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Evaluation the Biochemical, Enzymatic, and Immunological activity of *Viola odorata* methanolic extract on methotrexate treated albino male mice

Sura Alaa Saud

Department of Microbiology / College of Science / Al-Karkh University of Science / Baghdad- Iraq
Corresponding author: sura.alaa88@yahoo.com
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Abstract

Traditionally medicinal plants are used for its potential chemotherapeutic action and for safety upon the continued use of these plants. Many medicinal plants are containing great bioactive components that reported to preventive and therapeutic role on different body organs. Violet (*Viola odorata*) is a medicinal plant that had different groups of compounds like cyclotides, flavonoids, alkaloids, triterpenoids, violin, quercitin, steroids, tannins, and saponins) which possessed different biological activity such as antifungal, antibacterial, anticancer, antioxidant, antiasthmatic. This study focused the line on the effect of different concentration of *Viola odorata* extract on blood cholesterol, triglyceride, total protein, albumin, creatinin, and immunological effect (IgM, IgG, IgA) in serum of white albino male mice as well as, toxic effects of methotrexate drug. the results indicated that *Viola odorata* had the ability to counteract the toxic effect on all parameters studied due to presenting of flavonoid compound (isorhamnetin and luteolin) belong to flavonols class which is well reported to have hepatoprotective activity and had high detoxification effect against MTX drug. Also, a substantial effect which explained the ability of plant extracts to interfere with the MTX and prevented its harmful effect. The plant is safe to be used as an effective remedy against different xenobiotic.

Keywords: Medicinal plant, *Viola odorata*, Antioxidant, Hepatoprotective, Methotrexate, Immunoglobulin.

Introduction

It was assessed that about 25% of altogether up-to-date medicines are directly or indirectly derived from advanced plants (1). Plants are deliberated to be the environment's green drugstore, which offer medicines to maintain decent fitness and to re-establish the deteriorating healthiness of humans (2). A therapeutic plant is slightly plant, which in one or more of its tissues contains active elements which able to be castoff for therapeutic determinations or contain foundation complexes that able to using for the production of valuable treatments. That produces definite physiological action on the human body (3). One of these medicinal plants is viola odertera

Viola odorata is plants belong to genus Viola instinctive growing in Europe and Asia, and then familiarised to North America and Australasia. It's frequently famous as wood violet (4). The curative plant *Viola odorata* Linn belongs to family violaceae, is a generally known as Banafshah and sweet violet in Asia and Europe respectively

V. odorata are largely used for therapy for coughs and sore throat, croakiness and tonsillitis. *V. odorata* esteemed as a cough medicine, diaphoretic, antipyretic, and antibacterial, diuretic and as a purgative, ad have other friendliness (5). It have be used either unaided or in combination with anther basils for catarrhal and respiratory scrapes and for calculous regards, It have different groups of compounds like cyclotides, flavonoids, alkaloids, triterpenoids, violin, quercitin, steroids, tannins, and saponins) conventional as antifungal, antibacterial, anticancer, antioxidant, antiasthmatic, anti-inflammatory, anti-HIV and antipyretic agents Effective for the management of headaches, migraine and visominia (6).

This study aimed to assess the effect of different concentration of *Viola odorata* extract on the concentration of blood cholesterol, triglyceride, total protein, albumin, creatinin, and immunological effect (Igm, Igg, and Iga) sera of white albino male mice.

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Materials and Methods

Plant collection and identification

The aerial parts of plant were collected from the local markets of Baghdad| Iraq during September (2020), which had been previously identified by National Herbarium of Iraq.

Preparation of plant extract

Plant parts were cleaned with deionized water and dried at shade for a week, then, they were cut into minor pieces; shade dried and grounded using a coffee grinder. The ground material was relocated and extracted in the soxhlet extractor using 80% methanol for 72 h (7). The extract was filtered through a Whatmann filter paper No. 3 and concentrated using a rotary evaporator in the water bath which was set at 40°C (8). The powdered residue were transferred into vials and stored at 4°C in airtight vials before biological analysis.

