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ORIGINAL ARTICLE

In vitro and in vivo evaluation of antioxidant activity and phytochemical screening of Jordanian Gomphocarpus sinaicus boiss (Apocynaceae) extract

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Abstract

Gomphocarpus sinaicus Boiss, belonging to the *Apocynaceae* family, is a medicinal plant known for its therapeutic properties. Although it has a history of traditional use, comprehensive studies investigating its phytochemical constituents and antioxidant potential are still limited. This study aimed to evaluate the phytochemical composition and antioxidant activities of various solvent extracts from the aerial parts of *G. sinaicus* Boiss, utilizing both *in vitro* and *in vivo* approaches. *G. sinaicus* Boiss has been traditionally used for various medicinal purposes. Therefore, this study sought to investigate the phytochemical constituents and assess the antioxidant potential of different solvent extracts of the plant's aerial parts. Aerial parts of *G. sinaicus* were extracted using solvents of varying polarities. Phytochemical screening was performed to identify secondary metabolites, and the total phenolic and flavonoid contents were quantified. We assessed antioxidant activity through DPPH•, $ABTS^{+}$, ferrous ion chelation, and hydroxyl radical scavenging assays. Additionally, the *in vivo* antioxidant effects and acute toxicity of the butanolic extract were evaluated in BALB/c mice. *G. sinaicus* Boiss has been traditionally used for various medicinal purposes. This study aimed to investigate its phytochemical constituents and evaluate the antioxidant potential of different solvent extracts from the plant's aerial parts. The solvent extracts were analyzed for their phytochemical constituents, and the total phenolic and flavonoid contents were determined. Antioxidant activity was measured using DPPH•, $ABTS^{+}$, ferrous ion chelation, and hydroxyl radical scavenging assays. The *in vivo* antioxidant effects and acute toxicity of the butanolic extract were assessed using BALB/c mice. Phytochemical screening revealed the presence of anthraquinones, terpenoids, flavonoids, saponins, alkaloids, tannins, and cardiac glycosides. The butanol and aqueous methanol extracts exhibited the highest contents of phenolics and flavonoids, displaying the strongest antioxidant activity across all assays. In the *in vivo* tests, the butanolic extract demonstrated an LD_{50} of 116.64 mg/kg (intraperitoneally). Treated mice showed increased total glutathione levels and reduced protein carbonyl levels in both serum and liver homogenates.The butanolic extract of *G. sinaicus* Boiss exhibited potent antioxidant activity and favorable safety *in vivo*, indicating its potential as a natural source of antioxidants and therapeutic agents.

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1. Introduction

Oxygen plays a crucial role in the metabolic reactions that generate energy in all living organisms. However, oxygen free radicals can trigger a series of reactions that may harm cells. The inherent instability of these free radicals is significant in the development of cancer cells through a continuous chain of reactions. For centuries, plants have served as vital sources of medicinal drugs due to their content of active compounds. Along with that, Many plants produce secondary metabolites that serve as excellent sources of antioxidants. These active compounds are part of the plant's defense mechanisms [5, 16, 18]. There are several classes of secondary metabolites, including terpenoids, alkaloids, phenylpropanoids, polyketides, fatty acids, and peptides, all interconnected by complex metabolic pathways. These substances function as antioxidants, which are defined as compounds that significantly inhibit or prevent the oxidation of other substances at low concentrations. Antioxidants are known for their diverse biological effects, including anti-inflammatory, antibacterial, antiallergic, and vasodilatory properties [8]. Research, both *in vivo* and *in vitro*, has evaluated various extracts of *G. sinaicus* Boiss (*Apocynaceae,* subfamily *Asclepiadoideae*) for their total phenolic and flavonoid content, as well as their phytochemical constituents. The results indicate that *G. sinaicus* Boiss exhibits high antioxidant activity, suggesting that it could be a valuable source of natural antioxidants and other therapeutic compounds [13,22].

