Phytochemical analysis and in vivo toxicity study of Dianthus orientalis Adams crude extract

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Abstract

Background

Dianthus must be well investigated for its phytochemical content and biological and medicinal activities, including *Dianthus orientalis* Adams.

Objectives

To assess the phytochemical composition and determine the toxicity of Dianthus orientalis Adams in an animal model.

Materials and Methods

The whole plant materials were collected from July to August 2021 from Penjween district, Sulaimaniyah, Kurdistan of Iraq and then identified, authenticated, shadow-dried, and extracted using ethyl acetate and pure methanol to collect the crude extract of leaves and flowers separately. The GC-MS was run to determine the chemical composition and phytochemical content. Subsequently, the methanolic extract of the leaves was selected to be tested further for its toxicity in the male Sprague Dawley rat model using various techniques.

Results

The GC-MS results of the methanolic extract of Dianthus orientalis Adam's leaves and flowers present the higher phytocomponents (no.=34). Some medicinal compounds have been identified in leaves and flowers with various concentrations. The animal toxicity study revealed body weight gain, no clinical signs of toxicity, and no mortality, with non-significant changes in treated animals' haematological, biochemical and histopathological profiles.

Conclusions

Various huge compounds have been identified in the studied plant parts corresponding to their safe use in an animal model.

Introduction

Herbs and medicinal plants have been used for centuries as promising sources of therapeutic agents as they have played significant roles in the development of modern drugs [1]. Approximately 25% of medical drugs are produced from herbal ingredients and their derivatives in developed countries [2]. Also, the plant's

crude material or pure compounds have been used to treat various diseases by generations of conventional therapists [3]. According to the World Health Organization (WHO), 80% of people in developing countries rely on traditional medicine for therapy [4].

Medicinal plants produce small bioactive molecules called secondary metabolites. Their roles remain unknown in primary metabolic processes such as growth, photosynthesis and reproduction, while they increase the ability of plants to survive and overcome their local challenges [5, 6]. In addition, secondary metabolites can be used to manage various diseases such as anticancer, antioxidant, antimicrobial, anti-inflammatory, and anti-ulcer activities [7–9]. These bioactive compounds can be extracted from different parts of plants and classified as terpenoids, alkaloids, nitrogen-containing compounds, organosulfur compounds, and phenolic compounds [5].

Dianthus (carnations and pinks) genus belongs to the *Caryophyllaceae* family, which has over 300 species [10]. The major bioactive compounds plants in this family are polyphenols, flavonoids, saponins, phytoecdysteroids, lignans, peptides, anthraquinones, phenylpropanoids, phytoecdysteroids, amides, essential oils, N-containing substances such as alkaloids, vitamins, and cyclic peptides, which contributes to their pharmacological properties [11–13].

Plants belonging to the genus *Dianthus* were used in traditional medicine to treat stomach aches, toothache, throat and gum infections, wounds, cardiovascular ailments, and alexiteric and vermifugal diseases. Traditionally, they were also used to cure injuries and gastrointestinal conditions in China, Japan and Korea. In addition, its essential oil was also applied to improve memory and restore forces, heal wounds, relieve dizziness and lift appetite [14]. The main centre of *Dianthus* genus diversity is the Mediterranean area, including France, Mainland Spain, Italy (including Sicily and Sardinia), South/East of Turkey, Greece, and the Kurdistan regions of Iraq and Iran [15–17].

Dianthus orientalis A. (Georgian pink or called mexaka kewi in the Kurdish language) is a perennial wild species belonging to *the Dianthus* genus that is found in Iraq [18], Turkey, Iran, Tibet, China, Russia, Pakistan, Himalaya, and Assam [19, 20]. It is mainly seen in rocky areas, cliffs and lime-free rocks, which have the structure of a schist or gneiss. It is particularly blooming in autumn when there are few pink-coloured flowers (Fig. 1) [21].

Several published papers provided information about different medicinal plants of the family Caryophyllaceae and their biological, biochemical, pharmacological, and ethnomedicinal properties [14, 22]. Still, only a few of them focused on *Dianthus orientalis* A. However, it is reported that this species had antibacterial [23], antispasmodic, and anti-toothache activities [24].

Thus, *Dianthus orientalis* A. are not well investigated for its phytochemical content and biological and medicinal activities; therefore, this study aimed to collect the profound Dianthus species in the Kurdistan region of Iraq, assess its phytochemical composition, and its toxicity in an animal model (Fig. 2).

Materials and Methods

Dianthus orientalis A. collection and identification

The plant material (leaves and flowers) of *Dianthus orientalis* A. were collected from July to August 2021 from Penjween district, Sulaimaniyah, Kurdistan of Iraq. A certified taxonomist made proper identification and authentication at the Herbarium Department, Kurdistan Botanical Foundation, American University of Iraq Sulaimani (AUIS). A herbarium voucher with reference number (No. 265-12-02-2021-AUIS) was already deposited at the AUIS campus herbarium.

Dianthus orientalis A. extraction

The harvested plant (leaves and flowers) were dried in a shaded area for two weeks and powdered separately at the Biochemistry Laboratory, College of Medicine, University of Sulaimani, Iraq. Then, 50 gm of each of the dried powder material was macerated with different solvents (methanol and ethyl acetate) (1:10; w:v) for 72 hours at room temperature (25º C) with constant shaking. The extracts were filtered using Whatman No. 1 filter paper. The filtrates were concentrated by evaporating the solvents and were used to prepare the samples for further analysis. The yield product of the crude extracts was calculated as the weight of natural extract/weight of fresh plant) × 100%. All sections were stored at 4º C until needed.

GC-MS analysis of Dianthus orientalis A. extract

At the Biochemistry Laboratory, College of Agricultural Sciences, University of Basrah, Iraq, the crude extracts were subjected to chromatography–mass spectrometry (GC-MS) separation and eluted stepwise with various ratios of hexane, hexane/ethyl acetate, ethyl acetate, ethyl acetate/methanol and methanol to give the fractions.

Saponins test

About 50 mg of crude plant extract was added to 5.0 mL distilled water and shaken vigorously, then checked for a layer of foam formation [25].

Animals

Male Sprague Dawley rats, aged 6–8 weeks, weighing 100–150 gm, were provided by the Department of Biology, College of Education, University of Sulaimani, Iraq. The rats were acclimatized on distilled water and a standard rat chow for seven days and kept in a well-ventilated room with a 12 hours' dark/light cycle at 25 ± 3º C before the study.

Sub-chronic toxicity study

A toxicity study was performed according to the Organization for Economic Cooperation and Development (OECD) Guideline No. 407 for testing a chemical [26, 27]. Animals were randomly divided into

four groups (No.=6), including Group 1: Normal control rats received normal tap water (CN: Control Negative); Group 2: Low dose *Dianthus orientalis* extract (DOE) (300 mg/kg body weight); Group 3: Median dose DOE (600 mg/kg body weight); and Group 4: High dose DOE (900 mg/kg body weight). All drenching processes were done using force-feeding for four consecutive weeks.

