



## Evaluation of the antioxidant efficacy of Terpenes isolated from the *Zingiber officinale* Roscoe in treating experimentally alloxan-induced diabetes in Male Albino Rats

Ansaf Saleh Abar<sup>1</sup>, Saher Mahmood Jwad<sup>2\*</sup>

<sup>1</sup>Department of Biology, College of Education for Girls, University of Kufa, Iraq

<sup>2</sup>Professor, Department of Biology, College of Education for Girls, University of Kufa, Iraq

\*Corresponding author: Saher Mahmood Jwad, Professor, Department of Biology, College of Education for Girls, University of Kufa, Iraq

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### ABSTRACT

Current study was directed in AL- ameen center for research and advanced biotechnologies and the Faculty of science / branch of Natural science / animal dynasty, and 49 male albino rats be appropriate to the Sprague Dawley rinsing were charity. They assessed 200-245 g, and were distributed into seven clusters, 7 males in every cluster. The main encompassed regulator cluster that was verbally medicated with aqua of physiological salt 0.9% sodium chloride . The another cluster was vaccinated dermally with alloxan 100 mg/kg. The next and final clusters were orally administrated with terpene extract at the two concentrations (150,300) mg/kg respectively. The fifth and sixth clusters were vaccinated with alloxan 100 mg/kg, and then was orally dosed with terpene extract 150 and 300 mg/kg respectively. The last cluster was vocally acquiesced to the amyral solution 0.1 mg/kg after being injected with alloxan 100 mg/kg. It is worth mentioning that the dosage process continued for 30 days, just the once per day. The results of the statistical analysis chronicled a significant elevation ( $P < 0.05$ ) in levels of blood glucose, malondialdehyde (MDA) and MCP-1 in the cluster vaccinating with alloxan hypodermically and also the cluster of alloxan +amyral when related with the regulator cluster and the additional investigational clusters, otherwise the planes of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) exposed a significant decrease ( $P < 0.05$ ) in the two clusters mentioned . As for the clusters that were orally managed with terpene extract at the two concentrations 150 and 300 mg/kg revealed a notable reduction ( $P < 0.05$ ) in the equal of blood glucose ,MDA and MCP-1 when equated with the regulator cluster then other study clusters .On the other hand, the levels of SOD and CAT and GPX1 exposed an observable increment ( $P < 0.05$ ) in the cluster that treated with terpene quotation in the concentration 300 mg/kg in equated to the regulator cluster and other clusters of study.

**Keywords:** *Zingiber officinale*, Terpene , Alloxan, Diabetes mellitus, Antioxidants, MCP-1

## INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder branded by hyperglycemia, and a low level of insulin. It is one of the furthestmost mutual chronic diseases in the biosphere [1,2]. Diabetes mellitus is characterized by many biomarkers that indicate the pathogenesis such as change of enzyme systems, lipid peroxidation, carbohydrate, fats and proteins metabolism disorders, damage of DNA and oxidative stress, which induces a high risk of diabetic complications, including cardiac arrest, neuropathy, retinopathy, and nephropathy [3,4,5]. Modern medicine has not yet found an effective treatment for diabetes. In addition, there are side possessions accompanying with the available drugs including insulin and oral diabetic drugs. Meanwhile, natural medical drugs are safe and do not toxic influences [6]. Therefore, medicinal herbs were used in traditional medicine to treat diabetes in most countries of the world, and currently a wide spread is noticed in alternative drugs as treatment of diabetes. Some clinical studies confirmed that medicinal plant extracts show anti-diabetic activity and restore the functions of pancreatic beta cells [7]. Terpenes are secondary metabolic compounds extracted from turpentine oil, a viscous balm with a pungent aromatic odor flows when cutting or carving the bark or new wood in some species of pine trees, so it is known as pine trees resin. Turpentine covers resin acids and certain hydrocarbons, which were previously denoted to as terpenes. All natural composites are composed of subunits known as isoprene. Many applications have been discovered of these compounds, including pharmaceutical preparations, fragrances and food industries. Moreover, they are used as anti-microbial, anti-fungal, antitumor, anti-inflammatory, and antioxidant [8]. Thus, the aim of the current study is to evaluate the efficiency of terpenes isolated from the ginger plant in the treatment of experimental diabetes mellitus that encouraged by alloxan and to compare the impacts of these plant compounds with the widely used chemical drug amaryl in regulating the level of glucose in the blood.