Laboratory animals and sample collection:

A total of 20 albino male mice were purchased from Biotechnology Research Centre/ Al-Nahrain University/ Baghdad\ Iraq. Weighting 25-30 gm. aged of 2-3 months was used in this study. They were divided into five groups, each group contain 4 animal, all animals were housed per cage with ad libitum access to water and food pellets. The firs group treated with 100 mg / kg /day of *Viola Odorata* extract, the **second** group treated with 200 mg / kg / day of *Viola odorata* extract. The third which have had received 40 mg / kg /day of Methotrexate drug**.** The four group included interaction between MTX drug 40 (mg / kg) and plant extract at dose of 200 (mg / kg) of Viola *odorata* extract. And the five groups was the control group which had not received any material, Each investigated group was injected intraperitoneally (i. p.) with a single dose per day (0.1mL, The mice were killed by cervical decapitation after 7 days of treatment (scarified at day 8) (9).

The samples of collected blood were left for 15 minutes at room temperature for clotting, and then centrifugation by cooling serum collected. The serum measurements at the same day of collection of total cholesterol, triglyceride, total protein, albumin, and creatinin, and immunological test (IGM, IGG, and IgA) in serum of white albino male mice were done in control and treated animal groups.

Table (1): Laboratory tests and number of animals in the investigated groups of experiments

No	Groups	Tested material	Dose (mg/kg)	Laboratory Tests and Number of Animals
.1	Group (I)	Viola *Odorata*	100	4
.2	Group (II)	Viola *Odorata*	200	4
.3	Group (III)	Methotrexate Drug	40	4
.4	Group (IV)	Viola *Odorata* + Methotrexate	40+ 200	4
.5	Group (V)	Control Group	------	4
Total of animal				20

Assay methods:

Total cholesterol was determined in serum by enzymatic colorimetric method using kit cholesterol MR CE (linear chemicals S.L.).

Triglyceride was measured by using kit CE triglyceride GPO-POD Enzymatic colorimetric method (spinreact S.A.U.). The intensity of the color formed is proportional to the triglyceride concentration in the sample.

Total serum protein was measured by Biuret reaction using kit method CE colorimetric method for total protein (linear chemicals S.L.).

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Serum albumin was measured by kit CE colorimetric method of albumin (linear chemicals S.L.).

Serum creatinin concentration (CK) was estimated kit (Agappe diagnostics LTD in india) (10).

1. Statistical analysis;

Statistical analysis selected were mean ± SD coefficient of varation (CV) and un paired students test (11).

Result and Discussion

1. Biochemical tests

Table 2 show the result of effect of different concentration of on different biochemical assays including and proteins in sera albino male mice.

Table 2: The mean± SD values of Serum Total Cholesterol, Triglyceride, total protein, creatinine, albumin, and concentration in groups of treated and untreated mice

Treated Groups	Doses (mg\k)	Total Cholesterol (mg\dl) Mean± S.E.	Triglycerides (mg\dl) Mean± S.E.	Total Protein (g\dl) Mean± S.E.	Creatinine (mg\dl) Mean± S.E.	Albumin (g\dl) Mean± S.E.
Viola Odorata (I)	100	158.1667 ^{c} ± 1.73333	106.6333 ^{b} ± 1.79939	7.9100 ^{b} ± 0.16773	1.3100 ^{cd} ± 0.1528	5.3067 ^{b} ± 0.08647
Viola Odorata (II)	200	142.6667 ^{d} ± 1.74770	98.4167 ^{c} ± 1.53361	8.4633 ^{a} ± 0.17947	1.2267 ^{d} ± 0.02333 ^{d}	6.0033 ^{a} ± 0.08570
Methotrexate Drug (III)	40	199.1667 ^{a} ± 1.03665	138.5100 ^{a} ± 2. 62930	4.4267 ^{d} ± 0.20899	1.7633 ^{a} ± 0.02906	3.5267 ^{d} ± 0.08090
Viola + Methotrexate(IV)	40+ 200	162.3000 ^{bc} ± 2.215716	171.1433 ^{b} ± 2.51119	6.9167 ^{c} ± 0.11348	1.4067 ^{b} ± 0.0764	4.2067 ^{c} ± 0.08413
Control Group (V)	-------	164.8667 ^{b} ± 2.94637	102.0733 ^{bc} ± 1.24239	7.8600 ^{b} ± 0.09644	1.3933 ^{bc} ± 0.04842	5.1433 ^{b} ± 0.08762

a. Determination of total Cholesterol

As shown in figure 1, the result of determination of total cholesterol showed good result for effect of different treated groups of Viola Odorata in lower the concentration of cholesterol in albino male mice serum, Also administration of MTX Drug 40mg| kg recorded *(*199.2^{a} ±1.0), followed by MTX Drug *40mg| kg* and viola extract 2.00 that recorded (162.3^{bc}±2.2) as compared with result of control that recorded (164.9 ^{b} ±3.0), the viola (100 mg| kg) recorded (158.2 ^{c}±1.7), and finely the viola extract at concentration (200 mg| kg) that recorded (142.7 ^{d} ± 1.7).