Clinical observations and body weight measurements

The animals were observed for clinical and behavioral abnormalities, toxicological symptoms, feed consumption, and gross appearance twice each day throughout 28 day's treatment. In addition, the body weights of rats were recorded before treatment (zero-day) and on days 7, 14, 21, and 28.

Haematological study

At the end of the experiment (on day 29), all rats were sacrificed under deep anaesthesia using chloroform inhalation. Blood samples (5.0 mL) were obtained from the caudal vena cava. Half of it was collected into ethylene-diamine tetra-acetic acid (EDTA) vacuumed blood collection tubes, mixed well immediately, and was analyzed directly to estimate total white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT) and platelets (PLT) using automatic hematology analyzer (Cell Dyn, 3700, Abbot, USA).

Biochemical parameters

Another 2.5 mL of the blood was used for serum collection by centrifugation (3500 rpm for 10 min) at room temperature. The concentrations of serum total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, and creatinine (CREA) with sodium, potassium, chloride, calcium, and phosphate were determined using standard diagnostic kits (Roche) in an automatic biochemistry analyzer (Hitachi 902, Japan).

Histopathological examination

After animal scarification, the liver and kidneys were removed and processed for histological examination using previously established methods [28]. Briefly, collected tissues were rinsed in phosphate buffer saline, fixed and preserved in 10% neutral buffered formalin for at least two days, processed (using various concentrations of ethanol), trimmed (using microtome), embedded in paraffin, sectioned to a thickness of approximately 5.0 µm, stained with hematoxylin and eosin, mounted (using DPX), dried on a hot plate, then examined under ordinary light microscope and photos were captured.

Statistical analysis

All data were expressed as means ± SD. One-way analysis of variance (ANOVA; SPSS 25.0 for Windows; SPSS Inc., Chicago, IL, USA) was used to test for differences between treatment concentrations. Significant differences at $P \leq 0.05$ and highly significant differences at $P \leq 0.001$ were identified by the Student-Newman-Keuls multiple-range tests.

Results

GC-MS analysis

The GC-MS results of the methanolic extract of *Dianthus orientalis* Adam's leaves reflect 40 peaks which present its 34 phytocomponents (Table 1, Supplementary 1). In comparison, the GC-MS profile of the flowers reflects 38 peaks of biomolecules, which means the presence of about 34 phytocomponents (Table 2, Supplementary 2). On the other hand, the GC-MS profile of the *Dianthus orientalis* Adams's leaves extracted by ethyl acetate reflects 38 peaks of biomolecules that presents 31 phytocomponents (Table 3, Supplementary 3), and the GC-MS profile of the flowers reflects 35 peaks of biomolecules that stands for the 31 phytocomponents (Table 4, Supplementary 4).

Table 1
List of phytochemicals identified in methanolic extract of *Dianthus orientalis Adam's* leaves.

No.	Phytochemical's name	Molecular Formula	Area (%)	Retention Time
1	3-Hydroxypyridine-N-oxide	$C_5H_5NO_2$	0.24	3.166
2	Chloromethylmethyl sulfide	C_2H_5CLS	0.10	3.200
3	Cyclohexanone, 4-methyl-	$C_7H_{12}O$	0.10	3.560
4	Cyclohexene,1-(1,1-dimethylethoxy)-3-methyl-	$C_{11}H_{20}O$	7.34	5.063
5	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	0.03	5.940
6	2-Cyclohexen-1-one, 2-methyl-	$C_7H_{10}O$	0.64	6.488
7	3-cis-Methoxy-5-cis-methyl-1R-cyclohexanol	$C_8H_{16}O_2$	1.70	6.784
8	2H-Tetrazole, 2-(1,3-dioxolan-4-ylmethyl)-	$C_8H_{13}N_5O_3$	0.61	6.867
9	Tetrazole, 1-(1,3-dioxolan-4-ylmethyl)-	$C_6H_{10}N_4O_2$	0.91	7.058
10	4-Heptanone, 2,3:5,6-diepoxy-2,6-dimethyl-	$C_9H_{14}O_3$	0.54	7.121
11	Tricosane-2,4-dione	$C_{23}H_{44}O_2$	0.54	7.190
12	3-Methyl-2-butenoic acid, hexadecyl ester	$C_{22}H_{42}O_2$	0.06	7.415
13	1,6-Anhydro-2,4-dideoxy-.beta.-D-ribo-hexopyranose	$C_6H_{10}O$	0.38	8.379
14	Hexanoic acid, 3-hydroxy-5-methyl-, methyl ester	$C_8H_{16}O_3$	0.10	8.780
15	Z,Z,Z-1,4,6,9-Nonadecatetraene	$C_{19}H_{32}$	0.11	9.886
16	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	1.24	10.777
17	Phenol, 2-methoxy-4-(1-propenyl)-	$C_{10}H_{12}O_2$	0.11	11.327
18	2-Pentyne-1,4-diol, 1-(2-furanyl)-4-methyl-	$C_6H_{10}O$	0.29	14.494
19	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	0.73	17.521
20	Methyl 8-methyl-nonanoate	$C_{11}H_{22}O_2$	0.07	18.560
21	Pentadecanoic acid	$C_{15}H_{30}O_2$	4.13	19.763
22	2-Hydroxyhexadecyl butanoate	$C_{20}H_{40}O_3$	0.85	20.599
23	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	1.20	20.900

No.	Phytochemical's name	Molecular Formula	Area (%)	Retention Time
24	17-Octadecynoic acid	$C_{18}H_{32}O_2$	6.73	21.376
25	1,E-11,Z-13-Octadecatriene	$C_{18}H_{32}$	3.60	21.717
26	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	4.08	22.067
27	6-Ethoxy-6-methyl-2-cyclohexenone	$C_9H_{16}O_2$	2.32	22.350
28	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	6.43	22.683
29	Hexanoic acid, 4-hexadecyl ester	$C_{22}H_{44}O_2$	6.84	22.933
30	4-O-Methylmannose	$C_7H_{14}O_6$	44.42	23.984
31	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{25}H_{46}O_6$	0.65	24.579
32	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	$C_{21}H_{40}O_4$	1.06	26.142
33	1-Octacosanol	$C_{28}H_{58}O$	0.66	27.564
34	Vitamin E	$C_{29}H_{50}O_2$	0.82	29.485

Table 2
List of phytochemicals identified in methanolic extract of *Dianthus orientalis* Adam's flowers.