## *Substantial and Approaches*

### *Grounding of the research of animals Training*

Current work involved 49 albino male rats be appropriate to the strain Sprague Dawley, type *Rattus rattus*, considered (200-245) g, were its foundation from the animal community of the Branch of natural science.

### *Plants Selection*

#### *Purchasing and drying plants*

The rhizome stems of the ginger plant stood acquired from the indigenous markets in AL-Najaf Governorate after cleaning with drying of the plant were crushed in an electric mill, and then the fine ginger powder was reserved in airtight flute vials.

#### *Preparation of alcoholic extract*

The method of [9] was used in preparing the alcoholic extract by placing 10 g of ground ginger stems powder in an extraction thimble to obtain the extracted materials sequentially by the Soxhlate extraction device using 100 ml of hexane alcohol concentration (99%) for (24 hours). Then, the extract was dried in the electric oven at 45 °C to obtain the dry substance.

### *Methods of isolating and purifying plant materials*

#### *Isolation by the column chromatography technique*

After preparing the extract, it was isolated by column chromatography method of [10]. A burette was used and glass wool was placed at the bottom of the column. Then a small amount of sand was applied, after that 1 g of silica gel was placed with a porosity of 230-400 silica gel pore size 60 Å, 0.5 g of Na<sub>2</sub>SO<sub>4</sub> and hexane solvent was added. Then, it was left for half an hour to penetrate into the silica gel. Finally, the ginger extract was added to isolate the terpenes, hence three parts formed.

#### *Thin layer chromatographic analysis (TLC)*

The methanol - hexane - ethyl acetate solvent system was used at (20: 20: 60) to separate and

transport the active terpene compounds. Pre-coated (p-e) silica gel sheets manufactured from E. Merck German Danustade were used, 0.25 mm and 20 × 20 cm. The plate was placed in an electric oven at a temperature of 50 °C for 10 minutes. Then, 2 cm<sup>3</sup> of the isolated terpene extract containing crude essential oils was taken and concentrated in the form of spots using fine capillary tubes at equal distances of 2 cm from the beginning of the chromatographic layer [11].

### **Gas chromatography mass spectrometry (GC-Mas)**

According to the [12] method, the terpene extract that was isolated from the rhizome stems of the ginger plant was analyzed by a GC device of the type (GC clarus 500 perkin elmer) and a mass-spectrometer, at the College of Agriculture / Basra University / Food Research and Consumer Protection Unit.

### **Solutions preparation**

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:

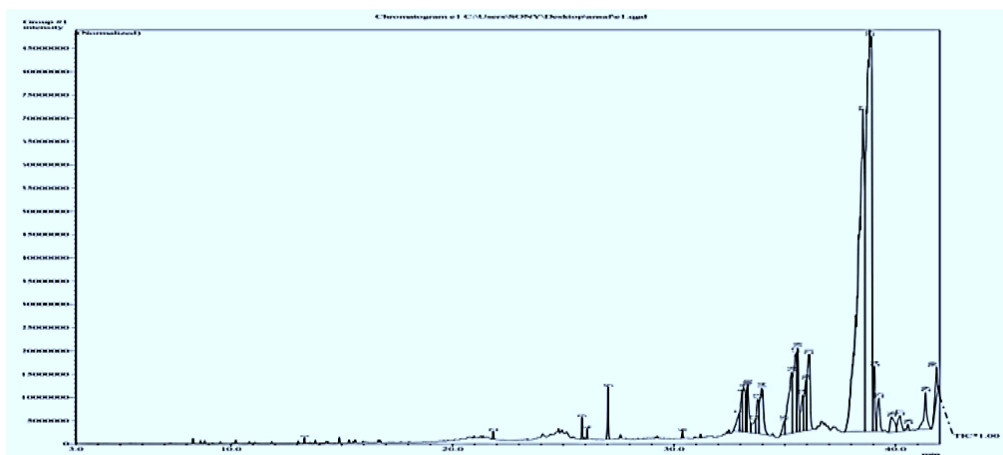
$$V2 \times N2 = V1 \times N1$$

Then Alloxan solution study was prepared ,then statistical analysis as same method in studies [14-16]

