41 U/L and 141.20 ± 14.34 U/L, respectively. In addition, diabetic ASP-treated rats showed a significant increase in ALT, AST and ALP serum levels 106.40 ± 2.96 U/L, 223.00 ± 6.00 U/ and 227.20 ± 2.58 U/L, respectively, compared to the control group 74.40 ± 11.92 U/L, 165.60 ± 21.41 U/L and 141.20±14.34 U/L, respectively.

Similarly, Table 3, 4 and 5 show that there was a significant increase in ALT, AST and ALP serum levels 116.40 ± 9.31 U/L, 227.80 ± 9.01 U/L and 229.60 ± 13.27 U/L in the diabetic control group as compared to the control group 74.40 ± 11.92 U/L, 165.60 ± 21.41 U/L and 141.20 ± 14.34 U/L respectively.

The results of the current study, as shown in Tables 3, 4 and 5, show that the SCG-treated rats showed no significant change in ALT, AST and ALP serum levels of 74.00 ± 11.72 U/L, 164.40 ± 7.12 U/L and 140.80 ± 14.54 U/L, respectively, compared to the control group 74.40 ± 11. 92 U/L, 165.60 ± 21.41 U/L and 141.20 ± 14.34 U/L, respectively. Consequently, the animals treated with SCG extract with ASP showed a significant decrease in ALT, AST and ALP serum levels 77.60 ± 27.18 U/L, 168.20 ± 13.06 U/L and 142.00 ± 24.80 U/L compared to normal ASP rats 110.60 ± 4.56 U/L, 218.60 ± 4.21 U/L and 232.00 ± 8.54 U/L, respectively. Diabetic SCG extract rats showed no significant change in ALT, AST and ALP serum levels 79.20 ± 8.10 U/L, 168.60 ± 14.63 U/L and 145.60 ± 37.18 U/L compared to normal control group 74.40 ± 11.92 U/L, 165.60 ± 21.41 U/L and 141.20 ± 14.34 U/L respectively. The results showed that there was a significant decrease in ALT, AST and ALP serum levels 79.20 ± 8.10 U/L, 168.60 ± 14.63 U/L and 145.60 ± 37.18 U/L, respectively, compared to the diabetic control group 116.40 ± 9.31 U/L, 227.80 ± 9.01 U/L and 229.60 ± 13.27 U/L, respectively.

Table 1: Effects of a single clove garlic extract (SCG) against aspartame-induced body weight changes in normal and diabetic rats.

	Mean ± SD Change in body weight (gm)		
Change of weight	**Initial weight body(g) 0th Day**	**Final weight body(g) 30th Day**	**Body weight gain(g)**
Normal control	152.60 ± 3.78	193.80 ± 7.01	+41.2
Normal SCG extract	220.53 ± 6.94	280.40 ± 9.47	+59.87
Normal SCG extract &aspartame	155.00 ± 4.24	195.80 ± 4.76	+40.8
Normal aspartame	168.80 ± 27.17	163.80 ± 26.12*	-5
Diabetic control	169.20 ± 7.25	161.80 ± 7.39*	-7.4
Diabetic SCG extract	246.60 ± 3.28	286.40 ± 9.86	+40
Diabetic SCG extract &aspartame	234.40 ± 17.41	274.80 ± 10.59	+40.4
Diabetic aspartame	234.20 ± 18.67	219.20 ± 20.82*	-15

Table 2: Effect of single clove garlic (SCG) extract against aspartame induced on relative liver weights (g) in normal and diabetic rats.

Relative weighs Group	**Relative liver weight (g) Mean ± SD**	**%Change**
Normal control	3.92±0.247	0.00
Normal SCG extract	3.59 ±0.256	-8.41
Normal SCG extract &aspartame	3.60 ±0.258	-8.16
Normal aspartame	4.47±0.539 a**b***	14.03
Diabetic control	4.03 ±0.296	2.80
Diabetic SCG extract	3.56 ±0.043	-9.18
Diabetic SCG extract &aspartame	3.50±0 .273 c**	-10.71
Diabetic aspartame	4.66 ±0.287 a**d**	18.87

Data were presented as mean ± standard deviation (SD) of 6 animals| group (n = 6) = Number of rats/groups. a: significant values compared to normal control. *p < 0.05; **p < 0.01; and *** p< 0.001.