No.	Phytochemical's name	Molecular Formula	Area (%)	Retention Time
1	2,4-Hexadienoic acid, methyl ester, (E,E)-	$C_7H_{10}O_2$	0.10	3.164
2	Cyclohexene,1-(1,1-dimethylethoxy)-3-methyl-	$C_{11}H_{20}O$	1.11	4.305
3	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	0.36	5.642
4	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	0.19	5.867
5	2-Cyclohexene-1,4-dione #	$C_6H_6O_2$	0.09	6.359
6	Decanoic acid, 3-methyl-	$C_{11}H_{22}O_2$	0.71	6.665
7	2H-Tetrazole, 2-(1,3-dioxolan-4-ylmethyl)-	$C_8H_{13}N_5O_3$	0.26	6.877
8	Bicyclo[2.2.1]heptane-2-carboxylic acid isobutyl-amide	$C_{12}H_{21}NO$	0.15	7.085
9	Pyrimidine-4,6-diol, 5-methyl-	$C_5H_6N_2O_2$	1.87	7.773
10	Silane, ethenyldiethylmethyl-	$C_7H_{16}Si$	0.13	8.517
11	5-Methyl-2-ethylamino-2-thiazoline	$C_6H_{12}N_2S$	0.27	8.611
12	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	12.15	10.647
13	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	0.16	17.486
14	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	2.02	19.424
15	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	0.64	20.398
16	2-Hydroxyhexadecyl butanoate	$C_{20}H_{40}O_3$	0.33	20.533
17	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	0.71	20.673
18	6-Ethoxy-6-methyl-2-cyclohexenone	$C_9H_{14}O_2$	1.70	20.875
19	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{28}H_{38}O_2$	13.79	21.254
20	Octadecanoic acid	$CH_3(CH_2)_{16}COOH$	3.91	21.431
21	2-Cyclopentene-1-undecanoic acid, (+)-	$C_{16}H_{28}O_2$	7.21	21.639
22	2-Hexadecenoic acid, methyl ester, (E)-	$C_{17}H_{32}O_2$	6.90	21.825

No.	Phytochemical's name	Molecular Formula	Area (%)	Retention Time
23	1,E-11,Z-13-Octadecatriene	$C_{18}H_{32}$	6.97	21.997
24	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	30.03	22.493
25	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	0.37	24.490
26	Sulfurous acid, 2-propyl tetradecyl ester	$C_{17}H_{36}O_3S$	0.43	25.855
27	1,2–15,16-Diepoxyhexadecane	$C_{16}H_{30}O_2$	0.32	26.855
28	Cyclohexane, 1-(1-tetradecylpentadecyl)-	$C_{35}H_{70}$	0.29	27.210
29	Fumaric acid, 2,2,2-trichloroethyl tridecyl ester	$C_{16}H_{26}Cl_2O_4$	2.05	27.512
30	26-Hydroxycholesterol	$C_{27}H_{46}O_2$	0.33	28.195
31	Ergost-4,7,22-trien-3.alpha.-ol	$C_{28}H_{44}O$	1.96	28.388
32	2,3,4,5,6,7-Hexahydro-3,6-dicyclohexyl-10,11-diphenyl-bis-[1, 3]-oxazino[6,5-f	$C_{36}H_{44}N_4O_2$	1.88	28.828
33	Vitamin E	$C_{29}H_{50}O_2$	0.42	29.013
34	1-Octacosanol	$C_{28}H_{58}O$	0.12	29.142

Table 3
List of phytochemicals identified in ethyl acetate extract of *Dianthus orientalis* Adam's leaves.

No.	Phytochemical's name	Molecular Formula	Area (%)	Retention Time
1	5-Chloro-5-methylnonane	$C_{10}H_{21}CL$	3.84	3.099
2	2-Hexen-1-ol, (E)-	$C_6H_{12}O$	3.05	3.498
3	5-Dodecene, (E)-	$C_{12}H_{24}$	0.15	8.356
4	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	0.16	13.778
5	Tridecanoic acid	$C_{13}H_{26}O_2$	0.19	17.062
6	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	0.4	17.576
7	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	7.09	17.975
8	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	4.79	19.422
9	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	0.22	20.304
10	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	0.16	20.378
11	3-Tetradecyn-1-ol	$C_{14}H_{26}O_2$	8.48	21.230
12	Octadecanoic acid	$C_{18}H_{36}O_2$	0.92	21.388
13	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	0.92	22.867
14	Tritetracontane	$C_{43}H_{88}$	0.95	24.156
15	2- Bromopropionic acid, pentadecyl ester	$C_{18}H_{35}BrO$	0.36	24.457
16	Ethanol, 2-(octadecyloxy)-	$C_{20}H_{42}O_2$	0.63	24.999
17	Cholesta-22,24-dien-5-ol, 4,4-dimethyl-	$C_{29}H_{48}O$	1.09	25.683
18	1-Heneicosanol	$C_{21}H_{44}O$	4.12	25.891
19	Cyclohexadecanone	$C_{16}H_{30}O$	1.32	25.996
20	1-Heptadecanol, acetate	$C_{19}H_{38}O_2$	1.08	26.755
21	Pentadecanal-	$C_{15}H_{30}O$	8.41	27.014
22	Triacontyl pentafluoropropionate	$C_{33}H_{61}F_5O_2$	2.99	27.128

No.	Phytochemical's name	Molecular Formula	Area (%)	Retention Time
23	1-Octadecanesulphonyl chloride	$C_{18}H_{37}ClO_2S$	1.37	27.420
24	1-Octacosanol	$C_{28}H_{58}O$	20.47	27.552
25	Disulfide, didodecyl	$C_{24}H_{50}S_2$	2.53	27.842
26	Lanost-7-en-3-one, (9.beta.,13.alpha.,14.beta.,17.alpha.)-	$C_{30}H_{50}O$	1.72	28.200
27	Hexacosyl acetate	$C_{28}H_{56}O_2$	3.94	28.321
28	3,4-Seco-5.alpha.-cholestan-3-oic acid, 4-hydroxy-4-methyl-, .epsilon.-lactone, (4R)-	$C_{28}H_{48}O_2$	0.87	28.683
29	Octadecane, 1-chloro-	$C_{18}H_{37}CL$	0.34	29.017
30	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-, acetate, [2R-]	$C_{31}H_{46}O_3$	16.56	29.498
31	Cholestan-3-one, cyclic 1,2-ethanediyl acetal, (5.alpha.)-	$C_{29}H_{50}O_2$	0.56	29.596

Table 4
List of phytochemicals identified in ethyl acetate extract of *Dianthus orientalis* Adam's flowers.