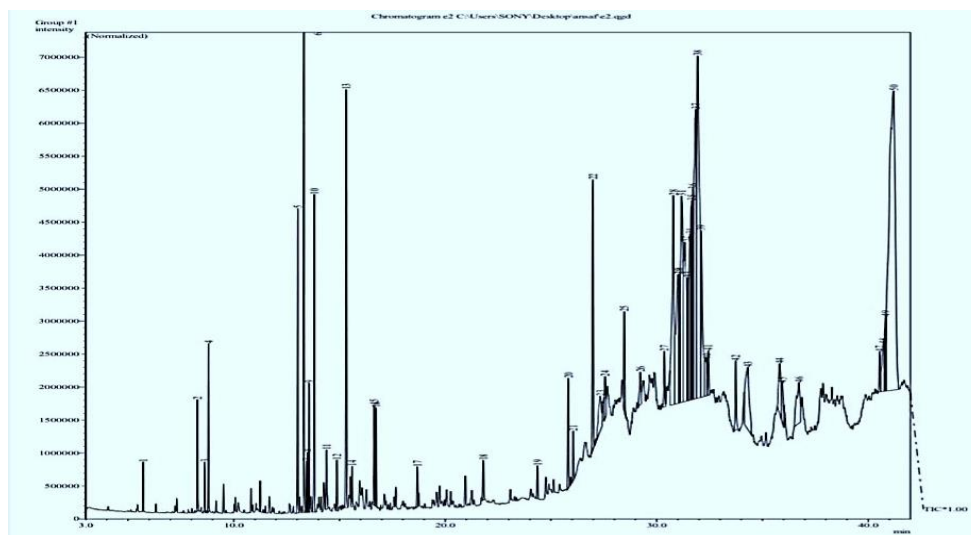
## **RESULTS**

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

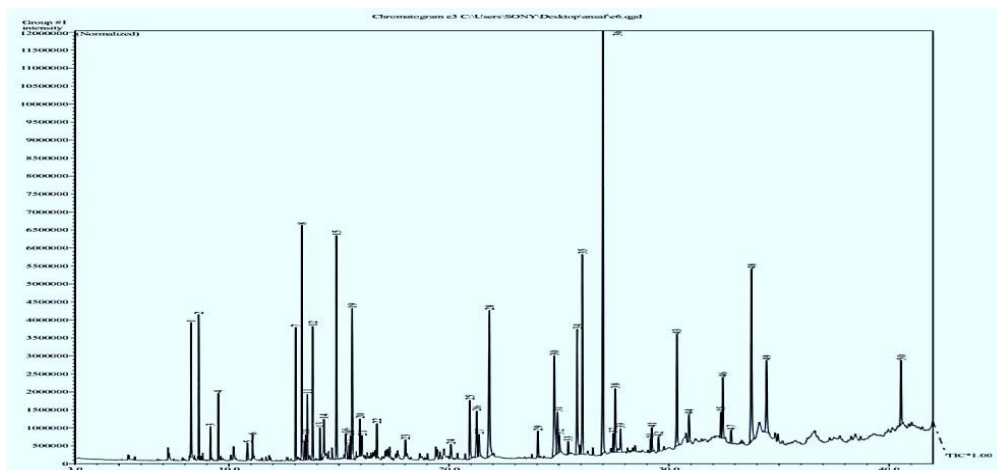
Our results of current analysis of the GC-Mass technique for parts (1-4) exposed twelve chemical compounds and 30 peaks. The highest area reached 32.78% at peak 23 in a retention time of 38.838 minutes for the active compound gamma-Sitosterol. The lowest area was 0.06. % at peak 1 in a retention time of 13.319 minutes for the active compound 2-Propanone, as shown in figure (1). As for parts (5-9) revealed 23 chemical compounds, with 50 peaks, where the highest area was 18.33% at peak 50 in a retention time of 41.200 minutes for the active compound Citronellol. The lowest area was 0.24% at peak 1 in a retention time of 5.718 minutes for the active compound Octanal, as shown in figure (2). The parts (10-12) exposed 19 chemical compounds and 50 peaks, where the highest area reached 13.17% at peak 36 in a retention time of 27.002 minutes for the active compound Geraniol. Whereas the lowest area was 0.41% at peak 23 in a detention time of 18.015 minutes of the active compound Oleic acid as in figure (3), and then all parts were combined.



