A Physiological Study for Evaluating of Terpenes Efficacy Isolated from Zingiber Officinale Roscoe, On Lipid Profiles and Some Biochemical Parameters in Alloxan-Induced Diabetic Male Albino Rats

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Abstract

The current study was conducted at the AL-ameen center for research and advanced biotechnologies and the Faculty of science / department of Biology / animal house. Forty nine male albino rats belonging to the strain Sprague Dawley, species Rattus rattus, were used. They weighed (200 – 245) g, and were divided into 7 groups, 7males in each group. The first included control group that was orally dosed with a solution of physiological salt 0.9% NaCl. The second group was injected subcutaneously with alloxan 100 mg/kg. The third and fourth groups were orally administrated with terpene extract at the two concentrations 150 and 300 mg/kg respectively. The fifth and sixth groups were injected with alloxan 100 mg/kg, then was orally subjected to terpene extract 150 and 300 mg/kg respectively. The last group was orally given amaryl solution 0.1 mg/kg after being injected with alloxan 100 mg/kg. It is worth mentioning that the dosage process lasted for 30 days, once per day. Regarding the results of the present study, the levels of biochemical parameters including blood glucose, total cholesterol(TC),triglycerides (TG), low-density and very low-density lipoprotein (LDL, VLDL), urea and creatinine, in addition to the liver enzymes activity of AST, ALT and ALP all showed a significant increase ($P< 0.05$) at the treated group with alloxan and the amaryl group, on the contrary, the levels of high-density lipoprotein (HDL) revealed significant decrease($P< 0.05$) in the same two groups, while the groups that were dosed with the terpene extract in two concentrations (150 and 300) mg /kg revealed notable decrement ($P< 0.05$) in the levels of total cholesterol (TC), triglycerides (TG), and low-density and very low-density lipoprotein (LDL, VLDL), in addition to the liver enzymes activity of AST, ALT and ALP, also levels of urea and creatinine, contrastively, the levels of HDL showed a observable elevation ($P< 0.05$) in the group that administrated with terpene at concentration of 300 mg ⁄ kg when compared with the control group and other experimental groups.

Keywords: Zingiber officinale, Terpene , Alloxan, Diabetes mellitus, Lipid profile

Introduction

Diabetes mellitus is a chronic metabolic disorder diagnosed by hyperglycemia, and a decreased insulin levels [1,2]. It is one of the most common chronic diseases in the world [3,4]. Researchers used medicinal extracts from plants and their secondary compounds treating diabetes. Many extracts have been discovered to control diabetes. Hence, medicinal plants and their extracts have been used as popular prescriptions for the treatment of diabetes since ancient times all over the world. There are many medicinal plants with sugar-lowering properties such as Aloe vera, Bitter melon, Cinnamon, Fenugreek, Okra and Ginger. They have the ability to restore pancreatic tissue function by increasing insulin secretion or reducing intestinal absorption of glucose, which means that herbal medicines have the feature of protecting pancreatic β cells and mitigate fluctuations in the level of glucose [5]. Terpenes are a large group of hydrocarbon compounds found in essential oils and in different parts of the plant. They are composed of smallest units called isoprene, which contain five carbon atoms and eight hydrogen atoms C5H8 linked in double bonds.

The simplest form of terpenes are the monoterpenes, which contain two units; isoprene and sesquiterpenes, which contain three isoprene units, and diterpenes, which contain four units of isoprene and others. The available terpenes are either hydrocarbons or compounds of oxygen in which case they are called terpenoids [6]. There is a plenty of research on terpenes isolated from plants, because many terpenes contain a variety of plant chemicals that show useful and important effects in promoting humans' health. They are less toxic compared to chemical compounds. Therefore, terpenes are considered alternative medicine for many diseases, including

chemo-preventive treatment of cancer, anti-hyperglycemia, anti-fungal and anti-viral, anti-inflammation, active anti-parasitic, and treatment of neurological diseases such as Alzheimer's, epilepsy and depression. It is an antioxidant and scavenger of free radicals, as well as enhances immunity and skin permeability [7, 8]

Aim of study

The study aims to diagnose the potential therapeutic effects of terpenes isolated from ginger plant on the glucose level, lipid profile, and some biochemical parameters in alloxan-induced diabetic rats, and compare the impacts of these plant origin compounds with the widely used chemical drug (amaryl) in regulating the level of blood glucose and preventing the development of microvascular and macrovascular complications.

Material and Methods

1-Preparation of the laboratory animals of study

Forty nine albino male rats weighing 200-245 g and their ages three months were used for this study. They were its source from the animal house of the department of Biology/ College Faculty of science / University of Kufa. Animals were kept in plastic cages and brushed with sawdust. The rats were maintained under standard laboratory conditions of 12 h light–dark cycle, temperature (22-28C), relative humidity, standard diet and water.

2-Plants selection

A- Drying Plants

The rhizome stems of ginger plant were cleaned and dried in the shade, away from moisture, after being purchased from the local markets in AL-Najaf Governorate ,then the dried stems of the plant were crushed in an electric mill, and the ginger powder was kept in in airtight glass vials

B- Preparation of alcoholic ginger extract

In preparing the alcoholic extract by placing 10 g of ground ginger powder in an extraction thimble to obtain the extracted materials sequentially by the Soxhlate extraction device using 100 ml of hexane alcohol for (24 hours), the method was used [9] .