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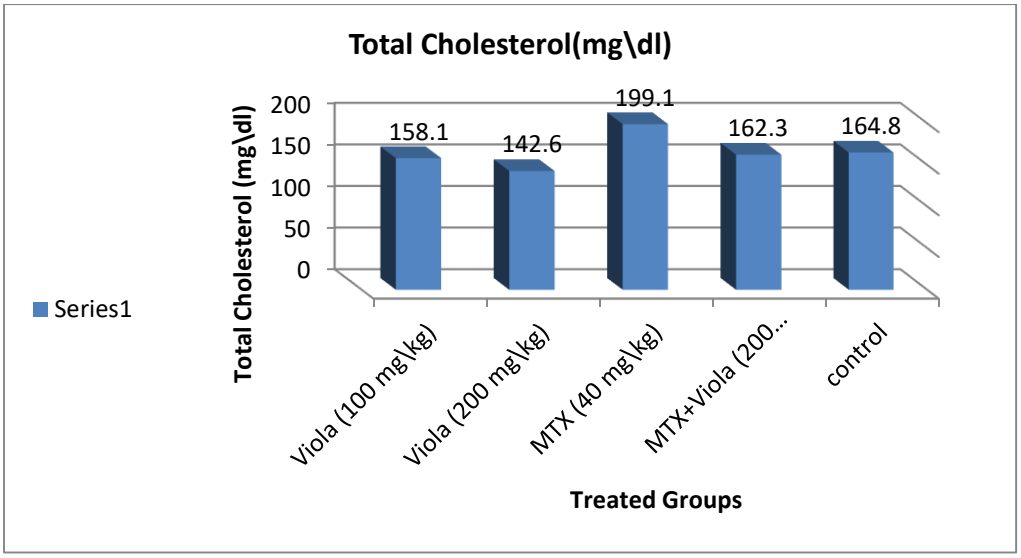

Figure 1: Effect of *viola odorata* extract on total Cholesterol

b. Determination of triglyceride concentration

The result of effect of different treated groups of *Viola Odorata* on triglyceride of in albino male mice serum recorded best result for MTX Drug and viola (40+200 mg\kg) respectively, with value reached to (171.1 ^{b}±2.5), for *voila odertea* extract 100 (mg / kg) the result recorded 106.6333 b±1.79939, and for voila odertea extract 200 (mg / kg) the result recorded (98.4c±1.5), and recorded (138.5 a±2. 6) for MTX drug 40 (mg / kg).