**FIGURE 1:** Peaks of the chemical compounds of terpenes isolated from the rhizome stems of the Zingiber officinale



**FIGURE 2:** Peaks of the chemical compounds of terpenes isolated from the rhizome stems of the Zingiber officinale



**FIGURE 3:** Peaks of the chemical compounds of terpenes isolated from the rhizome stems of the Zingiber officinale

***Effect of treatment of alloxan , terpene extract with (150, 300) mg / kg and amaryl on blood glucose level of albino male rats***

The consequences of the arithmetical analysis indicated that creation of experimental diabetes mellitus in the animals led to a momentous increment ( $P < 0.05$ ) in the equal of glucose by ( $485.4 \pm 51.9$  mg / dl) equated with the healthy regulator cluster by ( $117.8 \pm 5.38$  mg / dl) and the experimental clusters, as well the cluster treated with alloxan 100 mg / kg + amaryl 0.1 mg / kg had a momentous increase ( $P < 0.05$ ) in blood glucose level by ( $215 \pm 9.09$  mg / dl) compared

to the regulator cluster. On the additional pointer, the clusters that preserved with the terpene extract 150 mg / kg, the terpene extract 300 mg / kg, the alloxan 100 mg / kg + the terpene quotation 150 mg / kg and the alloxan 100 mg / kg + the terpene extract 300 mg / kg, no significant differences ( $P > 0.05$ ) were noticed, they were by  $92.4 \pm 2.83$  ,  $88.2 \pm 2.33$ ,  $118.4 \pm 4.33$ , and  $106.8 \pm 3.15$  mg / dl, respectively, in the level of blood glucose when compared with the regulator cluster and when comparing the clusters with each other [17-20], as in table (1).

**TABLE 1:** Effect of alloxan ,terpene extract (150, 300 mg/kg), and amyral drug on the blood glucose level

Blood glucose glassy (mg/dl )	
experimental clusters	Glucose level
Regulator cluster	117.8±5.38c
Cluster of alloxan	485.4±51.9a
Cluster of the extract of terpene (150 mg /kg)	92.4±2.83c
Cluster of the extract of terpene (300 mg /kg)	88.2±2.33c
Cluster of alloxan (120 mg/kg) + terpene (150 mg/kg)	118.4±4.33c
Cluster of alloxan (120 mg/kg) + terpene (300 mg/kg)	106.8±3.15c
Cluster of alloxan (120 mg/kg) s + amyral (0.1 mg/kg)	215±9.09b
LSD	58.5

Analogous eruditions mean no momentous alterations at (P <0.05) between clusters.

Diverse cultivations mean substantial variances at (P <0.05) between clusters.

Morals are articulated as mean and typical aberration.

***Effect of treatment of alloxan and terpene extract with (150, 300) mg / kg and amaryl on the antioxidants levels of albino male rats***

The statistical analysis of the data pointed to a noticeable decrement (P<0.05) in the catalase level in the cluster of alloxan induced diabetes with concentration 100 mg/kg by (0.029 ± 0.007 ng/ml) compared to the regulator cluster by (0.229 ± 0.03 ng/ml) and other experimental clusters. Moreover, a significant decline (P < 0.05) was also observed in the CAT level in the alloxan treated cluster 100 mg / kg + amaryl 0.1 mg/kg by (0.114 ± 0.017 ng / ml) compared with the regulator cluster. The results exposed a substantial increase (P<0.05) in the CAT level in the cluster treated with the terpene extract concentration of 300 mg / kg by (0.320 ± 0.019 ng/ml) compared to the regulator cluster and the cluster treated with alloxan in concentration 100 mg/kg + the terpene extract concentration of 150 mg / kg by 0.199 ± 0.013 (ng / ml). While no significant difference (P>0.05) was observed in the level of CAT when the other experimental clusters were compared with each other . On the other hand, the cluster treated with alloxan concentration of 100 mg / kg + terpene extract concentration of 150 mg / kg exposed a significant decrease (P<0.05)) in the level of CAT compared to the study clusters treated with, terpene 150 mg / kg, terpene 300 mg / kg and alloxan 100 mg/kg + the terpene extract 300 mg /kg by 0.298 ± 0.02 , 0.320 ± 0.019, and 0.263 ± 0.02 ng/ml, whereas no significant differences