No.	Phytochemical's name	Molecular Formula	Area (%)	Retention Time
1	5-Chloro-5-methylnonane	$C_{10}H_{21}Cl$	1.19	3.080
2	o-Xylene	C_8H_{10}	0.13	3.296
3	2-Hexen-1-ol, (E)-	$C_6H_{12}0$	1.75	3.519
4	2-Hexen-1-ol, (Z)-	$C_6H_{12}0$	0.21	3.567
5	Hexanoic acid	$C_6H_{12}0_2$	1.60	7.028
6	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}0$	0.15	17.469
7	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	6.33	19.530
8	9-Tetradecenal, (Z)-	$C_{14}H_{26}O$	10.97	21.332
9	Octadecanoic acid	$C_{18}H_{36}O_2$	1.45	21.495
10	trans-2-undecenoic acid	$C_{11}H_{20}O_2$	1.35	23.757
11	10-Heneicosene (c,t)	$C_{21}H_{42}$	0.95	23.917
13	2- Bromopropionic acid, pentadecyl ester	$C_{18}H_{35}BrO_2$	0.29	24.464
14	Acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester	$C_{22}H_{44}O_2$	0.23	24.779
15	Octadecane, 1-chloro-	$C_{18}H_{37}Cl$	0.48	25.537
16	Tritetracontane	$C_{43}H_{88}$	23.61	25.978
17	5-Ethyl-1-nonene	$C_{11}H_{22}$	2.63	26.283
18	Hexacosane, 13-dodecyl-	$C_{38}H_{78}$	0.36	26.442
19	Sulfurous acid, 2-propyl tridecyl ester	$C_{16}H_{34}O_3S$	0.77	26.647
20	1-Heptadecanol, acetate	$C_{19}H_{38}O_2$	0.44	26.767
21	Hexacosane, 9-octyl-	$C_{34}H_{70}$	0.74	27.143
22	Cyclohexane, 1-(1-tetradecylpentadecyl)-	$C_{35}H_{70}$	2.15	27.238
23	Heptadecane, 9-octyl-	$C_{25}H_{52}$	3.71	27.452

No.	Phytochemical's name	Molecular Formula	Area (%)	Retention Time
24	2-Pentacosanone	$C_{25}H_{50}O$	0.80	27.608
25	Octacosyl heptafluorobutyrate	$C_{23}H_{57}F_7O_2$	0.65	27.977
26	Oleyl Alcohol	$C_{18}H_{36}O$	1.47	28.611
27	Thiophene, 3-methyl-5-(2-methyldecyl)-2-tridecyl-	$C_{29}H_{54}S$	26.21	28.971
28	Eicosane, 10-methyl-	$C_{21}H_{44}$	3.50	29.132
29	1-Octacosanol	$C_{28}H_{58}O$	1.14	29.225
30	Vitamin E	$C_{29}H_{50}O_2$	2.19	29.458
31	Triacontyl pentafluoropropionate	$C_{33}H_{61}F_5O_2$	0.85	29.797

Some of the identified compounds has biomedical activities such as 4-O-Methylmannose (44.42%; Table 1) reported to had antibacterial activity [29, 30], 17-octadecynoic acid (6.73%; Table 1) has been reported to possess antihypertensive properties [31], 3-O-Methyl-d-glucose (30.03%; Table 2) has preservative activity [32], 1-Octacosanol (20%; Table 3) has insecticidal activity and increased mortality of the *Spodoptera frugiperda* in the larval stage, including the pupal stage. Also, this compound caused a decrease in the body weight of *S. frugiperda* pupae [33]. Moreover, stable form of vitamin E; 2H-1-Benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)- acetate, [2R-] (16%; Table 3) was identified and reported to has antioxidant properties that can be used in cosmetic formulations for skin care benefits [34], n-hexadecanoic acid (palmitic acid) (6.33%, Table 4) has antioxidant, anticancer and antibacterial activities [35], Thiophene, 3-methyl-5-(2-methyldecyl)-2-tridecyl (26.21%; Table 4) has antimicrobial activity [36].

Saponins determination

A layer of foam formation indicated the presence of saponins after vigorously shaking a crude extract with distilled water.

Clinical features and body weight measurements

Daily oral administration of the methanolic extract of *Dianthus orientalis* Adam's leaves at doses of 300, 600, and 900 mg/kg for 28 consecutive days did not significantly change the overall behavior of treated rats compared to controls. No animals died from either group, which indicates that the LD_{50} (The lowest dose that kills more than 50% of treated animals) was higher than 900 mg/kg body weight. Significant differences ($p < 0.05$) in body weight gain were observed in control negative and all treated groups for all timelines (Table 5).

Table 5
The mean body weight of studied animals before and after treatments with various doses of *Dianthus orientalis* Adam's methanolic leaf extract.

Animal Group	Body Weight (gm) (Mean ± SD)					P-value
	Day 0	Day 7	Day 14	Day 21	Day 28	
Control Negative	105.75 ± 4.53	116.75 ± 3.09	126.0 ± 2.94	133.75 ± 3.3	146.50 ± 2.08	< 0.05*
Low dose DOE	103.38 ± 7.89	121.63 ± 11.69	137.13 ± 19.28	149.75 ± 23.68	160.0 ± 26.87	< 0.05*
Median dose DOE	113.0 ± 3.65	119.5 ± 4.2	139.2.5 ± 2.5	151.0 ± 3.56	162.25 ± 6.9	< 0.05*
High dose DOE	102.5 ± 4.43	113.0 ± 4.24	125.25 ± 4.11	133.5 ± 6.16	150.5 ± 5.33	< 0.05*

Values are mean ± SD that have been analyzed using post hoc comparison test one-way ANOVA. *: Significant difference Increasing of body weight after 28 days in all treated groups when compared to that of 0.0 day. DOE: *Dianthus orientalis* Adams extract. Low dose (300 mg/kg), median dose (600 mg/kg) and high dose (900 mg/kg) were used.

Haematological analysis

The results showed that PLT was significantly decreased ($p = 0.016$) in all DOE-treated groups compared to the negative control group. Also, the HCT level was significantly ($p = 0.031$) decreased in low and medium DOE dose groups compared to a control group. Meanwhile, the WBC, RBC, and Hb did not record any significant ($p < 0.05$) changes compared to the control group (Table 6).

Table 6
Hematological analysis of studied animals before and after treatments with various doses of *Dianthus orientalis* Adam's methanolic leaf extract.

	Hematological Parameter (Mean ± SD)				
Animal Group	**WBC ($\times10^3$/µL)**	**RBC ($\times10^{12}$/L)**	**Hemoglobin (g/L)**	**Hematocrit (L/L)**	**Platelet ($\times10^{12}$/µL)**
Control Negative	10.35 ± 2.56	6.43 ± 1.01	13.55 ± 0.31	40.42 ± 1.15	736.25 ± 101.5
Low dose DOE	10.55 ± 2.08	6.58 ± 0.73	13.03 ± 1.43	38.78 ± 3.99	568.5 ± 86.74
Median dose DOE	8.87 ± 0.95	6.36 ± 0.74	12.10 ± 2.03	36.3 ± 3.15	586.67 ± 51.19
High dose DOE	8.73 ± 0.71	6.61 ± 0.40	13.43 ± 0.66	41.4 ± 3.0	543.25 ± 32.23
P-value	0.4	0.96	0.44	0.031*	0.016*

DOE: *Dianthus orientalis* Adams extract, WBC: White Blood Cell, RBC: Red Blood Cell. Data have been analyzed using Repeated Measurement ANOVA test, *: Significant difference using Independent Samples T test. Low dose (300 mg/kg), median dose (600 mg/kg) and high dose (900 mg/kg) were used.