3- Methods of isolating and purifying plant materials

A- **Column chromatography technique**

After preparing the extract, it was isolated by column chromatography technique of [10]. Finally, hence three parts formed from the terpenes isolated of the ginger extract.

B- Thin layer chromatographic analysis (TLC).

The method was used [11].

C - Gas chromatography mass spectrometry (GC-Mas)

According to the [12] method.

4.Solutionspreparation

A- Terpene solution

In two concentrations, 150 and 300 mg ⁄ kg, the terpene was dissolved in 10 ml of distilled water [13] by preparing the original stock solution to prepare the terpene extract (150 mg ⁄ kg) depending on the weight rate of the rat according to the following equation

B- Alloxan solation monohydrate drug

The alloxan provided from India (CDH Company) with dose of 120 mg/ kg, and the method that mentioned by [14] was followed.

C.Glucosesolution

The glucose equipped by Chemistry Department in (Faculty of Education for girls) with dose of 5 g, and prepared according to the method of [15].

D- Preparation of amyral (Glimepiride) drug

The drug that was used in this study provided from France (Sanofi Company) with concentration 0.1 mg/kg according to [16] was followed.

5- Induction of diabetes mellitus

To induce the diabetes mellitus experimentally, the method of [17] was followed.

6- The experimental groups of study

The rats were divided into seven groups of seven rats for each one .

Group I: control rats orally dosed with normal saline (0.9%) daily for 30 days.

Group II: diabetic rats submitted to glucose solution (5%) that was prepared previously for 30 days [16]

Group III: orally treated with terpene extract (150 mg/kg) daily for 30 days [13].

Group IV: orally given terpene extract (300 mg/kg) daily for 30 days.

Group V: diabetic rats administered with terpene extract (150 mg/kg) daily for 30 days.

Group VI: diabetic rats subjected to terpene extract (300 mg/kg) daily for 30 days.

Group VII: diabetic rats orally dosed with standard drug amaryl (0.1 mg/kg) daily for 30 days.

7- The collection of blood samples

After the end of experiment (30 day), the animals were fasted for 24 h, and they were anesthetized with xylazine and ketamin at a ratio of 3: 1. The blood collected (2ml) by directed heart puncture to estimate the levels of blood biomarkers.

8-The evaluation of biochemical blood parameters

A- Estimation of glucose level in the serum

The method [18] was used to assess the glucose levels in the blood.

B- Estimation of lipid profile levels in the serum

The serum TC, TG, HDL, LDL, and VLDL profiles were estimated in the serum according to [17,18] . Moreover, the kits and reagents were provided from France (Biolabo Company) .In addition, the absorbance read at (500 nm) by spectrophotometer.

C- **Estimation of liver enzyme activity (AST, ALT and ALP) in the serum**

To evaluate the serum AST ,ALT and ALP activities the methods that mentioned by [18 ,19] were used ,and the kits ,as well as reagents purchased from Syria(Syrbio Company). Moreover, the absorbance read at (546, 546, 510 nm) respectively by spectrophotometer.

D- Estimation of urea and creatinine levels in the serum

The serum urea and creatinine levels were assessed according to [17,20] . The kits were provided from France (Biomerieux Company) and the absorbance read at (580,460 nm) respectively by spectrophotometer.

9-The statistical analysis

The findings of the current study were analyzed statistically by the Statistical Package Social Sciences (SPSS) program, and to extract the mean ,as well as standard error (Mean ± SE) for all the criteria included in the study, the Descriptive Analysis was used , then the study groups were compared with each other's by ANOVA and Least Significant Differences (LSD) at $P<0.05$ level [21].

Results

1- Identification of bioactive terpene compound by Gas Chromatography Spectrometry Mass (GC-Mass)

The results of the GC-Mass technique analysis for parts (1-4) showed 12 compounds. The highest area was 32.78% at peak 23 in a retention time of 38.838 minutes for the active compound gamma-Sitosterol, while the lowest area was 0.06% at peak 1 in a time retention of 13.319 minutes for the active compound 2-Propanone, detected by TLC and IR chromatography as shown in table (1).

Table (1): GC-MS analysis of terpenes isolated from the stems of Zingiber officinale for parts of (1-4)

NO	Chemical Names	Classification	Mol-Formula	Mol- Weight
1	2-Propanone	Monoterpene	C10H12O3	180
2	Pentadecanoic acid	Sesquiterpene	C15H30O2	242
3	Zingiberene	Sesquiterpene	C15H24	204
4	Cyclohaxene	Sesquiterpene	C15H24	204
5	Octanal	Monoterpene	C8H16O	128
6	Campesterol	Triterpene	C28H48O	400

7	Stigmasterol	Triterpene	C29H48O	412
8	gamma- Sitosterol	Triterpene	C29H50O	414
9	Cycloartanol	Triterpene	C30H52O	428
10	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	Monoterpene	C10H16O	152
11	Stigmastadiene-3-one	Triterpene	C29H46O	410
12	Testosterone cypionate	Triterpene	C27H40O3	412

On the other hand, the analysis for parts (5-9) diagnosed 23 chemical compounds. The highest area was 18.33% at peak 50 in a retention time of 41.200 minutes for the active compound Citronellol, while the lowest area was 0.24% at peak 1 in a retention time of 5.718 minutes for the active compound Octanal detected by TLC and IR chromatography as shown in table (2).