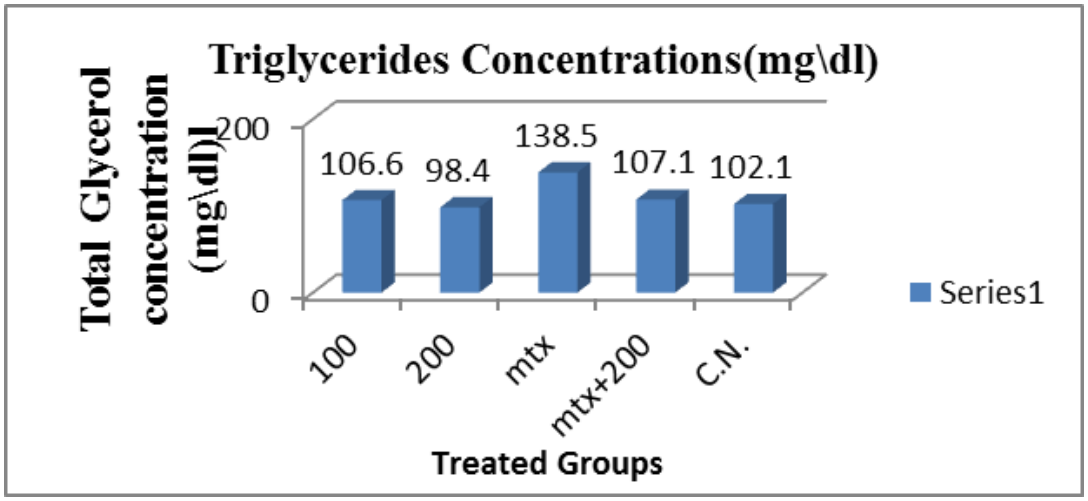

Figure 2: Effect of *viola odorata* extract on triglyceride concentration

c. Determination of total protein concentration

The result of determination of total protein concentration showed best activity of *Viola Odorata* extract (200mg| kg) in total protein concentration with value reached (8.5^{a}± 0.1), followed by viola (100 mg| kg) with value ranged (7.9 ^{b}±0.1) as compared with control that recorded (7.9^{b}± 0.1), followed by the (MTX Drug + Viola) with concentration (40+200 mg\kg) that recorded (6.9 ^{c} ± 0.1), and finely the MTX Drug (40 mg\ kg) that recorded (4.4 ^{d} ± 0.2), as in figure 3.

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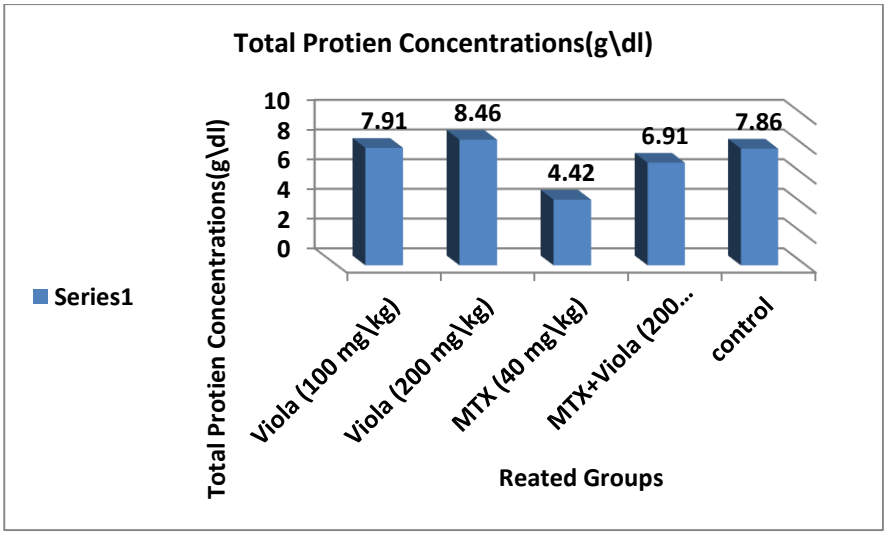

Figure 3: Effect of *viola odorata* extract on total protein concentration

d. Determination of creatinin concentrations

As show in figure 4, the effect of different treated groups of *Viola Odorata* on albino male mice serum is different with type of enzymes or proteins that need to measurement their concentration, in the figure (4), the MTX Drug (40 mg\ kg) recorded the highest value (1.8 ^{a} ± 0.02), followed by MTX Drug + Viola (40+200 mg\kg), that recorded (1.4 ^{b}± 0.8) and control that recorded the convergent value with (MTX + Viola) with value reach to (1.4^{bc} ± 0.04), followed by viola (100mg|kg) that recorded (1.3^{cd} ± 0.6) and finely the viola extract (200 mg | kg) that recorded (1.2^{d} ± 0.02^{d}).