2. Materials and Methods

All reagents used in this study were of analytical grade. Distilled water was used for common chemical solution preparation. DPPH, ABTS, ferrozine, ferric chloride ($FeCl_3$), Folin- Ciocalteu, bovine serum albumin (BSA), potassium persulphate ($K_2S_2O_8$); ascorbic acid; aluminum chloride ($AlCl_3$); sulphuric acid; sodium carbonate (Na_2CO_3); sodium hydroxide (NaOH); sodium nitrite ($NaNO_2$); sodium chloride (NaCl); Iron (II) chloride ($FeCl_2$); Iron (II) sulfate ($FeSO_4$) and hydrogen peroxide (H_2O_2) were purchased from Sigma Co. (St. Louis, MO, USA). All solvents n-hexane (99.8%), chloroform (99.8%), ethyl acetate (99.8%) and n- butanol (99.8%) used were of analytical grade and purchased from Merck Co. (Darmstadt, Germany). Distilled and deionized water ($d.H_2O$ and $dd.H_2O$) was prepared by an ultrapure water purification system (Department of Chemistry, Faculty of Science, Yarmouk University).

G. sinaicus samples collection, extraction and fractionation

The *G. sinaicus* plant was collected in the spring of 2016 in the Aqaba region**.** Specimens were identified by location. Fresh plant material was dried at room temperature (RT) in a shady place for one month. The plant material was powdered and then extracted in a Soxhlet extractor with petroleum ether to remove fatty acids and wax. Then methanol was added to the residual extract in the Soxhlet. Each time before extraction with the next solvent, the material was dried. After that, the extracts were concentrated by a rotary vacuum evaporator and then dried. The final product was added to chloroform ($CHCl_3$): $d.H_2O$ (1:1) and mixed solvent system. Then the phases $CHCl_3$ and $d.H_2O$ were separated. The $CHCl_3$ fraction was dried and then partitioned between 10% aqueous methanol and hexane, polar organic compounds were extracted from the water phase by using n-butanol and then, the extracts were used directly to estimate total phenolic content and to assess antioxidant potential by various chemical assays [2].

Phytochemical analysis

Qualitative analysis of butanol, aqueous methanol, hexane, and water extracts of *G. sinaicus* to determine their phytochemical constituents (alkaloids, tannins, anthraquinones, terpenoids, flavonoids, saponins and cardiac glycosides) and other secondary metabolites according to [26].

The determination of total phenolic content (TPC) in all types of extracts was analyzed using the Folin Ciocalteu method as described by [21].

Determination of total flavonoid content (TFC) of *G. sinaicus* in all types of extracts was determined using the aluminum chloride assay through colorimetry assay according to [21].

DPPH• free radical scavenging assay

The total radical scavenging capacity of the extracts was determined and compared to the standards of ascorbic acid and α-tocopherol. The modified method from reference [4,10] was used to evaluate the DPPH• free radical scavenging capacity of the extracts. In this method, 2 mL of 0.1 mM DPPH• solution was mixed with 1 mL of methanolic extract at various concentrations (0.005, 0.01, 0.05, 0.1, and 0.5 mg/mL). The mixtures were incubated in the dark for 30 minutes, after which the absorbance was measured at 517 nm using methanol as a blank. All experiments were repeated three times. A standard curve was prepared using different

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concentrations of DPPH•. The ability to scavenge the DPPH• radical was calculated according to the following Equation 1:

$$\text{DPPH• scavenging effect} = \left(\frac{1- AS}{AC}\right) \times 100\ \% \quad (1)$$

Where AC is the absorbance of the control and AS is the absorbance of the extract. DPPH• decreases significantly upon exposure to radical scavengers. Parameter introduced for the interpretation of the results from the DPPH method was used to calculate the IC_{50}. IC_{50} can be defined as the concentration of the substrate that causes a 50% loss of the DPPH• activity. The IC_{50} values were calculated by linear regression method by plotting the antiradical activity percent against the concentration of the tested compounds [17,19].