Table (2): GC-mas analysis of terpenes isolated from the stems of Zingiber officinale for parts of (5-9)

NO	Chemical Names	Classification	Mol-Formula	Mol- Weight
1	Octanal	Monterpene	C8H16O	128
2	endo–Borneo (Camphol) (Borneo camphor)	Monterpene	C10H18O	154
3	alpha.-Terpineol	Monterpene	C10H18O	154
4	Decanal	Monterpene	C10H20O	156
5	Zingiberene	Monterpene	C15H24	204
6	Bergamotene	Sesquiterpene	C15H24	204
7	alpha.- Farnesene	Sesquiterpene	C15H24	204
8	beta.-Bisabolene	Sesquiterpene	C15H24	204
9	beta-Sesquiphellandrene	Sesquiterpene	C15H24	204
10	Nerolidol	Sesquiterpene	C15H26O	222
11	Sesquisabinene	Sesquiterpene	C15H24	204
12	Farnesol	Sesquiterpene	C15H26O	222
13	cis-Sesquisabinene hydrate	Sesquiterpene	C15H26O	222
14	Uvaol	Triterpene	C30H50O2	442
15	Citronellol	Monoterpene	C10H20O	156
16	Geraniol	Monoterpene	C10H18O	154
17	Germacrene D	Sesquiterpene	C15H24	204
18	Phosphoric acid, tribornyl ester	Triterpene	C30H51O4P	506
19	Curcumene	Sesquiterpene	C15H22	202
20	Hexadecane	Tertraterpene	C40H82O2	594
21	gamma.-Sitosterol	Triterpene	C29H50O	414
22	Menthone	Diterpene	C16H22N4O4	
23	Gitoxigenin (Bigitaligenin)	Sesterterpene	C23H34O5	390

The analysis of parts (10-12) showed 19 chemical compounds. The highest area reached 13.17% at peak 36 in a retention time of 27.002 minutes for the active compound Geraniol, while the lowest area was 0.41% at peak 23 in a retention time of 18.015 minutes for the active compound Oleic acid, detected by TLC and IR chromatography as shown in table (3). Later, the parts were collected and mixed together to have the terpene extract.

Table (3): GC-mas analysis of terpenes isolated from the stems of Zingiber officinale for parts of (10-12)

NO	Chemical Names	Classification	Mol-Formula	Mol- Weight
1	endo-Borneol	Monoterpene	C10H18O	154
2	alpha.-Terpineol			
3	Nerolidol	Sesquiterpene	C15H26O	222
4	alpha.-Farnesene	Sesquiterpene	C15H24	204
5	beta.-Bisabolene	Sesquiterpene	C15H24	204
6	Cyclohexene	Sesquiterpene	C15H24	204
7	Hedycaryol	Sesquiterpene	C15H26O	222
8	Sesquisabinene hydrate	Sesquiterpene	C15H26O	222
9	Germacrene	Sesquiterpene	C15H26O	222
10	Geranic acid	Monoterpene	C10H16O2	168
11	2-Propanone	Monoterpene	C10H12O3	180
12	Farnesol	Sesquiterpene	C15H26O	222
13	Citronellol	Monoterpene	C10H20O	156
14	Oleic Acid	Faty acid	C18H34O2	282
15	Uvaol	Triterpene	C30H50O2	442
16	Geraniol	Monoterpene	C10H18O	154
17	beta.-Eudesmol (beta.-Selinenol)	Sesquiterpene	C15H26O	222
18	n-Decanoic acid	Monoterpene	C10H20O2	172
19	Curcumene	Sesquiterpene	C15H22	202

2- The effect of treatment of alloxan , terpene extract with (150 and 300) mg ⁄ kg and amaryl on glucose level of albino male rats

The results of the statistical analysis indicated that the inducing experimental diabetes in the rats led to a significant increase (P<0.05) in the level of glucose by (485.4 ± 51.9 mg ⁄ dl) compared to the healthy control group by (117.8 ± 5.38 mg ⁄ dl) and the other experimental groups. Moreover, the group treated with alloxan (100 mg / kg + amaryl 0.1 mg / kg) had a substantial increment (P<0.05) in blood glucose level by (215 ± 9.09 mg / dl) compared to the control group. Meanwhile, the groups treated with: the terpene extract 150 mg ⁄ kg, the terpene extract 300 mg ⁄ kg, alloxan 100 mg ⁄ kg + the terpene extract 150 mg ⁄ kg and alloxan 100 mg ⁄ kg + the terpene extract 300 mg ⁄ kg, did not show any significant differences in the blood glucose level (P> (0.05) by 92.4 ± 2.83, 88.2 ± 2.33, 118.4 ± 4.33, and 106.8 ± 3.15 respectively, compared with the control group and when comparing these groups with each other, as in table (4).

Table(4) : Effect of alloxan ,terpene extract (150, 300 mg/kg), and amyral drug on the blood glucose level

Blood glucose level (mg/dl)	
Experimental groups	**Glucose level**
Control group	117.8±5.38c
Group of alloxan	485.4±51.9a
Group of the extract of terpene (150 mg /kg)	92.4±2.83c
Group of the extract of terpene (300 mg /kg)	88.2±2.33c
Group of alloxan (120 mg/kg) + terpene (150 mg/kg)	118.4±4.33c
Group of alloxan (120 mg/kg) + terpene (300 mg/kg)	106.8±3.15c
Group of alloxan (120 mg/kg) s + amyral (0.1 mg/kg)	215±9.09b
LSD	58.5

Similar letters mean no significant differences at (P <0.05) between groups.

Different letters mean significant differences at (P <0.05) between groups.

Values are expressed as mean and standard error.