(P>0.05) were recorded in the CAT level when compared with the regulator cluster. Moreover, no significant variation was observed among the remaining experimental clusters, nor with the regulator cluster, as in table (2).

In addition, the findings of the present trial revealed a significant decrease (P<0.05) in the level of superoxide dismutase (SOD) in the cluster in which alloxan induced diabetes mellitus experimentally by (0.29 ± 0.14 ng / ml) in comparing with the regulator cluster by (9.50 ± 0.35 ng / ml) and other experimental clusters. Besides, a significant decrement(P<0.05) was also observed in the level of SOD in the cluster that treated with alloxan 100 mg / kg + amaryl drug 0.1 mg/kg by (3.62 ± 0.5 ng / ml) compared to the regulator cluster, as in table (2). In addition to what have been preceded, the data exposed a significant elevation (P<0.05) in the level of SOD in the cluster treated with the terpene extract at 300 mg/kg by (24.76 ± 2.23 ng / ml) compared to the regulator cluster and the study clusters treated with the terpene extract at 150 mg/kg, the alloxan + terpene extract at 150 mg / kg and the alloxan + terpene extract at a concentration of 300 mg / kg by 11.74 ± 1.84 ,10.50 ± 0.19, and 9.65 ± 0.33 ng / ml respectively. Furthermore, the results of the statistical analysis revealed a significant increase (P<0.05)) in the SOD level in the cluster treated with terpene extract of the concentration 300 mg / kg by (24.76 ± 2.23 ng / ml) compared to the

regulator cluster and experimental clusters that treated with alloxan + terpene at a concentration of 150 mg/kg and the alloxan + terpene cluster at a concentration of 300 mg/kg. Meanwhile, the results exposed no significant difference ( $P>0.05$ ) between the two clusters administrated with alloxan + the terpene extract at 150 mg/kg and alloxan + the terpene extract at (300 mg/kg) compared to the regulator cluster or when associating the two clusters with each other, as in table (2).

On the other hand, a significant decrease ( $P<0.05$ ) was observed in the level of oxidized glutathione (GPX) in the alloxan induced diabetes cluster with concentration of 100 mg/kg by ( $0.44 \pm 2.350$  ng / ml) compared to the regulator cluster by ( $3.22 \pm 0.25$  ng / ml) and other experimental clusters. Also a notable reduction ( $P<0.05$ ) was obtained in the GPX level in the cluster treated with alloxan 100 mg/kg + amaryl drug 0.1 mg / kg by ( $1.52 \pm 0.07$  ng/ml) compared to the regulator cluster, as in the table (2). Conversely, the findings exposed a significant increment ( $P<0.05$ ) in the GPX level in the cluster submitted to the terpene extract at concentration of 300 mg / kg and the terpene extract concentration of 150 mg/kg, by ( $5.24 \pm 0.55$ ,  $3.87 \pm 0.71$  ng / ml) compared with the regulator cluster and the other clusters that treated with alloxan + the terpene extract at a