ABTS free radical scavenging assay

The total antioxidant activity by radical cation decolonization assay ($ABTS^+$) was determined using the [23] method with some modifications. The $ABTS^+$ cationic radical solution was diluted with $d.H_2O$ to obtain an absorbance reading at 734nm of 0.75. Three mL $ABTS^+$ solution was mixed with 1mL of extract at different concentrations (0.005, 0.01, 0.05, 0.1, and 0.5 $mg.ml^{-1}$). The absorbance was measured at 734nm by using a UV-VIS spectrophotometer. The blank was run in each assay and all measurements were taken after at least 5min. The $ABTS^+$ scavenging capacity of the extract was compared to ascorbic acid and α-tocopherol standards. The percentage inhibition was calculated according to the following Equation 2:

$$ABTS^+ \text{ scavenging effect} = \left(\frac{1- AS}{AC}\right) \times 100\ \% \quad (2)$$

Where AC is the absorbance of the control and AS is the absorbance of the extract.The IC_{50} values were calculated by the linear regression method of plots of the percent of antiradical activity against the concentration of the tested compounds.

Ferrous chelating effect

Ferrous chelation *G. sinaicus* was estimated according to [21] method with some modifications. *G. sinaicus* ability to ferrous chelating was monitored by the absorbance of the ferrous–ferrozine complex at 562 nm. Three mL of methanolic extract solution in different concentrations (0.005, 0.01, 0.05, 0.1, and 0.5 $mg.ml^{-1}$) were added to 250 µL 2 mM $FeCl_2$ solution. The reaction was initiated by adding 0.2 ml of 5 mM ferrozine. It was then shaken vigorously and left at room temperature for 10 min. The absorbance of the solution was then measured spectrophotometrically at 562 nm. Percent inhibition of ferrous–ferrozine complex was calculated according to the following Equation 3:

$$\text{Ferrous ion chelating effect} = \left(\frac{1- AS}{AC}\right) \times 100\ \% \quad (3)$$

Where AC is the absorbance of the control and AS is the absorbance in the presence of *G. sinaicus* or standards. The control contains only $FeCl_2$ and ferrozine.

Hydroxyl radical assay

A hydroxyl radical assay was detected using salicylic acid and was used to measure the hydroxyl radical formation according to [28] method. To measure the formation of hydroxyl radicals, 1 ml methanol extract solution at different concentrations (0.005, 0.01, 0.05, 0.1 and 0.5 $mg.ml^{-1}$) was added to 0.5 ml of 6 mM $FeSO_4$. Then 0.5 ml of 6 mM H_2O_2 was added, followed by vertexing the tubes and finally the tubes were left to stand for 10min. Afterwards, 1ml of 6mM salicylic acid was added to each tube and incubated for 30 min at room temperature. Ascorbic acid was used as a positive control. The absorbance was measured at 510 nm and then the hydroxyl radical scavenging capacity was calculated according to the following Equation 4:

$$\text{Hydroxyl radical effect} = \left(\frac{1- AS}{AC}\right) \times 100\ \% \quad (4)$$

Where AC is the absorbance of the control and AS is the absorbance of *G. sinaicus* extract or standards.

In Vivo

Experimental design and animals

Adult female BALB/c albino mice, aged 8-10 weeks and weighing approximately 24 grams, were used as experimental subjects in this study. The mice were sourced from the Animal House Department of Biological Sciences at the Faculty of Science, Yarmouk University. They were kept in a controlled environment with a temperature between 21-23°C and a light cycle of 12 hours of light followed by 12 hours of darkness. Standard pellet food and water were provided. The evaluation of the antioxidant capacity of *G. sinaicus* was divided into two experiments. The first experiment involved an assessment of acute toxicity. For this purpose, two stock solutions of butanol extract were prepared: Stock A consisted of 1 gram of butanol extract dissolved in 15 milliliters of normal saline, while Stock B contained 1 gram of butanol extract dissolved in 60 milliliters of normal saline. In this experiment, 40 female mice were divided into four groups, each group having 10 mice,