3- Effect of treatment of alloxan, terpene extract (150 and 300) mg / kg and amaryl on total cholesterol (TC) and triglyceride level (TG) of male albino rats

The findings showed that the induction of experimental diabetes in rats led to a remarkable elevation (P<0.05)) in the level of total cholesterol by (87.6 ± 5.22 mg / dl) compared to the control group by (45.94 ± 3.32 mg / dl). Also, the group treated with alloxan 100 mg/kg + amaryl 0.1 mg/kg did not show any significant change (P>0.05) in the level of total cholesterol, by (60.4 ± 24.2 mg/dl) compared to the control group. Meanwhile, the groups administrated with: terpene extract 150 mg / kg, terpene extract 300 mg/kg, alloxan 100 mg/kg + the terpene extract 150 mg/kg and alloxan 100 mg/kg + the terpene extract 300 mg / kg pointed to no significant differences (P<0.05) in total cholesterol levels, by (39.66 ± 3.25, 45.6 ± 2.37, 39.2 ± 2.87, and 41.2 ± 4.68) mg/dl, respectively, when compared to the control group and when comparing these groups with each other, as in table (5).

Furthermore, the statistical analysis indicated that the induction of experimental diabetes in animals resulted in a significant rise (P<0.05) in the TG level by (142.8 ± 6.71 mg / dl) compared to the control group by (68.28 ± 3.21 mg / dl). Moreover, the TG level of the group that submitted to alloxan 100 mg / kg + amaryl 0.1 mg / kg increased significantly (P<0.05) by (94 ± 4.84 mg / dl) compared to the control group.

On the other hand, the groups subjected to terpenes 150 mg / kg, and terpene 300 mg / kg recorded a significant decrement (P<0.05) in the TG level by (43.8 ± 3.72, 32.6 ± 3.76)mg/dl respectively, compared to the control group and the other experimental groups. Meanwhile, the alloxan group 100 mg/kg + terpene extract 150 mg/kg and alloxan 100 mg/kg + terpene extract 300 mg/kg, showed no noticeable variations (P>0.05) in the level of TG, by (55.2 ± 5.07,59.8 ± 5.74,) mg / dl when compared with the control group, as in table(5) .

Table(5) : Effect of alloxan ,terpene extract (150, 300 mg/kg), and amyral drug on the total cholesterol (TC) and triglycerides level

Total cholesterol& Triglycerides level (mg/dl)		
Experimental groups	**Total cholesterol (TC)**	**Triglycerides (TG)**
Control group	45.94±3.32b	68.28±3.21c
Group of alloxan	87.6±5.22a	142.8±6.71a
Group of the extract of terpene (150 mg /kg)	39.66±3.25b	43.8±3.72de
Group of the extract of terpene (300 mg /kg)	45.6±2.37b	32.6±3.76e
Group of alloxan (120 mg/kg) + terpene (150 mg/kg)	39.2±2.87b	55.2±5.07cd
Group of alloxan (120 mg/kg) + terpene (300 mg/kg)	41.2±4.68b	59.8±5.74c
Group of alloxan (120 mg/kg) s + amyral (0.1 mg/kg)	60.4 ±24.2b	94±4.84b
LSD	28.35	14.1

Similar letters mean no significant differences at (P <0.05) between groups.

Different letters mean significant differences at (P <0.05) between groups.

Values are expressed as mean and standard error.

4- Effect of treatment of alloxan, terpene extract (150 and 300) mg / kg and amaryl on the lipoprotein levels of albino male rats

The data of the present study detected a significant increase (P < 0.05) in the level of low-density lipoproteins (LDL) by (70.8 ± 8.28 mg / dl) in the group that administrated with alloxan compared to the control group by (20.6 ± 4.4 mg/dl). In addition, the level of low-density lipoproteins (LDL) increased significantly (P<0.05) by (35.7 ± 2.63 mg/dl) in the group treated with alloxan 100 mg/kg + amaryl 0.1 mg/kg compared to the control group. On the other

hand, the groups treated with: the terpene extract 150 mg/kg, the terpene extract 300 mg/kg, alloxan 100 mg / kg + the terpene extract 150 mg / kg and the alloxan 100 mg/kg + the terpene extract 300 mg/kg did not show any observable alterations (P<0.05) in (LDL), by (11.6 ± 2.29, 10 ± 2.25, 18.92 ± 3.95, and 22.2 ± 3.56) mg/dl, respectively, when compared with the control group and when comparing the these groups with each other, as in table (6).

Additionally, the results of the current study diagnosed that the induction of experimental diabetes in rats led to a significant rise (P<0.05) in the level of very low-density lipoproteins (VLDL) by (42.4 ± 3.85 mg / dl) compared to the healthy control group by (13.6 ± 0.5 mg / dl). Also, (VLDL) of the group treated with alloxan 100 mg/kg + amaryl 0.1 mg/kg increased significantly (P<0.05), by (22.6 ± 1.93 mg / dl) compared to the control group, while the statistical results did not show any significant decrease (P<0.05) in the level of very low-density lipoproteins (VLDL) in the groups dosed with: the terpene extract 150 mg/kg, , alloxan 100 mg/kg + the terpene 150 mg/kg and alloxan 100 mg/kg + the terpene extract 300 mg/kg by (9.8 ± 0.37, 10.52 ± 1.1, and 9.58 ± 1.23 mg / dl) respectively, when compared with the control group. In concern with the terpene extract 300 mg / kg, there was a notable decline in the VLDL level by 6.68 ± 1.45 mg/dl comparing to the control group. Further, there was no significant difference (P>0.05) in the level of VLDL when comparing the other groups with each other, as in table (6).