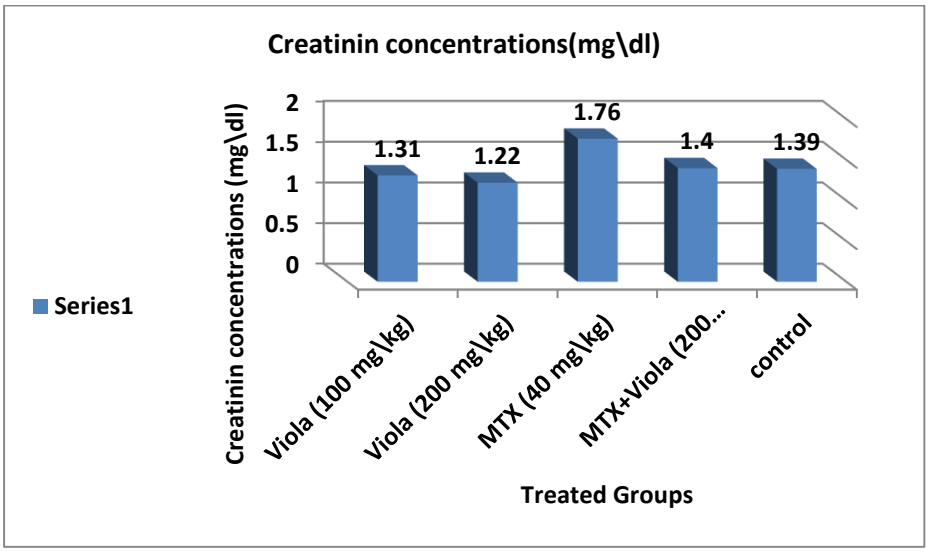

Figure 4: Effect of *viola odorata* extract on creatinine concentrations

e. Determination of Albumin concentrations

In this test there is un evident effect of viola extract (200 mg |kg) and viola extract (200 mg |kg) on Albumin concentration in albino male mice, in valio ranged from (6.0^{a} ± 0.9) to (5.3^{b} ± 0.9), followed by the MTX Drug + Viola extract (40+200 mg\kg), that recorded (4.2^{c} ± 0.9) and finally MTX Drug (40 mg\ kg) that give the value ranged (3.5\$^{d}$ ± 0.9).

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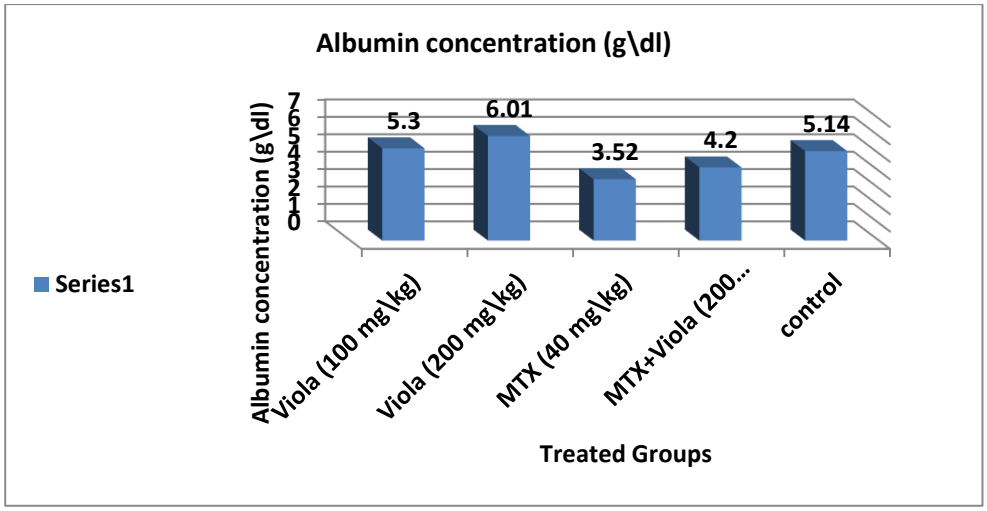

Figure 5: Effect of *viola odorata* extract on Albumin concentrations

2. Immunological test

The Viola Odorant treated gropes can also studied for their activity on their albino male mice serum, the Viola extracts give good result by increasing titter of immunoglobulin, as show in table 2.