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and the first group was regarded as a control group. And the other group used 0.1ml (200 mg.kg^{-1}) of stock solution A was injected intraperitoneally for 12 (dose/day) administration. And the other group used 0.2ml (100 mg.kg^{-1}) from stock B was injected intraperitoneally for the administration of 12 (dose/day). The last group used 0.1ml (50mg.kg^{-1}) from stock solution B and injected intraperitoneally for administration of 12 (dose/day). And another experiment was used to determine PC level and total GSH content. Butanol extract stock solutions were prepared as Stock A: 1g of butanol extract was dissolved in 60ml of normal saline. And Stock B: 10ml from stock A was mixed with 40mL normal saline. In this experiment, 21 female mice were divided into three groups, each group has 7 mice, and the first group was regarded as a control group. And the other group used where 0.2ml (20 mg.kg^{-1}) from stock B was administrated intraperitoneally for 12(dose/day) administration. The last group used 0.1ml (10mg.kg^{-1}) from stock solution B intraperitoneally for 12(dose/day) of administration.

Sample preparation for antioxidant evaluation

To preparation the serum sample, blood from treated mice was used, collected in Eppendorf tubes and allowed to clot at room temperature for 30 minutes. Then the tubes were centrifuged at 2000 xg for 15 minutes at 4°C. The serum was then transferred to new tubes and stored at -70 °C. And tissue homogenization used mice liver was collected from treated mice and stored at -70°C until use. Approximately 15mg liver tissue was homogenized in phosphate buffer using Tissue Lyser II (Talboys 930145. Kats Enterprise division, USA) at 20 Hz for 4min. After homogenization, the supernatant was transferred to a new tube and centrifuged for 20min at 13.4xg at 4°C. The supernatant was transferred to a new 1.5ml Eppendorf tube and stored at -70°C. About 30µL from the supernatant was transferred to a 1.5ml Eppendorf tube to measure the protein concentration.

Oxidative stress assessment

Protein carbonyl (PC) levels in serum and liver homogenate identified with the Protein Carbonyl Content Assay Kit (Sigma-Aldrich, USA) were used. Protein concentration of serum and liver homogenate was determined using a Bicinchoninic acid assay kit (Sigma-Aldrich, USA according to the manufacturer's instructions, using BSA as the standard. And the GSH content in the serum and liver homogenate identified by Glutathione Assay Kit (Cayman Chemical Item, USA) was used to measure the total glutathione level in serum and liver extracts [9].

Statistical analysis

The data were analyzed using SPSS software. Mean ± standard deviation (SD) was found from triplicate reading. *In vitro* antioxidant assays, one-way ANOVA test followed by Tukey's test ($P < 0.05$) were used to analyze the differences in antioxidant assays and the differences among IC_{50} of various fractions and the IC_{50} value. *In vivo*, biochemical data was analyzed by one-way ANOVA and the group's means ± SD. This was performed to compare the differences between the groups. A probability of ($P < 0.05$) was considered as significant.

3. Results

Revealed the analysis of the phytochemical activity of *G. siniacus* crude which is a qualitative analysis of the presence of many chemical constituents of the extracts, such as alkaloids, flavonoids, terpenes, glycosides, tannins, saponins and phenols, the chemical constituents of the plant it contains support therapeutic medicine. The chemical constituents were screened and their results are summarized as the presence or absence of the chemical, showing that the butanol and aqueous methanol extracts were rich in tannins, terpenoids, flavonoids, saponins, anthraquinones, alkaloids and glycosides[20]. While the water extract contained tannins, glycosides, terpenoids and anthraquinones and the hexane extract contained none of the chemicals. The determination of the total flavonoid content in the polar extract was higher than that of the non-polar extract. Where, the results demonstrated that *G. sinaicus* is rich in flavonoids; the highest value was in the aqueous methanol extracts 290.5 ± 7.740 mg.g^{-1} of Quercetin. Which differs significantly from other extracts ($P<0.05$).

The total phenolic content of all extracts was determined according to the Folin Ciocalteu method and the phenolic content was expressed in terms of Garlic acid equivalent (mg GAE.mL^{-1} extract)[18, 27]. The amount of total phenolic compounds in the polar extract were higher than those for non-polar extracts. The high value of total phenolic content was in *G. sinaicus* extracts in the butanol fraction (165.96 ±

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1.46) mg.g^{-1} Gallic acid. Which differs significantly from other extracts ($P<0.05$).