On the other hand, the findings revealed a remarkable reduction (P<0.05) in the level of high-density lipoproteins (HDL) in the group injected with alloxan at 100 mg/kg by (9.04 ± 2.64 mg/dl) compared to the control group by (45 ± 4.38 mg/dl), and the other groups. Moreover, the results of the statistical analysis did not indicate any significant change (P>0.05) in the level of HDL in the group treated with alloxan 100 mg/kg + amaryl drug 0.1 mg/kg by (34.38 ± 1.53 mg / dl) compared to the control group. In contrast to above, a significant elevation (P<0.05) was observed in the level of high-density proteins (HDL) in the group treated with the terpene extract at 300 mg/kg, by (83.8 ± 3 mg/dl) compared to the control group and other experimental groups .Furthermore, the terpene group 150 mg/kg showed significant increase (P<0.05) in the level of high-density proteins by (60 ± 2.96) when compared with the control group. However, the two groups administrated with alloxan 100 mg / kg + terpene extract 150 mg / kg, and alloxan 100 mg / kg + terpene extract 300 mg / kg, did not show any significant difference in the level of that index when compared with the control group and also when comparing these study groups with each other, as in table (6).

Table(6) : Effect of alloxan, terpene extract (150, 300 mg/kg), and amyral drug on the lipoprotein levels

Lipoprotein levels (HDL , LDL & VLDL) (mg/dl)			
Experimental groups	**HDL**	**LDL**	**VLDL**
Control group	45±4.38c	20.6±4.4c	13.6±0.5c
Group of alloxan	9.04±2.64d	70.8±8.28a	42.4±3.85a
Group of the extract of terpene (150 mg /kg)	60±2.96b	11.6±2.29c	9.8±0.37cd
Group of the extract of terpene (300 mg /kg)	83.8±3a	10±2.25c	6.68±1.45d
Group of alloxan (120 mg/kg) + terpene (150 mg/kg	53.2±8.63bc	18.92±3.95c	10.52±1.1cd
Group of alloxan (120 mg/kg) + terpene (300 mg/kg	54±4.57bc	22.2±3.56c	9.58±1.23cd
Group of alloxan (120 mg/kg) + amyral (0.1 mg/kg)	34.38±1.53c	35.7±2.63b	22.6±1.93b
LSD	13.05	12.66	5.35

Similar letters mean no significant differences at (P <0.05) between groups.

Different letters mean significant differences at (P <0.05) between groups.

Values are expressed as mean and standard error.

5- Effect of treatment of alloxan, terpene (150 and 300) mg / kg and amaryl on the liver enzyme activity (ALT AST, ALP) of male albino rats

The trial showed that the induction of experimental diabetes in rats yielded a significant increment (P<0.05) in the activity of the ALT enzyme, which reached (48.6 ± 9.09 units / liter) in comparison with the control group by (60 ± 11.8 units / liter) as well as the experimental groups .In regard with the group treated with alloxan 100 mg / kg + amaryl 0.1 mg / kg .The was an observable

increase (P<0.05) in the activity of the ALT enzyme up to (149 ± 11.2 units ⁄ liter) compared to the control group. However, the groups treated with: the terpene extract 150 mg ⁄ kg, the terpene extract group 300 mg ⁄ kg, the alloxan group 100 mg ⁄ kg + the terpene extract 150 mg ⁄ kg and alloxan 100 mg ⁄ kg + the terpene extract 300 mg ⁄ kg did not reveal any significant differences (P>0.05) in the activity of the ALT enzyme, they were (48.6 ± 9.09 , 17.8 ± 3.16, 64 ± 9.06,53.6 ± 9.44 units per liter), respectively when compared with the control group and when comparing these groups with each other, as in table (7).

Additionally, the results showed that the induction of experimental diabetes in rats caused a substantial elevation (P < 0.05) in the activity of the aspartate aminotransferase (AST) enzyme of up to (484.6 ± 73.3 units / liter) compared with the control group (110.7 ± 11.7 units / liter) and the experimental groups. The group treated with alloxan 100 mg ⁄ kg + amaryl 0.1 mg ⁄ kg recorded a significant increase (P<0.05) in the AST enzyme activity amounting to (202.4 ± 18.1 units ⁄ liter) compared to the control group. While the groups submitted to terpene extract 150 mg ⁄ kg, the terpene group 300 mg ⁄ kg, alloxan 100 mg ⁄ kg + the terpene extract 150 mg ⁄ kg and alloxan 100 mg ⁄ kg + the terpene extract 300 mg ⁄ kg no significant differences were found (P>0.05) in the enzyme activity AST amounted to 67.4 ± 12.2, 76.4 ± 8.12 , 113.4 ± 4.66 ,112.2 ± 4.01 units / liter respectively when compared with the control group and also when comparing these groups with each other, as in table (7). The experiment also indicated to a significant increase (P < 0.05) in the alkaline phosphatase enzyme (ALP) activity (312.4 ± 39.6 units / liter) in alloxan-injected group compared with the control group by (136.4 ± 10.4 units / liter) as well as the other experimental groups. The group treated with alloxan 100 mg ⁄ kg + amaryl 0.1 mg ⁄ kg recorded a noticeable rise (P<0.05) in ALP enzyme activity of up to (209.8 ± 7.88b units ⁄ liter) compared to the control group. Moreover, the results revealed a significant decrease (P<0.05) in the (ALP) activity in the group treated with the terpene extract at 300 mg ⁄ kg by (82.2 ± 6.24 units ⁄ liter) compared with the control group. However, in the other groups: the terpene extract group 150 mg ⁄ kg, alloxan 100 mg ⁄ kg + terpene extract 150 mg ⁄ kg and alloxan 100 mg ⁄ kg + terpene extract 300 mg ⁄ kg, there was no significant differences (P>0.05) in the ALP activity of up to 97.8 ± 6.08 , 124.8 ± 8.07, and127.8 ± 11.3, , units / liter, respectively when compared with the control group and when comparing these groups with each other, as in table (7).