In addition, the rats treated with diabetic SCG extract and ASP showed a significant decrease in ALT, AST and ALP serum levels 82.20 ± 12.93 U/L, 171.00 ± 5.47 U/L and 146.60 ± 2.40 U/L, respectively, compared to the diabetic ASP group 106.40 ± 2.96 U/L, 223.00 ± 6.00 U/L and 227.20 ± 2.58 U/L, respectively. The results also showed that there was a significant decrease in ALT, AST, and ALP serum levels 82.20 ± 12.93 U/L, 171.00 ± 5.47 U/L, and 146.60 ± 2.40 U/L compared to diabetic control rats 116.40 ± 9.31 U/L, 227.80 ± 9.01 U/L and 229.60 ± 13.27 U/L, respectively.

Table 3: Effects of single clove garlic (SCG) extract against aspartame-induced hepatotoxicity in normal and diabetic rats; levels of ALT.

Parameters Group	ALT (U/L)	% Change
Normal control	74.40 ± 11.92	**0.00**
Normal SCG extract	74.00±11.72	**-0.53**
Normal SCG extract &aspartame	77.60±27.18	**4.30**
Normal aspartame	110.60 ±4.56 ^{a***} ^{b***}	**48.65**
Diabetic control	116.40 ±9.31 ^{a***}	**56.45**
Diabetic SCG extract	79.20±8.10 ^{c***}	**6.45**
Diabetic SCG extract &aspartame	82.20±12.93	**10.48**
Diabetic aspartame	106.40 ±2.96 ^{a**} ^{d**}	**43.01**

G1: control group, G2: rats treated with SCG extract (0.5 g/kg body weight), G3: rats were treated with SCG extract (0.5 g /kg body weight), and aspartame (ASP) (200 mg/kg body weight) G4: rats treated with ASP (200 mg/kg body weight), G5: diabetic rats (Induced by alloxan (ALX) 120 mg/kg body weight), G6: diabetic rats treated with SCG extract (0.5 g /kg body weight), G7: diabetic rats treated with SCG extract (0.5 g /kg body weight), and ASP (200 mg /kg body weight) and G8: diabetic rats treated with ASP 200 mg /kg body weight.

Table. 4: Effect of single clove garlic (SCG) extract against aspartame-induced hepatotoxicity in normal and diabetic rats; levels of AST.

Parameters Group	AST (U/L)	% Change
Normal control	165.60 ±21.41	0.00
Normal SCG extract	164.40 ±7.12	-0.72
Normal SCG extract &aspartame	168.20±13.06	1.57
Normal aspartame	218.60±4.21^{a***}^{b***}	32.00
Diabetic control	227.80±9.01 ^{a***}	37.56
Diabetic SCG extract	168.60±14.63 ^{c***}	1.81
Diabetic SCG extract &aspartame	171.00±5.47	3.26
Diabetic aspartame	223.00±6.00 ^{a***} ^{d***}	34.66

G1: control group, G2: rats treated with SCG extract (0.5 g/kg body weight), G3: rats treated with SCG extract (0.5 g /kg body weight), and aspartame (ASP) (200 mg/kg body weight) G4: rats treated with ASP (200 mg/kg body weight), G5: diabetic rats (Induced by alloxan (ALX) 120 mg/kg body weight), G6: diabetic rats treated with SCG extract (0.5 g/kg body weight), G7: diabetic rats treated with SCG extract (0.5 g/kg body weight), and ASP (200 mg/kg body weight) and G8: diabetic rats treated with ASP 200 mg /kg body weight.

Table 5: Effect of single clove garlic (SCG) extract against aspartame-induced hepatotoxicity in normal and diabetic rats; levels of ALP.