Free radical scavenging capacity by the DPPH assay increased with increasing free radical inhibition percentage, and the antioxidant activity of the four *G. sinaicus* fractions was expressed in terms of (I%). The results of the two standard compounds (ascorbic acid and α-tocopherol) were compared with the antioxidant activity values. The data show that the highest percentage of DPPH inhibition occurred in the butanol extracts, followed by the methanol- aqueous extract, then the water extract and the hexane extract. The methanolic extract showed very good anti-radical activity compared to the ascorbic acid and α-tocopherol standards at the same concentration (Fig. 1) in the ABTS assay. Our data show that the highest ABTS^{+} scavenger activity was found in the butanol extract at 1 mg.ml^{-1}, then in the aqueous methanol, water and hexane extracts, respectively, compared to inhibition of ascorbic acid and α-tocopherol positive controls (Fig. 2).

Regarding the chelating effect of metals on ferrous ions. The chelating impacts of the four fractions of *G. sinaicus* were expressed in terms of the I% (Fig. 3). The results of the ascorbic acid and EDTA standards were obtained and compared with the values of plant extracts.

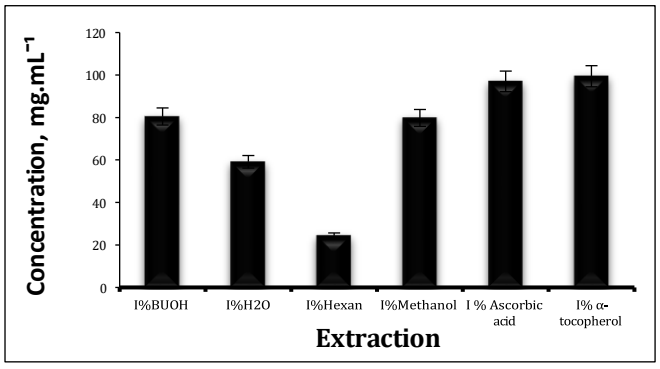

Figure 1. Antioxidant activity of the extract fractions of *G. sainicus* and positive controls (ascorbic acid and α-tocopherol) on DPPH• assay. Data are presented as the mean ± SD of triplicate samples (n=3)

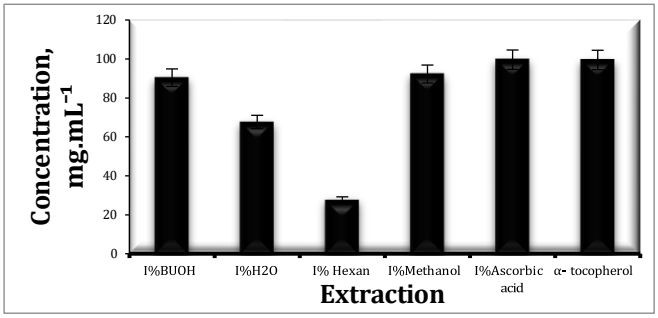

Figure 2. Antioxidant activity of the extract fractions of *G. sainicus* and positive controls (ascorbic acid and α-tocopherol) on ABTS$^{+\cdot}$ assay. Data are presented as the mean ± SD of triplicate samples (n=3)

Aqueous methanol was the highest ferrous ion chelating activity of the *G. sinaicus* extract fractions. The percentage of inhibition of other extracts was as follows: butanol, water and hexane in the same concentration.

In comparison, ascorbic acid and EDTA achieved the percentage chelating effect. The chelating effect obtained for the different fractions of the extract was less than that obtained with the standard antioxidants (EDTA and ascorbic acid).