Table 2: The mean± SD values of Serum Total immunological factor (IgM, IgG, and IgA) (mg\dl) and concentration in groups of treated and untreated mice

Treated Groups	Doses (mg\kg)	IgM (mg\dl) Mean± S.E.	IgG (mg\dl) Mean± S.E.	IgA (mg\dl) Mean± S.E.
Viola Odorata (I)	100	94.9333 ± 4.25846^{b}	1038.3333 ± 23.52731^{b}	238.8333 ± 15.60271^{b}
Viola Odorata (II)	200	131.1000 ± 4.78156^{A}	1279.5333 ± 38.63523^{a}	291.4333 ± 6.32201^{a}
Methotrexate Drug (III)	40	41.3333 ± 4.55790^{d}	218.4000 ± 22.84871^{d}	86.3333 ± 6.48854^{d}
Viola Odorata(II) + Methotrexate(IV)	40+ 200	86.7333 ± 2.54056^{c}	639.3333 ± 50.02367^{cd}	136.4333 ± 4.79247^{c}
Control Group (V)	---------	70.1667 ± 3.91847^{bc}	761.9000 ± 15.47331^{b}	218.6000 ± 5.68712^{bc}

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a. Determination of IgM concentration

Viola Odorata (200 mg |kg), have good effect on immunoglobulin types in albino male mice, that can be proved in this result, it give best value ranged (131.1±4.8[a]), and Viola Odorata (100 mg |kg), that recorded (94.9±4.3[b]), followed by control that recorded convergent value (70.2±3.9[bc]), and MTX+viola (40=200mg|kg) that recorded value (86.7±2.5[d]), the MTX drug give value ranged (41.3 ± 4.6).

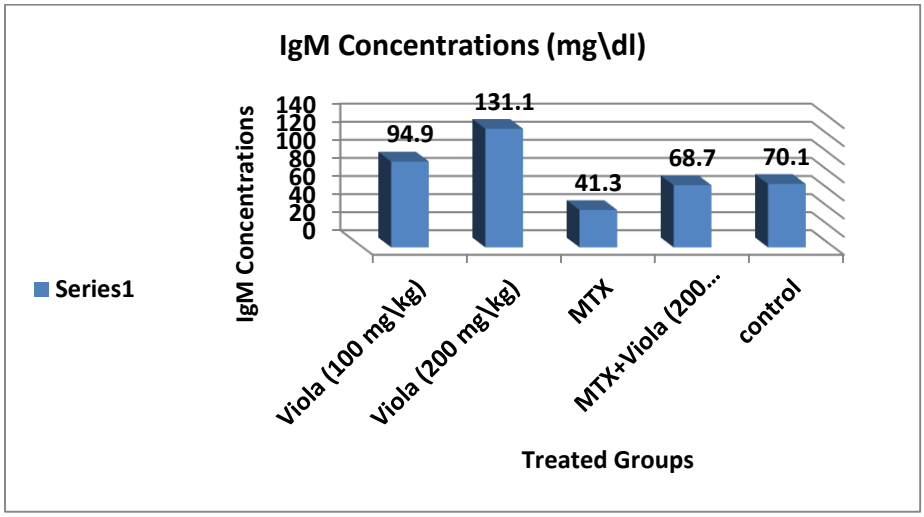

Figure 6: Effect of *viola odorata* extract on IgM concentrations

b. Determination of IgG concentration

The figure 6 show that the effect of *Viola Odorata* on IgG in albino male mice serum, the result show that the *Viola Odorata* (200 mg |kg), have good effect on IgG concentration, with value (1279.5± 38.6[a]), followed by *Viola Odorata* (100mg |kg) , with value (1038.3± 23.5[b]), and control groups that gave value (761.9± 15.5[b]), the Methotrexate Drug also have good effect with value ranged (218.4± 22.8[d]), and finely Viola 200 + Methotrexate40 (mg\ kg) that give value(639.3± 50.02[cd]).