Parameters Group	ALP (U/L)	% Change
Normal control	141.20 ± 14.34	0.00
Normal SCG extract	140.80±14.54	-0.28
Normal SCG extract &aspartame	142.00±24.80	0.56
Normal aspartame	232.00 ±8.54 ^{a***} ^{b***}	64.30
Diabetic control	229.60 ±13.27 ^{a***}	62.60
Diabetic SCG extract	145.60±37.18 ^{c***}	3.11
Diabetic SCG extract &aspartame	146.60±2.40	3.82
Diabetic aspartame	227.20 ±2.58 ^{a***} ^{d***}	60.90

G1: control group, G2: rats treated with SCG extract (0.5 g/kg body weight), G3: rats treated with SCG extract (0.5 g/kg body weight), and aspartame (ASP) (200 mg/ kg body weight) G4: rats treated with ASP (200 mg/kg body weight), G5: diabetic rats (Induced by alloxan (ALX) 120 mg/kg body weight), G6: diabetic rats treated with SCG extract (0.5 g/kg body weight), G7: diabetic rats treated with SCG extract (0.5 g/kg body weight), and ASP (200 mg/kg body weight) and G8: diabetic rats treated with ASP 200 mg /kg body weight.

4.4 Histopathological Examination

The liver cross-sections of the control group showed normal morphology, exhibiting normal hepatocytes with normal central vein and peripheral hepatic triads or tetrads embedded in connective tissue, as shown in Plate 1A, B & C. The liver cross sections of ASP (200 mg/kg body weight) treated rats showed congestion and some lesions such as hemorrhage compared to the control group as shown in panel 1D.

Plate 2A, B & C show the cross-sections of the liver of diabetic rats, which showed central vein (CV) and hepatocytes (HC), enlargement of many hepatocytes (arrows), congestion (C), and hydropic change. In the group treated with alloxan and SCG extract (0.5 g/kg body weight), the liver cross-sections showed slight congestion (C). The liver cross-sections of rats treated with ethanolic SCG extract, and ASP showed a slight hydropic change. The liver cross-sections of rats treated with alloxan, and ASP showed severe degeneration of many hepatocytes, as most cells had lost their cell borders (Plate 2d).

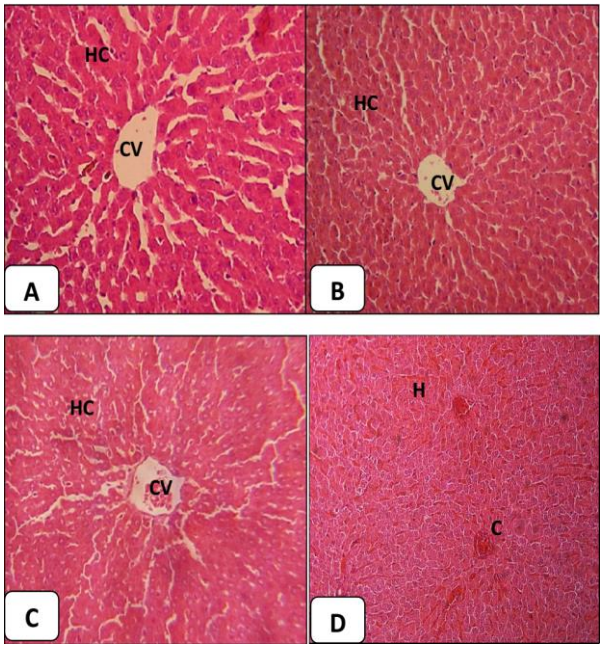

Plate 1: Photomicroscopic cross-sections of the liver of (A): G1 showing normal hepatocyte architecture (HC) and normal central vein (CV). (B): G2 showing normal central vein (CV) and hepatocyte (HC) architecture. (C): G3 showing normal central vein (CV) and hepatocyte (HC) structure. (D): G4 with congestion (C) and hemorrhage (H) of the central vein (CV) and hepatocytes (HC); H& E (X100) and H& E (X400).
G1: control group, G2: rats treated with SCG extract (0.5 g/kg body weight), G3: rats treated with SCG extract (0.5 g/kg body weight) and aspartame (ASP) (200 mg/kg body weight), G4: rats treated with ASP (200 mg/kg body weight).

5. Discussion

5.1 The Change in Body Weight

The results of the present study showed that body weight gain decreased significantly in the ASP-treated group compared to the normal control group. We also reported that administration of ASP in diabetic rats showed a significant decrease in body weight gain compared to that of the normal control group. Our findings are in agreement with the study of [30 ,31], Abd-Elfatah *et al*., (2012) who reported that albino rats receiving ASP showed a significant decrease in body weight. The observed decrease in body weight of treated animals could be a result of protein depletion due to the unavailability of carbohydrates as an energy source [32,33].