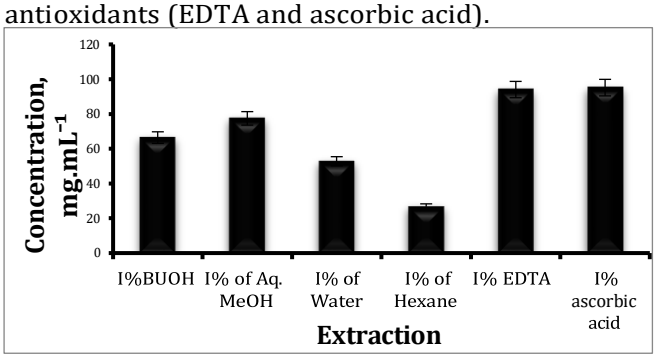

Figure 3. Metal chelating effect on ferrous ions in the extract fractions of *G. sainicus* and positive controls (ascorbic acid and EDTA). Data are presented as the mean ± SD of triplicate samples (n=3).

Activity to scavenge hydroxyl radicals also showed that butanol has the highest activity at a concentration of 1 mg.mL^{-1}. Percent inhibition of other extracts was as follows: water, aqueous methanol and hexane extract at the same concentration compared to EDTA and ascorbic acid standards at the same concentration (Fig. 4).

Therefore, the antioxidant activity of *G. sinaicus* extracts plays an important role through their scavenging activity which is a valuable in the treatment of many diseases and free radical scavenging activities were evaluated in comparison

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to DPPH•, $ABTS^{+}$, hydroxyl radical, and the ferrous ion chelating activity.

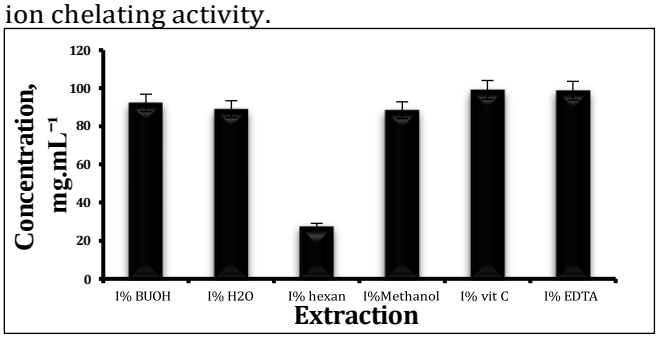

Figure 4. Hydroxyl radical effect of all extracts fractions for *G. Sinaicus* and standards.