Table (7): Effect of alloxan ,terpene extract (150, 300 mg/kg), and amyral drug on the liver enzyme activity(ALT AST, ALP)

The liver enzyme activity ALT AST, Alp (U/L)			
Experimental groups	**ALT**	**AST**	**ALP**
Control group	60±11.8c	110.7±11.7c	136.4±10.4c
Group of alloxan	466.4±88.6a	484.6±73.3a	312.4±39.6a
Group of the extract of terpene (150 mg /kg)	48.6±9.09c	67.4±12.2c	97.8±6.08cd
Group of the extract of terpene (300 mg /kg)	17.8±3.1c	76.4±8.12c	82.2±6.24d
Group of alloxan (120 mg/kg) + terpene (150 mg/kg)	64±9.06c	113.4±4.66c	124.8±8.07cd
Group of alloxan (120 mg/kg) + terpene (300 mg/kg)	53.6±9.44c	112.2±4.01c	127.8±11.3cd
Group of alloxan (120 mg/kg) + amyral (0.1 mg/kg)	149±11.2b	202.4±18.1b	209.8±7.88b
LSD	86.2	85.5	49.11

Similar letters mean no significant differences at (P <0.05) between groups.

Different letters mean significant differences at (P <0.05) between groups.

Values are expressed as mean and standard error.

6- Effect of treatment of alloxan, terpene extract (150 and 300) mg ⁄ kg and amaryl on the urea and creatinine levels of male albino rats

The results detected that the induction of diabetes in rats yielded in a significant increment of (P<0.05) in the urea level by (98.6 ± 11.5 mg/dl) compared

with the control group by (35.94 ±4.04 mg/dL) as well as the experimental groups .Regarding the group administrated with alloxan 100 mg ⁄ kg + amaryl 0.1 mg ⁄ kg ,the urea level increased significantly (P<0.05) by (64.74 units ⁄ liter) compared to the control group. On the other hand, the groups treated with terpene 150 mg ⁄ kg, terpene extract 300 mg ⁄ kg , alloxan 100 mg ⁄ kg + terpene extract 150 mg ⁄ kg and alloxan 100 mg ⁄ kg + terpene extract 300 mg ⁄ kg, revealed no significant differences (P>0.05) in urea levels by (22.94 ± 3.02 , 20.04 ± 3.87 , 29.32 ± 3.02, and 23.2 ± 1.99 mg/dl), respectively, when compared with the control group and when comparing the othese groups with each other, as in table (8).

According to the results, it was shown that the group of diabetes induction resulted in a remarkable elevation (P<0.05) in the creatinine level by (2.16 ± 0.25 mg/dl) compared to the healthy control group by (0.42 ± 0.05 mg/dl) and the experimental groups. The group treated with alloxan 100 mg / kg + amaryl 0.1 mg / kg showed a significant increase (P<0.05) in creatinine level by (1.3 ± 0.23 mg / dl) compared to the control group. Meanwhile, the groups treated with: terpene extract 150 mg / kg, terpene extract 300 mg ⁄ kg, alloxan 100 mg ⁄ kg + terpene 150 mg ⁄ kg, and alloxan 100 mg ⁄ kg + terpene extract 300 mg ⁄ kg showed no significant differences (P>0.05) in creatinine level by 0.28 ± 0.06 , 0.18 ± 0.03, 0.23 ± 0.05,and 0.24 ± 0.05 mg/dl, respectively when compared with the control group and when comparing the these groups with each other, as in table (8)

Table(8) : Effect of alloxan ,terpene extract (150, 300 mg/kg), and amyral drug on the urea and creatinine levels

Urea and creatinine levels (mg/dl)		
Experimental groups	**Urea**	**Creatinine**
Control group	35.94±4.04c	0.42±0.05c
Group of alloxan	98.6±11.5a	2.16±0.25a
Group of the extract of terpene (150 mg /kg)	22.94±3.02c	0.28±0.06c
Group of the extract of terpene (300 mg /kg)	20.04±3.87c	0.18±0.03c
Group of alloxan (120 mg/kg) + terpene (150 mg/kg)	29.32±3.02c	0.23±0.05c
Group of alloxan (120 mg/kg) + terpene (300 mg/kg)	23.2±1.99c	0.24±0.05c
Group of alloxan (120 mg/kg) + amyral (0.1 mg/kg)	64.74±11.3b	1.3±0.23b
LSD	19.48	0.41

Similar letters mean no significant differences at (P <0.05) between groups.

Different letters mean significant differences at (P <0.05) between groups.

Values are expressed as mean and standard error.