Biochemical analysis

Regarding the ALT level, the mean values were significantly lowered (p = 0.009) in low and median DOE treatments than in the control group. Similarly, AST levels were significantly decreased in treated rats with all amounts of DOE. However, the mean value of ALP was significantly increased in median doses (572.33 ± 138.48) compared to that of the control group (431.0 ± 147.62) but significantly decreased in low/high DOE dose treatment groups. Considering kidney function biomarkers, the mean urea level significantly lowered (p = 0.012) in all DOE-treated groups compared to a control group, while the level of creatinine was non-significantly (p = 0.557) increased in all treated groups. At the same time, the mean value of TP and albumin were significantly increased in DOE low and high-dose groups (Table 7). Regarding the electrolyte levels, there was a significant increase in calcium and a decrease in potassium in the groups treated with low and high DOE than in the control group. On the other hand, the phosphate level was decreased in the median dose treatment, and chloride levels were reduced in low/high DOE-treated groups (Table 8).

Table 7
Biochemical profile of male rats treated with different doses of *Dianthus orientalis* Adam's methanolic leaf extract after 28 days.

Animal Group	Biochemical Parameter (Mean ± SD)						
	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/L)	Albumin (g/L)	Urea (mmol/L)	Creatinine (µmol/L)
Control Negative	65.0 ± 12.95	250 ± 70.53	431.0 ± 147.62	5.95 ± 0.44	3.45 ± 0.13	38.63 ± 3.37	0.31 ± 0.03
Low dose DOE	44.0 ± 3.16	128.75 ± 18.52	391.5 ± 101.96	6.42 ± 0.27	3.55 ± 0.7	30.93 ± 4.6	0.35 ± 0.01
Median dose DOE	54.33 ± 7.77	207.0 ± 125.73	572.33 ± 138.48	5.86 ± 0.4	3.25 ± 0.93	30.2 ± 4.43	0.33 ± 0.04
High dose DOE	68.25 ± 7.27	135.25 ± 43.18	349.25 ± 99.5	6.31 ± 0.54	3.95 ± 0.37	26.73 ± 4.05	0.35 ± 0.06
P-value	0.081	0.744	0.61	0.29	0.44	0.012*	0.557

DOE: *Dianthus orientalis* extract, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, TP: Total protein. Data have been analyzed using Repeated Measurement ANOVA test, *: Significant difference using Independent Samples T test. Low dose (300 mg/kg), median dose (600 mg/kg) and high dose (900 mg/kg) were used.

Table 8
Blood electrolytes of male rats treated with different doses of *Dianthus orientalis* Adam's methanolic leaf extract after 28 days.

Animal Group	Blood Electrolyte (mmol/L) Mean ± SD				
	Sodium	Potassium	Chloride	Calcium	Phosphate
Control Negative	137.0 ± 1.63	8.40 ± 0.76	99.0 ± 2.16	11.18 ± 0.4	9.9 ± 0.96
Low dose DOE	138.5 ± 1.0	6.63 ± 1.21	96.5 ± 2.52	13.23 ± 0.49	9.6 ± 0.32
Median dose DOE	138.33 ± 0.85	7.33 ± 0.55	99.67 ± 2.08	12.89 ± 0.5	8.92 ± 0.83
High dose DOE	137.0 ± 2.94	6.8 ± 1.19	95.75 ± 2.22	13.77 ± 1.51	10.1 ± 0.99
P-value	0.618	0.109	0.109	0.618	0.325

DOE: *Dianthus orientalis* Adams extract. Data have been analyzed using Repeated Measurement ANOVA test. Low dose (300 mg/kg), median dose (600 mg/kg) and high dose (900 mg/kg) were used.

Histopathological examination

Regarding the liver sections of studied animals, the tissues of the negative control group reveal no apparent pathological lesions, signified by typically arranged radiated hepatocytes around the mildly congested central vein with distinctive sinusoidal capillaries and few scattered Kupffer cells attached to the capillary wall. Low dose group demonstrates moderate vacuolar degeneration, some area of typically radiated hepatocytes around the central vein and usually appeared sinusoidal capillaries. The medium dose group elucidates the presence of average to severe fatty degeneration, hydropic degeneration, and ordinarily radiated hepatocytes column around the typical central vein with some sinusoidal capillaries. Finally, the high-dose group demonstrates severe vacuolar and hydropic degenerations, some necrotic eosinophilic cells, and narrowing of some sinusoidal capillaries with disturbed morphological arrangement around the central vein with no apparent vascular congestion (Fig. 3).

In the kidney sections, tissues of the control negative animal group reveal no prominent lesions, evident by typical small glomeruli and regular Bowman's capsule. Renal tubules display no significant lesions except for slight and non-significant cellular swelling. Whereas in the low dose group, the renal tubular epithelia show non-significant and moderate vacuolar cellular swelling, many areas typically appeared renal tubules together with renal glomeruli with no noticeable morphological changes. In the medium dose group, moderate and significant hydropic degeneration within the renal tubular epithelium with mild glomerular atrophy was evident by moderate flaring of Bowman's space. Finally, the high-dose group reveals severe glomerular atrophy, collapse, and significant cellular swelling within the renal tubular epithelia. At the same time, other areas of the given section typically show renal tubules and some regular glomeruli (Fig. 4).

Discussion

The chemical constituents of the plant extracts are provided by phytochemical screening. The biological properties of these phytochemicals may provide the basis for new compounds with potential bioactivities [37]. GC-MS is an accurate and fast technique for detecting and identifying various combinations, including alkaloids, nitro compounds, alcohols, long-chain hydrocarbons, organic acids, steroids, esters and amino acids [38].

Hence, in the present study, the GC-MS technique was adopted for detecting and identifying phytochemical compounds of *Dianthus orientalis* A. (leaves and flowers) crude extract. The results indicated the presence of more than 100 various compounds in the tested extracts (methanolic and ethyl acetate), mainly including fatty acid derivatives, nitrogenous compounds, vitamin E, organosulfur compounds, alkaloids, flavonoids, and phenolic compounds. In this regard, another study in Iraq revealed that this species contained high amounts of phenolic compounds, including vanillic acid, coumaric acid, genistein, cinnamic acid, and oleuropein using TLC, HPLC, IRLC/Mass analyses [18]. Moreover, phytochemical analysis of *D. caryophyllus* showed that it contained fatty acid derivatives, benzenoids, phenyl propanoids, isoprenoids, and nitrogenous compounds [39–41]. At the same time, GC and GC-MS analyses of the flower's essential oil of *Dianthus rupicola* Biv from Sicily showed the presence of 66 components, including a high content of thymol and carvacrol derivatives, with a low amount of t-casino

[42]. Other compounds isolated from the plants of the *Caryophyllaceae* family include fatty acid derivatives, benzenoids, phenyl propanoids, isoprenoids, triterpenes, alkaloids, coumarins and cyanogenic glycoside [40, 43]. These variations in the phytochemical constituents between various species of Dianthus, or even between the same species, might be related to different analyses technique, chemicals used, plant chemistry, and even soil content, with environmental factors (temperature and rain).