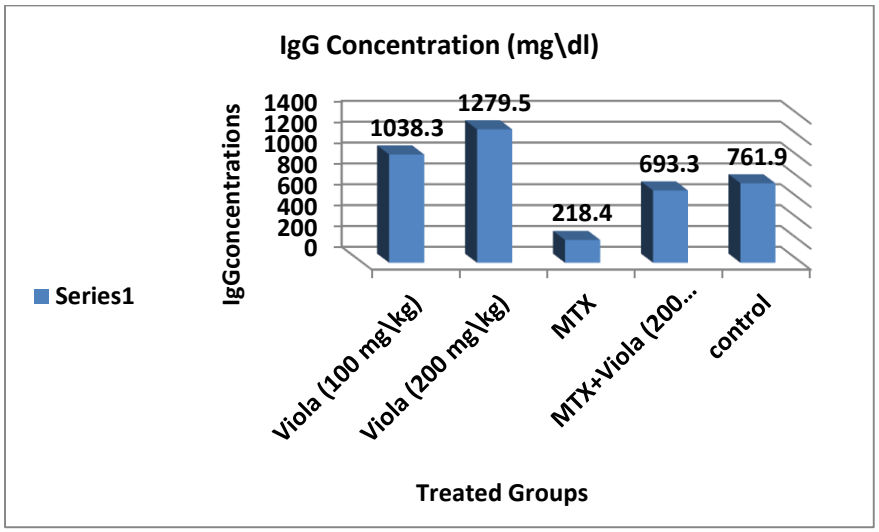

Figure 7: Effect of *viola odorata* extract on IgG concentrations

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c. Determination of IgA concentration

In figure 6, the *Viola odorata* (200 mg |kg), also have good effect on IgA concentration in albino male mice serum, that give good index of effect of viola extract on immunoglobulin types in albino male mice, the value ranged (291.4±6.3^{a}), followed by *Viola odorata* (100mg |kg) , with value (238.8± 15.6^{b}), the control recorded value ranged (218.6±5.7^{bc}),the Viola 200 + Methotrexate40 (200+ 40 mg\ kg) give value ranged (136.4± 4.8^{d}) and finely the MTX drug that recorded value (86.3± 6.5^{d}).

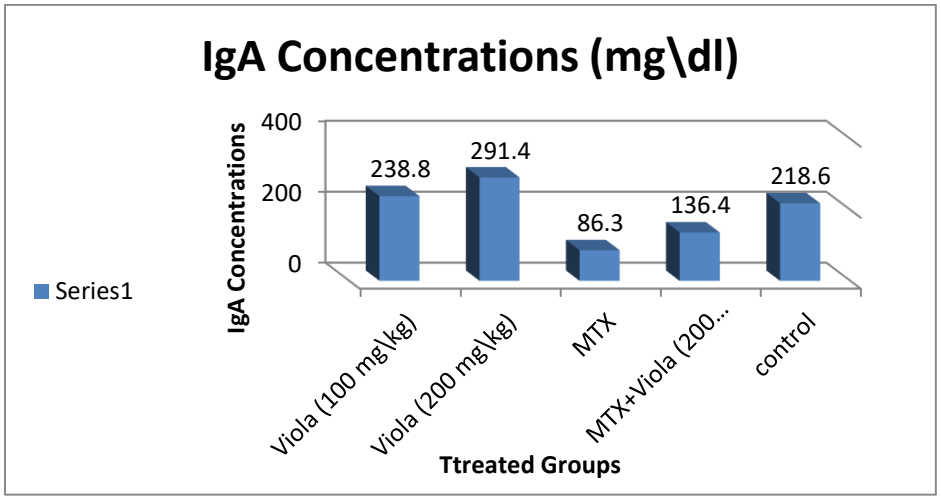

Figure 8: Effect of *viola odorata* extract on IgA concentrations

Discussion

Plants were used in traditional medication due its containing a huge collection of ingredients or active compounds (secondary metabolite) which used to delight durable or transmittable illnesses. Certainly, it using in considerable of the pharmacopeia in medicine was consequential from the herbal lure of natural people (12). this needed controlled to the detection of a large quantity of novel medications, and no medication ingredients. *Viola odorata* (Banafshah) contains numerous phytochemical institutes (alkaloids, violin, quercetin, steroids, tannins, flavonoids and saponins) in above ground parts of it (13).. *Viola odorata* is identified to have durable antiinflammatory and diuretic possessions and stimulate circulatory and immune system because flavonoids (one of the secondary metabolite presented in *viola odorata*) that decrease oxidative strain by right searching free-radicals, due to its ability to interfere through free-radical making appliances therefore; aggregate the role of endogenous antioxidants (14). In addition, flavonoids have stimulated other antioxidants with immune-enhancing action, such as vitamin e (15). And carotenoids (16). These plants similarly stated for using in liver protests in addition to, exhibited vital hepato protecting potential due to for antioxidant and membrane conservation end result (17). , In traditional medications custody in opinion the usage alongside flavonoids and polyphenolic insides of Viola species with ingrained character in contesting oxidative tension along with hepatotoxicity (18) . Accordingly, flavonoid complex (isorhamnetin and luteolin) were fit in flavones session with it testified to hepato protective commotion also present in *Viola odorata and* had high detoxification effect against MTX drug. Also, a substantial effect which explained the ability of plant extract to interfere with the MTX and prevented its harmful effect agreed with the result of other researchers who suggested that the drug generates DNA double strand breaks through its effect on the formed topoisomerase II cleavable complex, and such effect of plant mimics the action of ionizing radiation.