Discussion

1- Identification of bioactive terpene compound by Gas Chromatography Spectrometry Mass (GC-Mass)

The results are in line with the results of [22] when using iodine test on thin silica films TLC and examining them with the naked eye yielded in colors between (yellow-light brown to dark brown) and when examined under ultraviolet radiation with a wavelength of (254 nm) the stains were colored (yellow - light brown - brown - dark brown), respectively. This indicates that the compounds in the isolated terpenes under study express the positive detection of the terpene compounds in the alcoholic extract of ginger. The qualitative analysis was performed using the GC-Mass gas chromatography technique on the three parts of the terpenes that were collected after isolating from alcoholic ginger extract by column chromatography technique. Hence, many chemical compounds belonging to the terpene group were diagnosed, and they were identified in the GC-Mass scores on the basis of the preserved indicators of compound structures, literary data and previous studies of the authors which were consistent with the researcher [23].

2-The effect of treatment of alloxan and terpene extract with (150 and 300) mg ⁄ kg and amaryl on glucose of albino male rats

The high levels of glucose in rats may be attributed to the structural similarity of alloxan with glucose and the effectiveness of the absorption mechanism of beta

cells. This leads to damage to these cells via several mechanisms particularly the generation of toxic free radicals, oxidation of sulfhydryl groups, and inhibition of the glucokinase enzyme with disturbances of calcium balance in cells [24,25,26]. The results of the current study showed a significant decrease in glucose level in the groups treated with terpenes at two concentrations (150 and 300) mg ⁄ kg compared to the alloxan-injected group. Many studies have indicated that the curcumene compound, which is a terpene sesquiterpene - which was detected among the terpene compounds isolated from the ginger in the current study - plays a significant role in reducing glucose level by activating pancreatic beta cells and stimulating them to secrete insulin. This in turn plays an important role in enhancing the liver to store glucose as glycogen by activating the enzyme hexokinase, thus rapidly transferring glucose to the liver through the glucose transporter, GLUT2. Then the excess of glucose is transformed to adipose and muscle tissues, which contain the insulin-regulating transporter GLUT4. This causes a decrement in blood glucose levels subsequently [27, 28,29], , which is the case observed in groups treated with terpenes at two concentrations (150 and 300 mg ⁄ kg) compared to other groups. On the other hand, the drug used to reduce the level of glucose in the blood may be explained to the increased secretion of insulin by the pancreatic beta cells and stimulating the action of insulin from the peripheral tissues [30].

3-Effect of treatment of alloxan, terpene extract (150 and 300) mg ⁄ kg and amaryl on total cholesterol (TC) and triglyceride level (TG) of male albino rats

The data of the current study recorded a significant increment in the level of cholesterol in the induced-diabetes group, which is consistent with [31]. Hence, after the male rats were injected with alloxan, an elevation in the level of cholesterol (TC) was observed compared to the control group. The increase in cholesterol level may be attributed to various mechanisms and metabolic changes, including a decrease in the level of insulin, which is responsible for increasing the activity of the enzyme cholesterol acyl transferase, which in turn stimulates the absorption of total cholesterol from the intestine. This leads to an increase in the level of cholesterol in the blood [32]. Moreover, reduction in insulin levels causes a decrease in the production of the enzyme lipoprotein lipase (LPL) in the adipose tissue, which breaks down triglycerides under normal conditions, resulting in the production of TG from internal sources specifically in the liver, which coincides with the persistence of high blood glucose. It is worth mentioning that hypertriglyceridemia is common in diabetic patients and is responsible for vascular complications [33].

In relation with other groups dosed with terpenes at two concentrations (150 and 300) , the results showed a significant decline in compared to the alloxan-induced diabetic group. The finding is consistent with some studies [34] ,that the terpene compound of Menthone isolated from the mint plant ,which was also detected among the terpene compounds isolated from the ginger plant using GC-mas technology in the present study, has the ability to lower cholesterol levels and activate the secretion of bile acids in rats. Also, when administering terpene-induced diabetic rats with Cyclohaxene isolated from walnut leaves for 28 days (this compound was diagnosed when separating the terpene compounds from the rhizome stems of the ginger plant for the current study) it showed an effect in reducing the level of triglycerides TG and total cholesterol TC as indicated by the results. Moreover, an observable decrease was noticed in the two groups treated with terpenes (150 and 300 mg ⁄ kg), which indicates that the terpene extract possesses antioxidant compounds that have the ability to reduce lipid levels and lipoproteins production after improving the level of blood sugar [35,36]. As for the diabetic group treated with amaryl, the results revealed a significant decrease compared to those injected with alloxan. This could be because the drug belongs to the sulfonylureas family, which has the ability to lower blood sugar levels while stimulating insulin secretion, and thus reducing levels of total cholesterol and triglycerides consequently [37].

4-Effect of treatment of alloxan, terpene extract (150 and 300) mg ⁄ kg and amaryl on the lipoprotein levels of albino male rats

The results of the study revealed a notable reduction in the level of high-density lipoproteins (HDL) in the alloxan induced diabetic group, compared to the control group, which is consistent with [38]. Hence, when rats were injected with alloxan for a period of 6 weeks, the results showed a decrease in high-density lipoproteins (HDL) compared to low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL). The reason may be due to the decrease in the levels of the hormone insulin, which enhances the hormone-sensitive lipase enzyme to increase fat decomposition and the metabolic rates of fatty acids

in the blood of rats' induced-diabetic group and convert the surplus of free fatty acids in the liver into phospholipids and cholesterol. These substances form with large amount triglycerides a large mass in the form of lipoproteins [39].Conversely , and when diabetic animals were administered the terpene extract isolated from ginger plant, a significant rise in the level of HDL was observed, in particular the terpene extract at 300 mg/kg showing a substantial decrease in the level of low-density and very low-density lipoproteins.