concentration of 150 mg / kg and alloxan + the terpene extract at 300 mg / kg by ( $3.80 \pm 0.54$ ,  $4.09 \pm 0.23$  ng / ml), respectively. However, the results noticed that there was no significant difference ( $P>0.05$ ) in the level of GPX between the clusters treated with alloxan + terpenes at a concentration of 150 mg / kg and alloxan + terpenes at a concentration of 300 mg / kg compared to the regulator cluster and the two clusters with each other, as in the table (2). The statistical analysis revealed that the induction of diabetes mellitus in the animals resulted to a significant increase ( $P < 0.05$ ) in the level of malondialdehyde (MDA) by ( $13.47 \pm 0.31$  ng / ml) compared with the regulator cluster ( $3.20 \pm 0.53$  ng/ml) and the other clusters of experiment. The cluster treated with alloxan 100 mg / kg + amaryl 0.1 mg / kg increased significantly ( $P<0.05$ ) in MDA by ( $10.34 \pm 1.33$  ng / ml) compared to the regulator cluster, as in table (2). In addition, the results exposed that there was an observable decrement ( $P<0.05$ ) in MDA level in the cluster subjected to terpene extract at (300 and 150) mg/kg by ( $0.48 \pm 0.2$ ,  $1.48 \pm 0.12$  ng / ml) compared with the regulator cluster. However, the two clusters treated with alloxan + terpene extract at 150 mg / kg and alloxan + terpene extract at 300 mg/kg did not record any significant difference ( $P>0.05$ ) in MDA level by ( $2.45 \pm 0.45$ ,  $2.41 \pm 0.31$ , ng/ml) compared with the regulator cluster, as in table (2).

**TABLE 2:** Effect of alloxan ,terpene extract (150, 300 mg/kg), and amyral drug on the antioxidants levels

Antioxidants levels CAT, SOD,GPX, and MDA (ng/ml)				
experimental clusters	CAT	SOD	GPX	MDA
Regulator cluster	0.229±0.03bc	9.50±0.35b	3.22±0.25b	3.20±0.53c
Cluster of alloxan	0.029±0.007e	0.29±0.14d	0.44±2.35d	13.47±0.31a
Cluster of the extract of terpene (150 mg /kg)	0.298±0.02ab	11.74±1.84b	3.87±0.71ab	1.48±0.12cd
Cluster of the extract of terpene (300 mg /kg)	0.320±0.019a	24.76±2.23a	5.24±0.55a	0.48±0.2d
Cluster of alloxan (120 mg/kg) + terpene (150 mg/kg)	0.199±0.013c	10.50 ±0.19b	3.80±0.54ab	2.45±0.45c
Cluster of alloxan (120 mg/kg) + terpene (300 mg/kg)	0.263±0.02ab	9.65±0.33b	4.09 ±0.23ab	2.41±0.31c
Cluster of alloxan (120 mg/kg) s + amyral (0.1 mg/kg)	0.114±0.017d	3.62±0.5c	1.52 ±0.07c	10.34±1.33b
LSD	0.064	3.91	2.85	1.74

Analogous eruditions mean no momentous alterations at ( $P < 0.05$ ) between clusters.

Diverse cultivations mean substantial variances at ( $P < 0.05$ ) between clusters.

Morals are articulated as mean and typical aberration

***The effect of treatment with alloxan, terpene extract 150, 300 mg / kg and amaryl on the MCP-1 of albino male rats***

The findings indicated that the induction of diabetes mellitus experimentally in animals led to a significant increase ( $P < 0.05$ ) in the level of monocyte chemoattractant protein MCP-1 by ( $258.8 \pm 18.01$  ng/ml) compared with the healthy regulator cluster by ( $60.01 \pm 5.71$  ng/ml), and the other clusters of study. The alloxan treated cluster at 100 mg/kg + amaryl 0.1 mg/kg also recorded a significant rise ( $P < 0.05$ ) in this marker by ( $116.1 \pm 5.05$  ng / ml) compared to the regulator cluster, as in table (3). The statistical analysis exposed a remarkable decrease ( $P < 0.05$ ) in the level of MCP-1 in the cluster dosed with

terpene extract at a concentration of 150 mg/kg and terpene at a concentration of 300 mg/kg by ( $33.78 \pm 2$ ,  $21.22 \pm 2.65$  ng/ml) compared to the regulator cluster and the other clusters of trial: alloxan + the terpene extract at a concentration of 150 mg/kg and alloxan + the terpene extract at a concentration of 300 mg/kg by ( $62.5 \pm 5.73$ ,  $68.4 \pm 7.42$  ng/ml). On the other hand, the remaining clusters: alloxan + the terpene extract at a concentration of 150 mg / kg and alloxan + terpene extract at a concentration of 300 mg/kg did not show any significant difference ( $P > 0.05$ ) in compared with the regulator cluster [21-24], and when these two clusters were compared with each other, as in table (3).