Regarding the saponins content of *Dianthus orientals*, the result of this study is supported by other studies' outcomes [12, 18, 44, 45]. Furthermore, saponins have been shown to have significant medicinal properties such as expectorant, estrogenic, antimicrobial, anti-inflammatory, anticancer, cytotoxic, and hypocholesterolemic functions in humans or animals [46]. Thus, various saponins, including dianchinenoside (A and B), dianchinenoside (C and D), and aglycone saponin dianchinenoside-B (Dch-B), have been identified [12]. Consequently, further studies or analysis need to be done for *Dianthus orientals* A. to determine its exact saponins content, type, and formula, as it will be supportive data for future biomedical properties of this species.

The secondary metabolites are not entirely free of side effects. Therefore, the toxicity study of herbal medicines is necessary when considering them as a drug [47, 48]. In the current study, the sub-chronic 28-day oral toxicity study of the *Dianthus orientals* A. demonstrated no adverse clinical signs or negative influences on behavior and mortality in the treatment groups. Meanwhile, a gradual and significant ($p < 0.05$) increase in the body weight of all treated DOE groups was observed compared with day 0.0. As the body weight of laboratory animals is an indicator during *in vivo* studies to assess the animals' overall health [49], our outcomes confirmed that *Dianthus orientals* A. is safe to be used orally for human ailments after more intensive investigations of its biological, chemical, and medicinal properties. These outcomes also were reported for other plant species of genus dianthus that not produced toxicity after oral treatment at various concentrations for a stipulated time, including *Dianthus helenae* Vvad. in Uzbekistan (oral administration of 500–3000 mg/kg in mice and rats/14 days) [13] and *Dianthus basuticus* in South Africa (oral administration of 100–3200 mg/kg in rats/14 days) [50]. However, the latter study reported anorexia after 28 days of treatment of animals with oral plant crude extract at doses of (200, 400, and 800 mg/kg).

Estimating some haematological parameters plays a vital role in assessing the toxic effects of test substances [51]. There was no significant difference ($p \geq 0.05$) in WBC counts, RBC counts, and Hb between the DOE-treated groups and the negative control group, indicating that the DSE did not affect the circulating blood cells of the tested animals. Meanwhile, the HCT fluctuated significantly, and PLT decreased significantly ($p \leq 0.05$) in all treated groups. In this respect, we could not find any species of Dianthus in the literature to pose antiplatelet activities. Thus, we compared our outcomes to another plant species. Subsequently, these findings agreed with that found in other studies for some plant species, including *Evodia rutaecarpa*[52], *Scolopendra subsidies* mutilans [53], and *Dactylicapnos torulosa*[54]. The exact antiplatelet mechanisms are still unclear. However, different studies worldwide focus on medicinal plants with antiplatelet activity, and the same bioactive molecules for a significant number of these

investigations are unknown [55–57]. Based on the literature, the alkaloids isolated from various sources provoked antiplatelet activity, which is generally mediated through multiple mechanisms, different from aspirin, a cyclooxygenase inhibitor [58].

The effect of the methanolic extract of *Dianthus orientals* A. on the kidneys was investigated by measuring the serum level of urea and creatinine, which are specific indicators of kidney function. We found that the mean urea level significantly lowered ($p = 0.012$) in all DOE-treated groups, and the creatinine level was non-significantly ($p = 0.557$) increased in all treated groups. In this regard, another study in South Africa concluded that urea/creatinine was not altered after 14 days of oral treatment with 200–800 mg/kg of *Dianthus basuticus*[50].

Liver function tests help determine the toxicity effects of plant extracts, as the liver is involved in the excretion of foreign substances and detoxification [59, 60]. In this study, ALT level was significantly lowered ($p = 0.009$) in low and median DOE treatments, while AST levels were significantly decreased in all doses. However, ALP levels increased dramatically in median amounts but significantly reduced in low/high doses. At the same time, the mean value of TP and albumin increased dramatically in DOE low and high-dose groups.

Generally, ALT functions to convert alanine into pyruvate for cellular energy, AST functions on the metabolism of amino acids, and ALP functions to break down proteins. Thus in this study, the fluctuations in the concentrations of serums ALT, AST, and ALP are biologically irrelevant as there was no dose-response relationship because rats in the highest dose group were not affected. These findings agreed with another study for *Dianthus basuticus* in Wister rats [50]. Regarding the electrolyte levels, there was a fluctuation in serum calcium, potassium, phosphate, and chloride levels without significant differences ($p \geq 0.05$).

The histopathological observations of the examined tissues confirmed the liver and kidney findings. At high doses of DOE, severe liver (vacuolar and hydropic degenerations) and kidney (tubular atrophy) injuries were observed, which indicates that *Dianthus orientalis* A. is not safe to be used at 900 mg/kg for four consecutive weeks or more. Otherwise, all found histopathological alterations were mild and are not considered an indication of toxicity of the crude methanolic extract of this plant species. These findings agreed with that found in another study for *Dianthus basuticus* in Wister rats [50], which mentioned no treatment-related microscopic changes were observed in the tested organs.

Conclusions

Many precious phytochemical compounds are available in *Dianthus orientalis* A. that might reflect this plant's various biomedical, pharmacological, and biological properties. Also, multiple doses of the studied plant have not produced adverse effects in the treated animals for a given period that contraindicate its use in the future when planned to be a medical candidate to treat human ailments.

Declarations

Ethics approval

The proposal and study protocol were revised and approved by the scientific and ethical committees of the College of Medicine, University of Sulaimani, Iraq (No. 215/17/10/2021/UoS). All methods were performed in accordance with guidelines of Directive 2010/63/EU in Europe. Measures were taken to minimize pain and discomfort.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request

Competing interests

There is no conflict of interest related to this study.

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Authors' contributions

VAA: Methodology, data collection, data analysis, prepared tables and figures, written the original manuscript. HSR: Conceptualization, supervision, study registration, written the original manuscript. MOM: Supervision, resources, study administration, edit, revise, and correct the original manuscript. All authors agreed to submit the manuscript.