The present study is in agreement with [40], thus when the extract of the terpene compound alpha-Farnesene isolated from citrus fruits - and from the ginger plant - was subjected to rats, it had a hypolipidemic impact and an increment in the level of high-density protein lipids. Likewise, this effect was found when terpene extract was administered to the rats at 300 mg/kg. This may be because terpenes increase the level of adiponectin in the blood plasma, which is associated with an inverse relationship with VLDL and TG, and thereby stimulates the catabolism of low-density lipoproteins (VLDL) by increasing the activity of the enzyme lipoprotein lipase and the expression of VLDL receptors that are related with improving insulin. This mechanism mediates a decrease in the levels of lipoproteins and triglycerides in the blood [41]. Concern with the amaryl treatment, it revealed a significant decrease in the levels of low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL), with increase in high-density lipoproteins (HDL), compared to the alloxan-induced diabetic group. It likely to be due to the amaryl stimulation of insulin from beta cells, which suppresses the breakdown of glycogen and fats, and at the same time enhances the activity of the enzyme lipoprotein lipase and inhibits the enzyme hepatic lipase, which all work synergistically towards increasing the levels of high-density proteins HDL [42-45].

5-Effect of treatment of alloxan, terpene (150 and 300) mg / kg and amaryl on the liver enzyme activity(ALT AST, ALp) of male albino rats

In the current trial, a significant elevation was observed in the activity of liver enzymes (AST, ALT, ALP) in male rats of induced diabetes, compared to the control group. The increase in liver enzymes effectiveness may be attributed to the alloxan, which causes an imbalance in the metabolic processes of liver cells, with an increment in cellular metabolism rate and an enlargement of the hepatocytes. This results in stimulation of the internal plasma reticulum to produce a large amount of liver enzymes in line with the size of the liver cell. Moreover, the toxic effect of alloxan on the liver cells causes its enzymes to go out to the bloodstream. Hence, its negative influence extends to stopping the protein producing process and increasing the catabolism. This in turn results in damage to the hepatic cells with poor membrane selectivity, which allows the passage of enzymes from the cytoplasm of the cells into the blood and increases the activity of liver enzymes in the blood plasma accordingly. Thus, the high activity of these enzymes indicates to a dysfunction in the liver caused by high blood sugar, which induces liver toxicity. [46-48]

In contrast to what have been preceded, the terpene extract showed a significantly low effectiveness of liver enzymes in the group submitted to the terpene extract at 300 mg/kg compared to the control group. Moreover, the groups treated with the terpene extract revealed a remarkable decrement in compared to alloxan-induced diabetic group. The current study agreed with [49] that the β-Bisabolene isolated from the plant Duguetia gardneriana, - and β-Bisabolene among the terpene compounds isolated from the ginger plant by GC-mass technique - has the ability to inhibit the cytotoxicity of hepatocellular carcinoma cells and thus prevent the death of liver cells. This is because the compound β-Bisabolene has water solubility and penetrability, which improves tissue matrices significantly [50].In regard with the drug, it reduces the effectiveness of liver enzymes as a result of diabetes, possibly due to increased insulin production, stimulation of the fusion of amino acids into proteins, and elevated glycogen uptake by the liver tissues [51]

6-Effect of treatment of alloxan, terpene extract (150 and 300) mg/kg and amaryl on the urea and creatinine levels of male albino rats

The results of the current study pointed to a significant rise in the level of creatinine and urea in diabetic animals compared to the control group. This was consistent with many studies [52], which indicated high levels of urea and creatinine in the alloxan-induced diabetic group. They attributed this to the toxic effect of alloxan on the renal tissues, which results in inhibiting the nephrons to perform their functions and a defect in the histological structure of the kidney that leads to an increment in the volume of fluids resulting from the failure of the glomerular filtration mechanism .This leads to

accumulation of waste products such as urea and creatinine and increase their levels in the blood plasma significantly.

As for the groups administrated with the terpene extract, the results of the study indicated a noticeable decline compared to the induced-diabetic group. These results were supported by [53], who used the terpene extract of Geraniol in two concentrations (100 and 200 mg / kg) in doxorubicin-induced renal failure rats. Hence, Gas-mass technology showed that the terpene compound Geraniol had the highest surface area when isolating the terpenes of the ginger plant. Thus, the terpene extract tries to prevent renal damage caused by doxorubicin, improves the functions of renal glomeruli and reduce the high levels of some bio-indicators particularly creatinine, albumin, and urea in the blood [54-56]. The researchers attributed this to the fact that Geraniol is a strong antioxidant and anti-inflammatory compound, and it works on suppressing the deteriorative ROS generated by the toxicity of the drug doxorubicin, besides its inhibitory effect on the inflammatory pathway NF-κB. Moreover, it has a vital role in the balance of programmed death proteins. This was confirmed by some studies [57].

Conclusion: The terpenes isolated from the Zingiber officinale Roscoe regulate blood glucose level and ameliorate lipid profile because of their substantial contained of bioactive antioxidants., In addition, the induced diabetic group and treated with amaryl showed a significant decrease when compared to the group injected with alloxan only. These results were consistent with a studies who suggested this to the ability of the sulfonylurea group to penetrate pancreatic tissue and stimulate the remaining beta cells to secrete insulin, which in turn activates the cellular metabolism of these metabolites by special mechanisms within the tissues of body.

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