The result of the present study is in agreement with the study of Anton *et al.* (2010) and Abd-Elwahab *et al.* (2017) [34,35] who used data to find that sweeteners ASP can affect both fat and carbohydrate metabolism in addition to increasing energy expenditure. Consequently, the results showed a significant decrease in body weight in the diabetes control group compared to the body weight of the normal control group.

In contrast, the rats orally administered the normal SCG extract with ASP showed no change in body weight compared to the normal control rats. However, the rats given the normal SCG extract orally showed a significant increase in body weight compared to the normal control rats. The diabetic group treated with SCG extract and the group treated with SCG extract and ASP also showed a significant increase in body weight compared to the body weight of the diabetic control group.

The results of the current study are in agreement with the studies of [36,37] who treated rats with garlic extract before alloxan treatment and showed a significant increase in body weight of the diabetic rats, which is because the active ingredients of garlic such as S-allylcysteine and organic sulfur can lead to weight gain in alloxan-induced diabetic rats. These results confirm a study by [38]. The treatment of rats with aqueous garlic extract compensates for the reduction in body weight and causes a significant increase in body weight of alloxan-treated rats. These results show that the extract from a single clove of garlic has a protective effect on the changes in body weight caused by the administration of aspartame and alloxan.

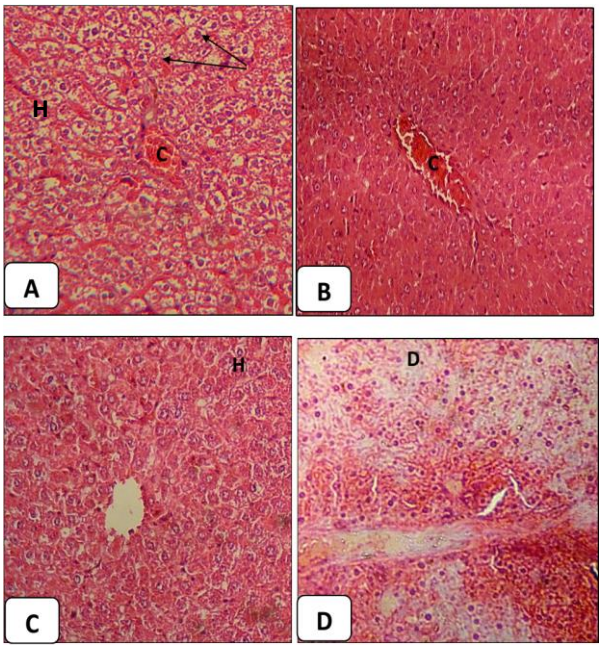

Plate 2: Photographic cross-sections of the liver of diabetic rats (A): G5 showing hydropic change, enlargement of many hepatocytes (arrows) and congestion (C), central vein (CV) and hepatocytes (HC). (B): G6 with mild congestion (C), central vein (CV) and hepatocytes (HC). (C): G7 with mild hydropic changes (H) of the central vein (CV) and hepatocytes (HC). (D): G8 shows severe degeneration of many cells and most cells have lost their cell borders (D) of the central vein (CV) and hepatocytes, H & E (X 100) and H & E (X 400).
G5: diabetic rats (induced by alloxan (ALX) 120mg/kg body weight), G6: diabetic rats treated with SCG extract (0.5 g/kg body weight), G7: diabetic rats treated with SCG extract (0.5 g/kg body weight) and ASP (200 mg/kg body weight) and G8: diabetic rats treated with ASP 200 mg/kg body weight.

5.2 The Relative Liver Weight

The results of the groupsreated group showed a significant increase in relative liver weight compared to the relative liver weight of the normal control group. In addition, the diabetes group treated with ASP showed a significant increase in relative liver weight compared to the normal control group. These results are consistent with the study of [39], in which diabetic rats with CCL_2 were evaluated for hepatotoxicity, as evidenced by increased liver weight relative to body weight and elevated liver enzymes. In the study [40], gentamicin was administered at a dose of 80 mg/kg, which also caused a significant increase in liver weight. The increase in liver weight in the ASP group was most likely due to inflammatory cells, enlargement, congestion, and hemorrhage in the liver, which were evident in the overall appearance of the liver. These symptoms were similar to the results of our study.