Also, flavonoids might production significant persons in signalling particles in mammals, over its capability to co-operate with a varied series of protein kinases, including mitogen-activated protein kinases (MAPK) that supplant significant paces of cell evolution and variation (17). this possessions are realized concluded numerous metabolic passageways, for requests, prevent the creation of free radicals, overpower chain opening and/or contravention chain proliferation reaction, accumulative the motion of cleaning

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enzymes such as glutathione transferase (GST) and superoxide dismutase (SOD) and remove anti-oxidant and version when the indicator for the creation and retort of free radicals development and transference of the anti-oxidant to the accurate position. It is actually significant to reference that, phenolic complexes have right antioxidant achievement, since for their redox possessions, which permit to their performance for instance plummeting factor, hydrogen givers and singlet oxygen quenchers (18). Finally, *viola odorata* had the ability to protect diverse tissues against free radicals deleterious effects which resulted to cytokine activation. This result was agreed with (19) showed that feeding animals with little alimental altitudes of Ammi visnaga and Artemisia herba-alba (Ammi visnaga) (Khella, AV) for three weeks with no any variations in serum AST, cholesterol and total lipids, while feeding on 2% AV for 6 or 9 weeks increased serum AST, cholesterol and decreased serum total lipid. (20).

Conclusion

viola odorata is safe to be used as an effective remedy for different diseases without side effect *V. odorata* is generally used as remedy for coughs and sore throat, hoarseness and tonsillitis. *V. odorata* is valued as an expectorant, diaphoretic, antipyretic, and antibacterial, diuretic and as a laxative, in bilious affections.

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تقدير فعالية الكيمياء الحياتية ، الانزيمية والمناعية للمستخلص الميثانولي لنبات ورد البنفسج على ذكور الفئران البيض المستحثة بعقار الميثوتروكسيت

سرى علاء سعود

قسم الاحياء المجهرية / كلية العلوم / جامعة الكرخ للعلوم / بغداد - العراق
Corresponding author: **sura.alaa88@yahoo.com**

الخلاصة

تستخدم النباتات الطبية تقليدياً كعلاج كيميائي بسبب التاثير الامن عند استخدام هذه النباتات. تحتوي العديد من النباتات الطبية على مكونات حيوية كبيرةاسهمت في دورها الطبي. واحد من هذه النباتات الطبية هو نبات البنفسج والذي يمتلك تاثير وقائي وعلاجي على أعضاء الجسم المختلفة. يحتوي البنفسج على العديد من المركبات مثل السيكلويدات، الفلافونويدات، قلويدات، triterpenoids، quercetin، وغيرها و التي تمتلك نشاط بيولوجي مختلف مثل مضادات الفطريات، المضادة للبكتيريا، مضاد للسرطان، المضادة للأكسدة، المضادة للاكساثات. ركزت هذه الدراسة على دراسة تأثير تراكيز مختلفه من مستخلص النبات على نسبة الكوليسترول في الدم، الدهون الثلاثية، البروتين الكلي، الكرياتينين، والتأثير المناعي (IgM، IgG، IgA) في مصل ذكور الفئران البيض ، والآثار السامة لعقار الميثوتريكسيت. أشارت النتائج إلى أن نبات البنفسج كان لديه القدرة على مواجهة التأثير السام على جميع المعلمات التي تمت دراستها بسبب احتواءه على مركب الفلافونويد التي تفيد بشكل جيد على نشاط الكبد وكان لها تأثير كبير على إزالة السموم ضد السموم. مستخلص نبات البنفسج هو آمن لاستخدامها كعلاج فعال ضد xenobiotics مختلفة.

الكلمات المفتاحية: النباتات الطبية، نبات الفيولا، مضادات الاكسدة ،التأثيرات الحامية للكبد ، عقار الميثوتروكسيت ، البروتينات المناعية.