Data are presented as the mean ± SD of triplicate samples (n=3).All extracts showed different radical scavenger activity ranging from 0.005– 0.5mg.ml^{-1} concentration. However, butanol is considered the best compound for exhibiting the strongest DPPH, ABTS, hydroxyl radical scavenger, and ferrous ion chelate activity compared to other extracts. A toxicological study (acute toxicity assessment) was conducted to evaluate the antioxidant capacity of *G. sinaicus*. Forty adult female BALB/c albino mice were used. The mice were divided into four groups and the results of this experiment showed no change registered in the control group, where all mice died (200mg.kg^{-1} intraperitoneal), half of the group (100 mg.kg^{-1} intraperitoneal) died while the other half survived. Finally, the fourth group (50mg.kg^{-1}- intraperitoneal) survives. These results indicate that *G. sinaicus* products can have toxic effects when injected in high concentrations. This experiment was evaluated to demonstrate *in vivo* antioxidant potential and to determine the median lethal dose (LD_{50}) of butanolic extracts of *G. sinaicus*.Percent mortality was calculated by counting the deaths of animals in each group to determine the LD_{50}. Symptoms of acute toxicity from *G. sinaicus* in mice were an increase in respiratory rate and heart rate, followed by heart failure and death after half an hour. The LD_{50} was found 116.64 mg/kg while the LD_{100} was found 194.40 mg.kg^{-1}. LD_{50} was used as an indicator of acute toxicity.Protein carbonyl levels in the serum and liver homogenate in the butanolic extract of *G. sinaicus* were used to determine PC levels in mice serum PC formation is an important marker of protein oxidation and be mediated by the direct free radical attack on some amino acid side chains of lipid peroxidation products that react with proteins [7]. In addition, the data show that the Protein carbonyl content decrease significantly in the group (20mg.kg^{-1} - intraperitoneally) and group (10mg.kg^{-1} - intraperitoneal) compared to control. In addition, the Protein carbonyl content in the liver tissue was also determined, and our data showed that the Protein carbonyl content in the (20 mg.kg^{-1} - intraperitoneal) group and the (10 mg.kg^{-1} - intraperitoneal) group was compared decreased significantly (Fig. 5). The results obtained from this study showed that administration of *G. sinaicus* extracts showed a significant decrease in protein carbonyl content of liver homogenate and serum of BALB/c mice. According to [1, 9] , the production of protein carbonyl at high levels indicates that oxidation significantly damages amino acid residues in proteins in human tissues and body fluids and this is due to increased free radicals. Therefore, measured an increase in protein carbonyls leads to a decrease in protein thiol groups, which is indicated by significant oxidative modification of proteins, suggesting that protein carbonyl groups are considered a biomarker of oxidative stress. Total glutathione (GSH) content in the serum, the highest total GSH concentration value was the increase significant in the group (20 mg.kg^{-1} - intraperitoneal) followed by the group (10 mg.kg^{-1} - intraperitoneal) compared to the control group (Fig. 6).In the liver, the total GSH concentration was highest in the 20 mg.kg^{-1} - intraperitoneal group and then in the 10 mg.kg^{-1} - intraperitoneal group compared to the control. In addition, the results of the present study showed that the content of the GSH in the liver and serum increased significantly with increasing concentration of the extracts compared to the control. [14, 22] found that the total concentration of glutathione, a low molecular weight tripeptide thiol, is considered an important antioxidant popular for its ability to minimize the effects of oxidative stress. Glutathione is widely known to minimize the lipid peroxidation of cell membranes and other similar targets known to occur during oxidative stress [11, 24]. Finally, evaluating the toxicity profile of each compound is important in drug discovery. In the present study, the acute intraperitoneal toxicity of butanol extraction from *G. sinaicus* showed that the LD_{50} value when administered intraperitoneally in mice was 116.64 mg.kg^{-1} body weight.

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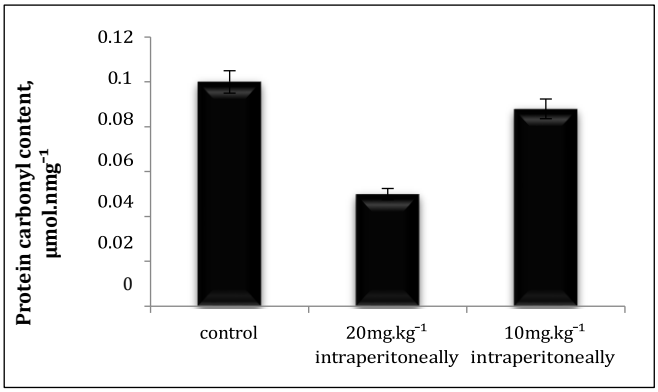

Figure 5. Protein carbonyl content in the serum of mice treated with butanol extracts of *G. sinaicus.* Values are means ± SD of 7 animals in each group. *$p < 0.05$ compared to the control.

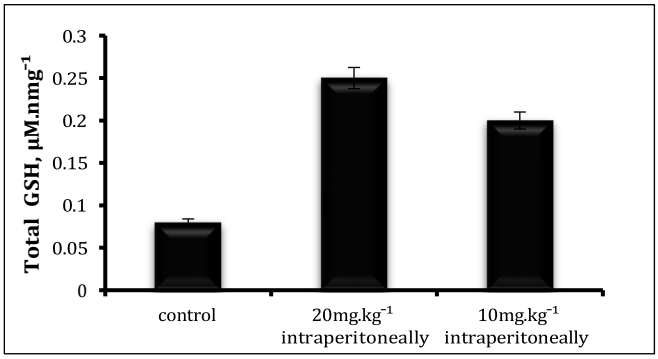

Figure 6. Total GSH in serum of mice treated with butanol extracts of *G. sinaicus*, Values are means ± SD of 7 animals in each group. * $p < 0.05$ compared to the control.