The current results are not consistent with the study by [41], who reported a significant decrease in some organ weight of the liver of the aspartame group (40mg/kg body weight). This may be attributed to the difference in dose, treatment duration, and sex or size of the rats. The results of the current study showed that there were no changes in relative liver weights. On the contrary, there was no significant difference between the relative liver weight in the SCG extract treated group and the SCG extract with ASP compared to the normal control group.

However, there was a significant decrease in relative liver weight in the diabetic group treated with SCG extract alone and in the group treated with SCG extract and ASP compared to the diabetic control group and the normal control group. In a study [42], the effects of garlic at a dose of (500 mg/kg body weight) administered to experimental animals for 4 weeks and the general morphological effects on the liver were observed. A low concentration of allicin has a limited role in lowering cholesterol levels by inhibiting the metabolic pathways of cholesterol biosynthesis. In a previous study [43], it was reported that administration of black garlic (200 mg/kg body weight) resulted in a significant decrease in liver weight. These results demonstrate the protective effect of SCG extracts on the changes in relative liver weight induced by aspartame and alloxan in normal and diabetic rats.

5.3 The liver enzymes (ALT, AST, and ALP) levels

The results of the current study have shown that oral administration of ASP affects liver function tests by increasing the levels of liver enzymes (ALT, AST and ALP) compared to the normal control group. These results are in line with [44,45,46] who found that ASP causes a significant increase in the liver function markers AST, ALT and ALP, leading to the formation of free radicals that cause cell damage and release the marker enzymes into the bloodstream. Estimating the activities of the marker enzymes in serum allows an assessment of liver function. Excessive alcohol consumption has been reported to be associated with altered liver metabolism and liver injury with leakage of cytoplasmic liver enzymes into the bloodstream [47,48,49,50].

In the current study, the results showed that liver enzymes were significantly elevated in ALT, AST and ALP in the diabetes control group compared to the normal control group. Thus, these results are consistent with [51], who found that high blood glucose causes a significant increase in ALT, AST and ALP levels in the blood. Thus, as previously reported, our results can be explained by the oxidative stress caused by hyperglycemia, which plays an important role in the development of diabetes and its complications. The increase in ALT, AST and ALP activities after hypoglycemia is stimulated by the production of reactive oxygen species (ROS) and impaired antioxidant enzymes, leading to oxidative stress and organ dysfunction [52].

Our results have shown that there was a significant increase in ALT, AST, and ALP levels after administration of ASP to the diabetic group compared to the normal control group. These results are consistent with those of [53], whose results, as previously reported, can be explained by the oxidative stress caused by high blood glucose, which plays an important role in the development of diabetes and its complications. The results have also shown that after the administration of SCG extract alone and SCG extract and induction of ASP, there was a significant decrease in ALT, AST, and ALP blood levels compared to the group treated with ASP. This decrease was due to the efficacy of ALT, AST, and ALP after the administration of SCG extract, which is attributed to the protective effect of garlic-derived S-allylcysteine on liver damage and oxidative stress [54]. Moreover, the results of the SCG extract-treated group after induction of diabetes by ALX inhibited the higher increase of ALT, AST, and ALP compared to the diabetic control group. Certainly, these results are consistent with the study of [55], which indicates that diabetes can lead to liver dysfunction. Thus, the effect of garlic was reported in both alloxan- and streptozotocin-induced diabetic rats.

The study [56] suggests that the presence of polyphenols and flavonoids in garlic extract may be responsible for the antioxidant activity and the increase in serum levels of ALT, AST and ALP. From the present data, treatment with SCG extract significantly improves the impaired liver functions of ALX-induced diabetic rats. However, the results of the same study show that ALT, AST and ALP levels decreased significantly in the diabetic group treated with SCG extract compared to the diabetic control group. The researchers found that garlic extracts contain certain compounds such as germanium and selenium, which play an important role in normalizing oxygen utilization in the cells [57].