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Figures

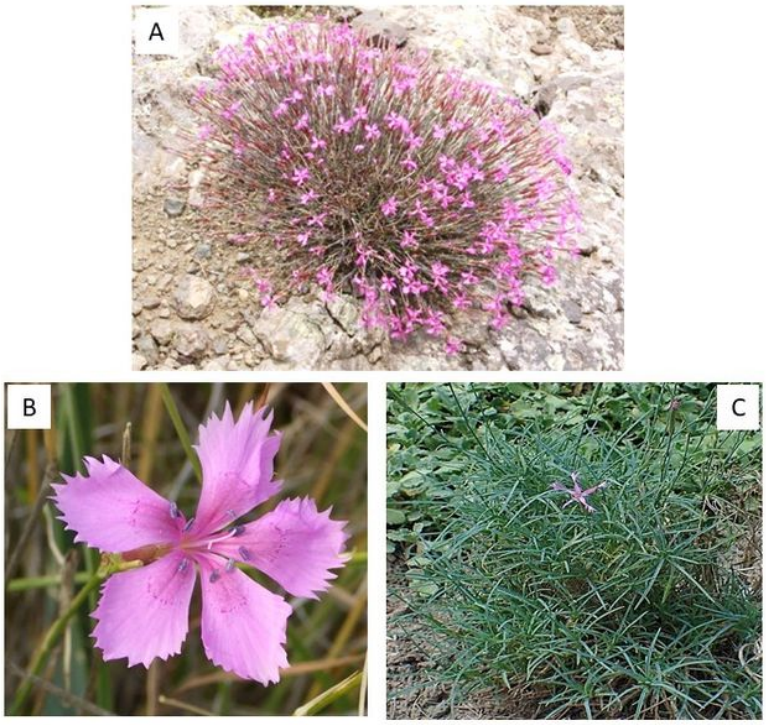

Figure 1

Dianthus Orientalis Adams whole plant (A), flower (B), and leaves (C).

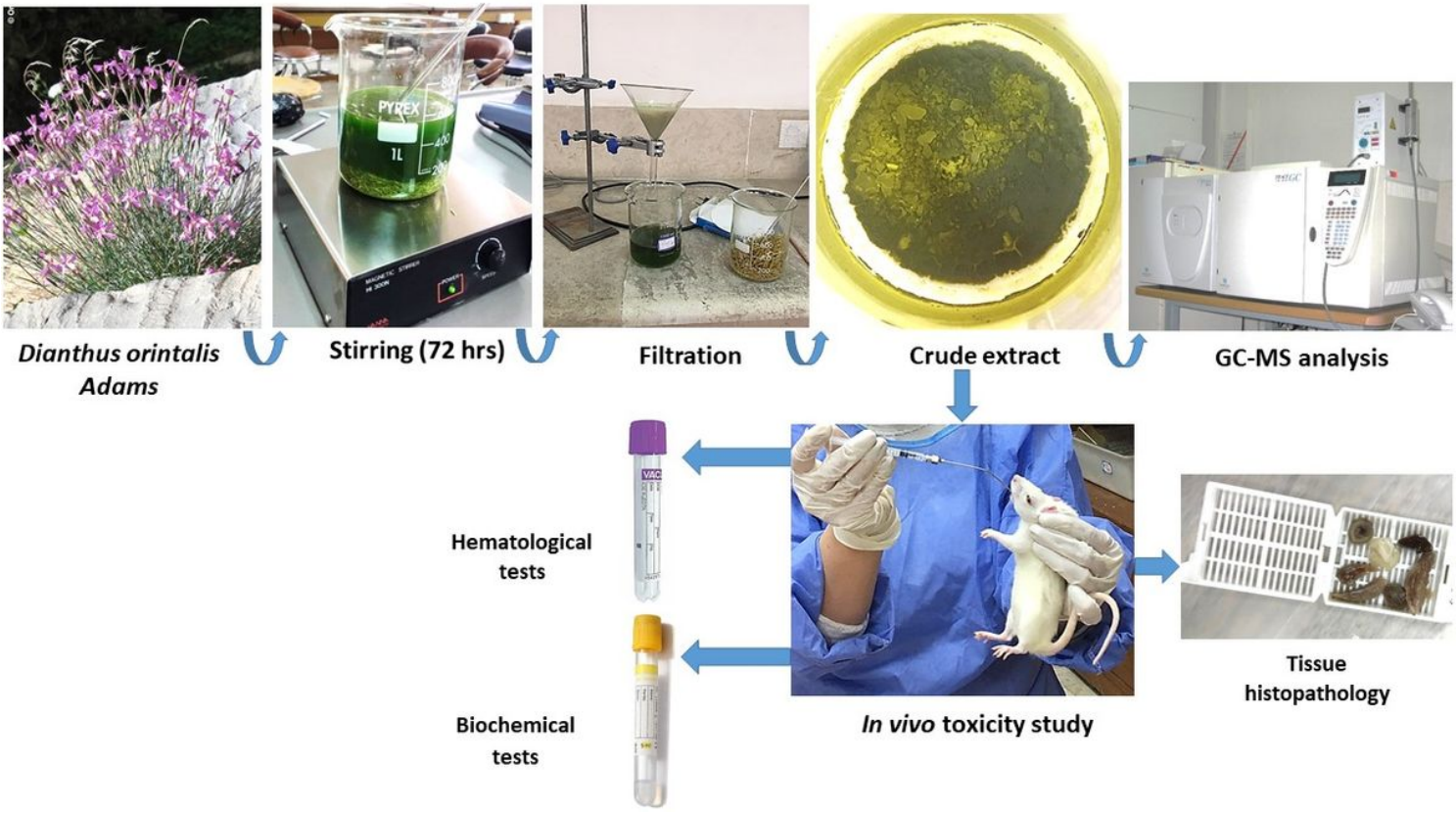

Figure 2

Dianthus orientalis Adams extraction, analysis, and *in vivo* study.