4. Discussion

G. siniacus offers superior analysis results as compared to many other plants in other studies such as *Capparis spinosa* L., *Capparis decidua* (Forssk.) Edgew [2,3,25F]C*alotropis procera* (Ait.) Ait.fil, *Peruglaria tomentosa* L. and *Pentatropis spiralis* (Forssk.) Decne. (Asclepiadaceae) [2,3,25].According to [2,3,25] to determination of the total flavonoid content in the polar extract was higher than those of the non- polar extract showed that the flavonoids of *Calotropis procera* (Ait.) Ait. fil, in the aqueous methanol extract was 339.0 ± 1.1 mg.g^{-1}, in the total flavonoids of butanol extract were found 1684.5 ± 0.9 mg.g^{-1} in the *Pentatropis spiralis*. While total flavonoid contents of butanol extract of the *Capparis spinosa L., Capparis decidua* (Forssk) Edgew was (306.93 ± 17.64 and 325.82 ± 8.39 mg.g^{-1} of Quercetin) respectively.

The total phenolic content of all extracts was determined using the Folin-Ciocalteu method, and the results were expressed as milligrams of Gallic acid equivalent (mg GAE per mL of extract). In a previous study, the aqueous methanol extract of *Calotropis procera* (Ait.) Ait. fil exhibited a higher total phenolic content of 377.2 ± 2.6 mg of gallic acid per gram of dry weight. Additionally, the butanol extract of *Pentatropis spiralis* (Forssk) Decne. (*Asclepiadaceae*) showed a total phenolic content of 113.2 ± 2.3 mg of gallic acid per gram of dry weight. In comparison, the butanol extracts *of Capparis spinosa* L. and *Capparis decidua* (Forssk) Edgew had total phenolic contents of 227.17 ± 7.29 mg and 311.50 ± 3.04 mg of Gallic acid per gram of dry weight, respectively.

All extracts demonstrated varying levels of radical scavenging activity. However, butanol is considered the best compound for exhibiting the strongest DPPH, ABTS, hydroxyl radical scavenger, and ferrous ion chelate activity compared to other extracts. These results correspond to [2,3,25] except for *Calotropis procera* (Ait) Ait. fil plant. According to [14,22] found that the total concentration of glutathione, a low molecular weight tripeptide thiol, is an important antioxidant recognized for its ability to reduce the effects of oxidative stress. Glutathione is well-known for its role in minimizing lipid peroxidation in cell membranes and other similar targets that are affected during oxidative stress. Additionally, assessing the toxicity. profile of each compound is crucial in the drug discovery process. According to [6,12] estimated that the intraperitoneal median lethal dose (LD_{50}) of *G. sinaicus* extract grown in Egypt was 49.82 mg per100g^{-1} body weight for intraperitoneal administration in mice. Where the mean lethal dose of butanol extraction of *G. sinaicus* was determined according to the classification of LD_{50} values [15].

5. Conclusions

The highest levels of total phenols and flavonoids content and antioxidant activity of *G. sinaicus* indicate the potential of using *G. sinaicus* in the pharmaceutical industry. In addition to the therapeutic effect of the active compound present in *G. sinaicus* increases the total GSH levels and decreases the PC levels. However, the toxicity and LD_{50} of *G. sinaicus* the butanolic extract were moderately toxic when tested in mice.

Abbreviations: ABTS – 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid); BSA - bovine serum albumin; DPPH – 2,2-diphenyil-1picrylhydrazyl; $FeCl_3$- ferric chloride; Ferrozine - 3-(2-pyridyl)-5, 6-diphenyl-1, 2, 4-tri-azine-4', 4"-disulfonicacid sodium salt); GAE –Gallic acid equivalent; GSH - total glutathione; I % - percentage inhibition; PC - protein carbonyl; SD - standard deviation; TFC - total flavonoid content.

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the Ethical Approval

Mahmoud A. Al-Qudah conceived the study, jameel Allaham developed the theoretical framework, and Salam Husam Sabri performed experiments, collected and analyzed data, interpreted the results, and drafted and all researchers revised the manuscript.

6. References

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