**TABLE 3:** Effect of alloxan ,terpene extract (150, 300 mg/kg), and amaryl drug on the MCP-1 level

MCP-1 (ng/ml)	
Experimental clusters	MCP-1 equal
Regulator cluster	60.01±5.71c
Cluster of alloxan	258.8±18.01a
Cluster of the extract of terpene (150 mg /kg)	33.78±2d
Cluster of the extract of terpene (300 mg /kg)	21.22±2.65d
Cluster of alloxan (120 mg/kg) + terpene (150 mg/kg)	62.5±5.73c
Cluster of alloxan (120 mg/kg) + terpene (300 mg/kg)	68.4±7.42c
Cluster of alloxan (120 mg/kg) s + amaryl (0.1 mg/kg)	116.1±5.05b
LSD	24.03

Analogous eruditions mean no momentous alterations at ( $P < 0.05$ ) between clusters.

Diverse cultivations mean substantial variances at ( $P < 0.05$ ) between clusters.

Morals are articulated as mean and typical aberration

**DISCUSSION**

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

A qualitative analysis was carried out using the GC-Mass gas chromatography technique on the three parts of the terpenes that were collected after isolation from the alcoholic ginger extract by the column chromatography technique. Accordingly, many chemical compounds belonging to the cluster of terpenes were identified. Hence, the results of the analysis by GC-Mass and TLC revealed Geranic acid,

Pentadecanoic acid, Borneol, Zingiberene, Cyclohexane, Campesterol, Stigmasterol,  $\gamma$ -Sitosterol, alpha- Farnesene, Uvaol and many other terpene compounds, which is consistent with the findings of other studies [25 ].

***Consequence of treatment of alloxan , terpene extract with (150, 300) mg / kg and amaryl on blood glucose level of albino male rats***

The fallouts of the recent work demonstrated a significant proliferation in blood glucose in the

alloxan induced diabetes cluster related to the regulator cluster, which is in agreement with the study [26-28] when diabetes was induced by alloxan, which caused hyperglycemia. The finding may be explained by the fact that alloxan is a toxic chemical compound that causes selective breakdown of pancreatic beta cells, leads to a deficiency in insulin secretion and thus a decrease in its levels in the blood. This in turn causes in high levels of blood glucose by stimulating gluconeogenesis in the liver and other tissues, which results in a partial or complete decrement in insulin levels [29]. In contrast, the present trial revealed a significant decrease in the blood glucose level in clusters dosed with terpenes at two concentrations (150 and 300) mg/kg compared to the cluster treated with alloxan. This result agreed with [30] when administering diabetic rats with the terpine camphor, isolated from *Cinnamomum camphora* L, for a period of 21 days, which was also diagnosed when terpenes were isolated from *Zingiber officinal* Roscoe, the treatment exposed an observable decline in blood glucose, which is due to its antioxidant properties and the high activity to scavenge the harmful ROS that generated by lipid peroxidation processes and oxidative stress because of alloxan injection and pancreatic tissues breakdown subsequently. It also promotes insulin secretion from beta cells and improves the transfer of blood glucose to tissues. As for the cluster treated with alloxan and amaryl, it exposed a lower decrease than the alloxan cluster only, because amaryl is highly absorbed by the tissues of the target organs, including the pancreatic tissues. Hence, it tries to reduce glucose levels by enhancing the remaining beta cells from the effect of alloxan to secrete insulin, which in turn reduces glucose in the blood [31-33].