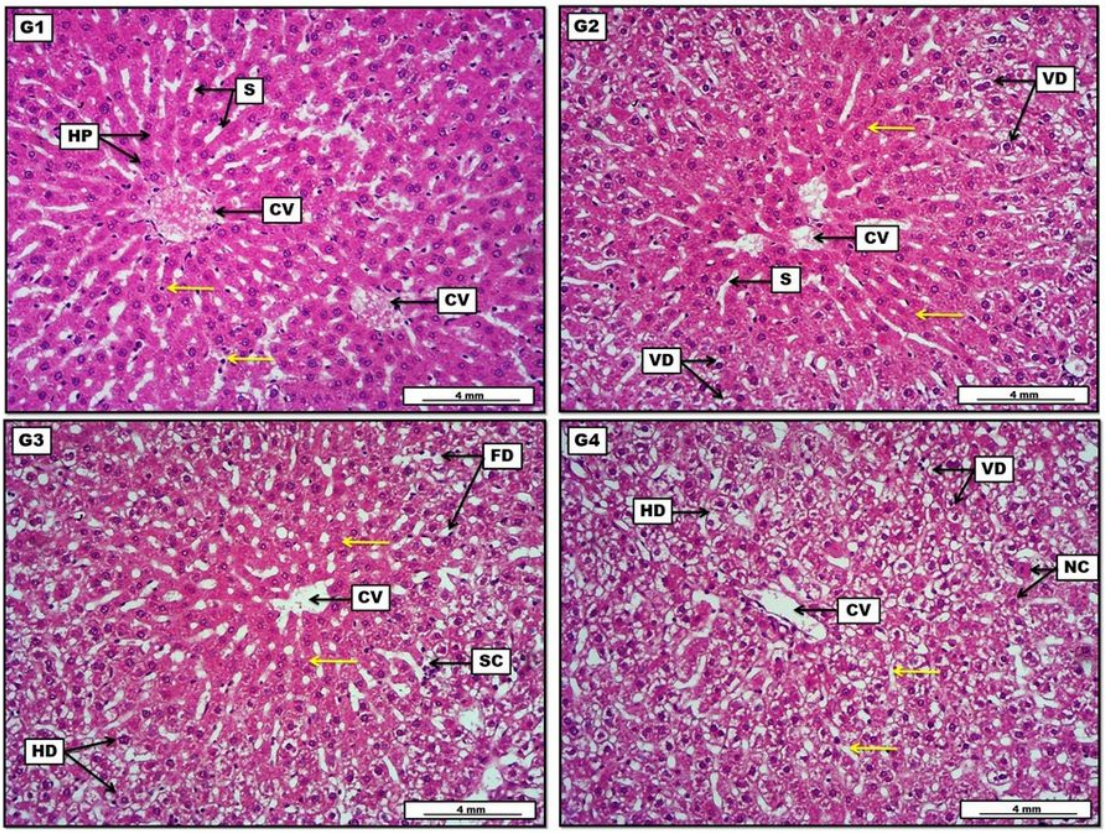

Figure 3

Photomicrograph of liver from; **G1:** Control negative group received normal tap water, liver section reveals no obvious pathological lesions, signified by typically arranged radiated hepatocytes (HP) around the mildly congested central vein (CV) with distinctive sinusoidal capillaries (S) and few scattered Kupffer cells attached to the capillary wall. **G2:** Low dose group treated with 300 mg/kg of *Dianthus orientalis* Adams, demonstrates moderate vacuolar degeneration (VD), which distributed throughout the given section. Moreover, the section reveals some area of typically radiated hepatocytes (yellow arrows) around the CV together with normally appeared sinusoidal capillaries (S). **G3:** Medium dose group treated with 600 mg/kg of *Dianthus orientalis* Adams, elucidates the presence of moderate to severe fatty degeneration (FD), together with hydropic degeneration (HD). On the other hand, the section shows ordinarily radiated hepatocytes column (yellow arrows) around the typical CV with some sinusoidal capillaries (SC). **G4:** High dose group treated with 900 mg/kg of *Dianthus orientalis*Adams, demonstrates the severe vacuolar and hydropic degenerations (VD, HD), together with some necrotic eosinophilic cells (NC). Narrowing of some sinusoidal capillaries (yellow arrows). Disturbed morphological arrangement around the CV with no apparent vascular congestion. H&E staining, scale bar of 4.0 mm.

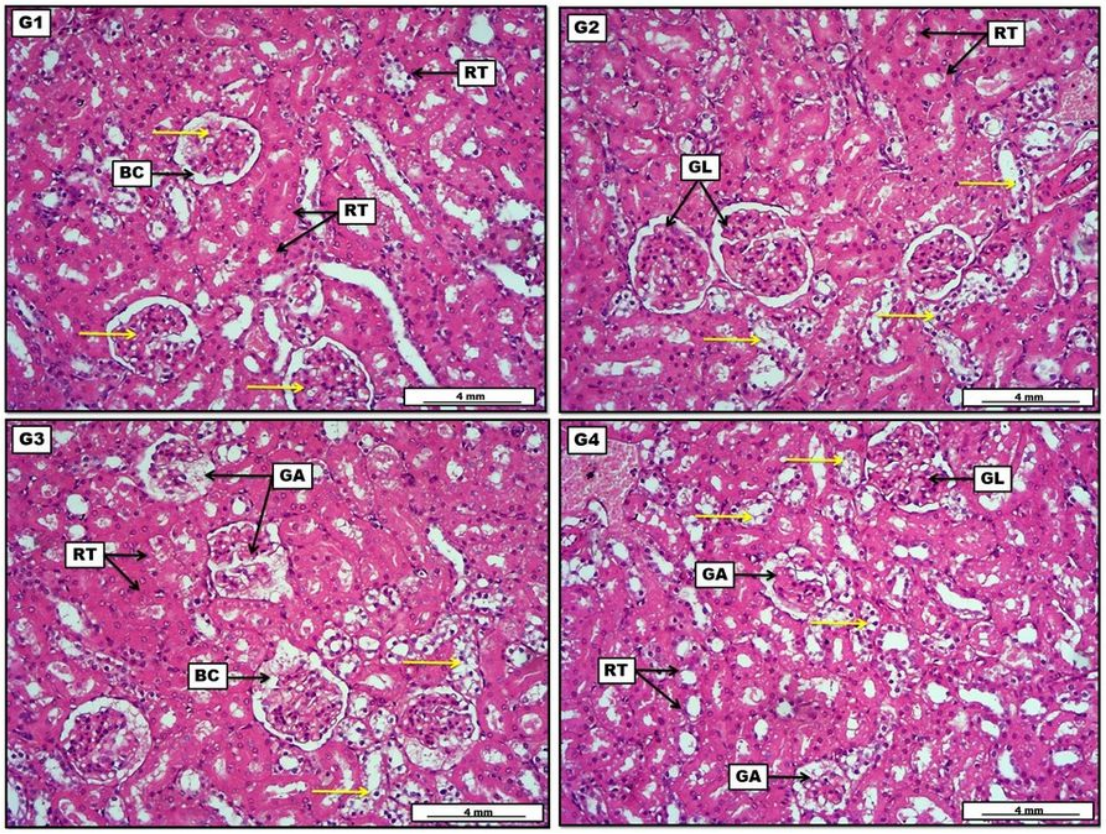

Figure 4

Photomicrograph of kidney from groups; **G1:** Control negative group received normal tap water, reveals no prominent lesions, evident by typical small glomeruli (yellow arrows) and normal Bowman's capsule (BC). Renal tubules (RT) display no significant lesions except for slight and non-significant cellular swelling. **G2:** Low dose group treated with 300 mg/kg of *Dianthus orientalis*Adams. Renal tubular epithelia show non-significant and moderate vacuolar cellular swelling (yellow arrows). The section also reveals many areas of typically appeared renal tubules (RT) together with renal glomeruli (GL) with no obvious morphological changes. **G3:** Medium dose group treated with 600 mg/kg of *Dianthus orientalis* Adams, shows moderate and significant hydropic degeneration (yellow arrows) within the renal tubular epithelium. The section also reveals moderate glomerular atrophy (GA) evident by moderate flaring of BC. **G4:** High dose group treated with 900 mg/kg of *Dianthus orientalis* Adams, reveals severe glomerular atrophy (GA) and collapse. Together with the presence of significant cellular swelling within the renal tubular epithelia (yellow arrows). Moreover, other areas of the given section, shows normally appeared RT as well as some regular glomeruli (GL). H&E staining, scale bar of 4.0 mm.

Supplementary Files

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