5.4 Histopathological

Histologic analysis provided reliable support for the data obtained by biochemical analysis in rats receiving ASP that exhibited severe histologic changes in the form of congestion and hemorrhage. In this study, male albino rats treated with ASF showed histopathological changes in their livers, namely disintegration of hepatocytes, depletion of cytoplasm and disorganization of cellular organelles. In addition, glycogen granules decreased while the amount of collagen fibers in the portal area increased. In this respect, the histopathological changes in the liver of male albino rats were similar to those of female albino rats in the study [30].

The results state that aspartame was rapidly metabolized with minimal toxicity and liver damage at the border, while other studies showed visceral vacuoles in the hepatocytes after administration of ASP. These toxic agents caused an imbalance in the arrangement of cytoskeletal components, leading to cytoplasmic discharges. The production of methanol and aspartic acid, which led to the release of free radicals, was due to the ingestion of ASP [58,59,60]. The researcher also found that the liver of the diabetic rats with ASP had other severe histologic changes compared to the control group, in which many cells degenerated and most cells lost their cell borders (D), as shown in (Plate 2d). Thus, the results are in agreement with [28], who found that the liver of diabetic-treated rats showed degeneration of many cells and most cells lost their cell borders. The results are also in agreement with [46], who found that consumption of ASP by diabetics could further aggravate the health condition of these individuals. These results can be explained by the fact that oxidative stress triggered by hyperglycemia plays a key role in the development of diabetes and its complications. This opinion is consistent with our study in which a significant increase in lipid peroxidase was observed in liver tissue of rats after 28 days of treatment with ASP. A similar result was reported [60]. Thus, the results are in correlation with our results which showed changes in liver functions and an increase in hepatotoxicity markers after administration of ASP to normal and diabetic rats [28,61].

In addition, the results of our study showed that the liver sections of diabetic rats exhibited hydropic changes, enlargement of many hepatocytes and congestion, as shown in (Plate 2a). These results are consistent with those of [28]. The diabetic-treated liver showed enlargement of many hepatocytes and necrosis in some areas of hepatocytes. It was also shown that administration of ALX to experimental rats selectively caused pancreatic β-cell membrane destruction and cytotoxicity after its intracellular accumulation [62]. In addition, the depletion of glycogen in diabetic rats would increase hyperglycemia and cause damage to the liver and the whole body [63].

In the comparison to the control group, histopathological abnormalities were detected in the liver of both the SCG extract and the SCG extract with ASP (see Table 1a, b and c). In contrast, mild hepatic congestion was observed in the central vein and hepatocytes of the diabetic rats with SCG extract (see panel 2b). Thus, the results of this study show that garlic has a high level of antioxidant activity. Recent in vitro studies by [64,65] have confirmed the vasoactive properties of garlic's sulfur compounds. The aged garlic extract contains the active and stable component S-allylcysteine, which allows standardization of S-allylcysteine. Table 2c shows that the liver of diabetic rats with SCG extract and ASP exhibited mild hydropic changes in the central vein and hepatocytes compared to that of the diabetic ASP group. Thus, the results are in agreement with the study [66], which reported that garlic such as allicin, alliin, and two major organosulfur compounds S-allylcysteine are potent free radical scavengers. In the present study, we reported that these compounds may be responsible for protecting tissues from damage and various diseases, as garlic extract has high antioxidant content and health-promoting potential. Furthermore, a study by [67] has shown that S-allylcysteine and organic sulfur compounds found in garlic scavenge hydroxyl radicals and radicals from oxidative and nitro stress in the laboratory.

6. Conclusion

The current study has shown that the administration of aspartame causes liver dysfunction in normal rats. At the same time, it was confirmed that treatment with a single clove of garlic (SCG) leads to a protective effect against the toxicity of aspartame on the liver. Aspartame caused further damage in diabetic rats compared to the effect of aspartame treatment in normal rats. The data obtained indicates that treatment with an extract of a single clove of garlic (SCG) could significantly improve impaired liver functions in diabetic rats. Our results suggest that single clove garlic extract (SCG) has a protective effect on aspartame and alloxan-induced changes in body weight and relative liver weight in both normal and diabetic rats.

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