***The effect of treatment of alloxan , terpene extract with (150, 300) mg/kg and amaryl on the antioxidants levels of albino male rats***

The results of the analysis pointed to a remarkable decrement in the level of glutathione peroxidase, catalase and superoxide dismutase (SOD) in the induced diabetes cluster, which was

similar to [29]. Meanwhile, the data of the current study recorded a significant increase in the levels of malondialdehyde (MDA) compared to the regulator cluster and the other experimental clusters. The results agreed with many studies [34], and this may be attributed to the fact that hyperglycemia in rats with induced diabetes liberates various reactive oxygen species (ROS), which causes cellular damage by oxidizing DNA, proteins and membrane lipids. It also changes the metabolism and results in accumulation of ketone bodies [35]. Moreover, the high levels of malondialdehyde in alloxan induced diabetes rats likely to be due to the induction of oxidative stress, which causes the accumulation of lipid peroxidation products and the high rate of free radicals production that destroy cell membranes rich in fatty acids consequently, this leads to cellular necrosis with tissue injury, and weak activity of the internal antioxidant defense systems, especially SOD, GPx, and CAT, which are unable to balance the toxic effect of deteriorative radicals, causing elevated levels of oxidative stress biomarkers particularly MDA [36].

Furthermore, the results also exposed a significant rise in the levels of antioxidants represented by glutathione peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD) in rats treated with terpene extract at a concentration of 300 mg 1/2 kg. In contrast, there was a noticeable decrease in malondialdehyde (MDA) in blood serum compared to the regulator cluster, which is in agreement with some studies [37]. The extract of the terpene compound Nerolidol from the sesquiterpene cluster, detected by GC-mass technique when isolating the terpene compounds of ginger plant , acts on reducing the oxidative damage and inflammation, as well as inhibits malondialdehyde MDA releasing, and thereby increases the effectiveness of antioxidant enzymes by activating the gene expression of superoxide dismutase and glutathione peroxidase with an increment in the levels of catalase enzyme [38]. On the other hand, the results of the statistical analysis revealed a significant decline in the level of malondialdehyde (MDA) in the amaryl submitted cluster, conversely, a



substantial increment in the level of antioxidant enzymes compared to the alloxan induced diabetes cluster. The current study agreed with some studies [39], possibly due to the increased stimulation of the activity of antioxidant enzymes represented by superoxide dismutase, which is responsible for the dissolution of the superoxide ion of both oxygen and hydrogen peroxide. This results in shielding cellular organizations from impairment triggered by alloxan toxicity [40]

***Consequence of behavior of alloxan , terpene extract with (150, 300) mg / kg and amaryl on MCP-1 level of albino male rats***

Regarding the level of MCP-1, a significant elevation was noted in the alloxan induced diabetes cluster, which is consistent with [41, 42], where high levels of monocyte chemoattractant protein were recorded in patients with diabetic nephropathy. The finding could be due to an imbalance between antioxidants and oxidants, which generates numerous reactive oxygen species. This was observed in the current study through the decrease in the levels of antioxidants and contrarily, the high levels of malondialdehyde, which is a strong stimulator of the transcription of the nuclear factor NF- $\kappa$ B, which activates the inflammatory mediators in particular nitric oxide and prostaglandin E2. In addition, it increases the activity of inflammatory cytokines especially IL-1, TNF- $\alpha$ , and inflammatory chemokines, that was confirmed by some studies [43-45]. With regard to the level of MCP-1 in the two clusters treated with the terpene extract at a concentration of (150 and 300) mg / kg, an observable decrement was detected and which agrees with [46, 47], who studied the anti-inflammatory effectiveness of terpenes in experimental models and found that all types of terpenes have compounds with the ability to reduce the levels of MCP-1 gene expression. This is conducted by reducing the levels of nuclear factor NF- $\kappa$ B, and decreasing the oxidative stress process ,as well inhibiting inflammation through the production of proinflammatory cytokines including IL-1, IL-6 and TNF- $\alpha$ , besides increasing the levels of anti-inflammatory associated with M2 macrophages.

This has been confirmed by many studies [48-50]. Concern to the cluster that treated with alloxan + amaryl, a significant decrease was observed in the level of MCP-1 compared to the cluster that induced diabetes mellitus with alloxan only. Perhaps the drug exposed antioxidant activity by stimulating the nitric oxide production with selective inhibition of the cyclooxygenase pathway, according to some studies [51, 52].

## CONCLUSION

The terpenes isolated from the Zingiber officinale Roscoe can ameliorate blood glucose level better than the chemical drug amaryl due to their potent antioxidant activity.

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