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Research Article

Chemical Characterization of *Chelidonium Majus* Flowers with Hepatoroxicity Protective Study in Male Albino Rats

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ABSTRACT

The aim of this study was to determine the biochemical characterization of *Chelidonium majus* flowers and to assess its antitoxic activity against carbon tetrachloride induced hepatotoxicity in rats. Extract from *Chelidonium majus* flowers was prepared then its total phenolic and flavonoid content were analyzed. Moreover, the reducing power and radical scavenging activity were determined. Male albino Wistar rats were randomly allocated into four groups of ten each: control negative; control positive; treated medium dose; treated high dose. Treatment was carried out simultaneously, for 3 consecutive weeks, on all groups. Animals were sacrificed 48 h after the last injection then hepatic tissues were collected and routinely processed for histopathological examination. The total polyphenol and flavonoid contents of *Chelidonium majus* flowers extract were 87.53mgGAE/g and 9.45mgQE/g, respectively. HPLC analysis identified from polyphenolic and flavonoid compounds were estimated. The antioxidant activity by using (FRAP) of methanolic extract plant flowers was 1.467 at the concentrations of 80mg/ml. While the IC_{50} using (DPPH) was 0.41. Microscopic examination of hepatic tissues from treated rats with *Chelidonium majus* flowers revealed anti hepatotoxic activity with improved histological tissue changes compared with carbon tetrachloride induced hepatotoxicity group. Moreover, Fibrotic change was prevented with both medium and high doses, while near normal tissue condition with microsteatosis and few necrotic cells was noted with the high dose. In conclusion, *Chelidonium majus* flowers possess beneficial effect as antioxidant and anti-hepatic toxicity.

Key words: *Chelidonium majus*, Antioxidant, Hepatotoxicity, HPLC, Rats

INTRODUCTION

Since ancient times, medical plants and simpler herbal remedies have been used in all parts of the world for the treatment and alleviation of various ailments. Although the use of medicinal plants is as old as mankind itself, their controlled application, the isolation and characterization of active substances, started only in the early nineteenth century. It is a known fact that the extractive plant isolates and isolated active substances played a major role in the development of modern pharmacotherapy. Many of the isolated compounds are still used today, or they have served as a model for the synthesis of a large number of drugs. Veeresham (2012).

Chelidonium majus Linn is the only species of the tribe *Chelidonieae* belonging to the *Papaveraceae* family. Previous chemical studies of C. majus have reported the isolation of isoquinoline alkaloids such as chelidonine, chelerythrine, sanguinarine, berberine, coptisine, and dl-stylopine. Among them, chelindonine and protopine exhibited anti-tumor activity, and protoberberine showed antibacterial and antiviral activity, while sanguinarine and chelerythrine had anti-inflammatory activity. Zuo *et al.* (2008).

Biswas *et al*. (2008), have reported that ethanolic whole plant extract of *Chelidonium majus*, has been tested for its possible anti-tumor, hepato-protective and antigen toxic effects in *p*-dimethylaminoazobenzene (*p*-DAB) induced hepatocarcinogenesis in mice through multiple assays: cytogenetically, biochemical, histological and electron microscopical.

Aim of work: This study was aimed to investigate the effect of *Chelidonium majus* as Antioxidant and as anti hepatotoxicity.

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MATERIALS AND METHODS

Samples of *Chelidonium majus*, was kindly obtained from Agricultural Research Center, Mansoura, Egypt. Samples were air dried in the shade and ground into a fine powder. The powdered air dried flowers which were divided into dry part and four extracts.

The powdered air dried flowers were divided into dry part and methanolic extract. Air dried sample was extracted six times using 10 litters of methanol, then it was concentrated to nearly dryness under vacuum using rotary evaporator at 45ºC, which kept under 4ºC, until use according to Farid *et al.,* (2016). All tests were conducted in were obtained from Science Academy of Experimental Researches, Mansoura, Egypt.

Plant sample
Name: *Chelidonium majus* or greater celandine
Kingdom: Plantae
Family: Papaveraceae

Total phenolic contents

Total phenolic contents of air dried flowers were determined by using Folin–Ciocalteu reagent method according to Lin and Tang (2007), about 0.1g of air dried sample was dissolved, separately in 1 ml distilled water. Aliquots of 0.1 ml from previous solution was taken and mixed with exactly 2.8 ml of distilled water, 2.0 ml of (2% w/v) sodium carbonate and finally 0.1 ml of 50% (v/v) of Folin–Ciocalteu reagent was added. Mixture was incubated for 30 minutes at room temperature and the absorbance of the resulting color was measured at 750 nm against distilled water as blank, using a Spekol 11 (Carl Zeiss -Jena) spectrophotometer. For quantitatively determination a standard curve of gallic acid (0- 200mg/l) was prepared in the same manner. Total phenolic contents were expressed as milligram gallic acid equivalent (GAE)/g based on dry weight.

Total flavonoid contents

Total flavonoid contents of air dried flowers were determined calorimetrically using aluminum chloride as described by Chang *et al.,* (2002), about 0.1g of air dried sample was dissolved in 1ml of distilled water. Resulting solution (0.5 ml) was mixed with 1.5 ml of 95% ethyl alcohol, 0.1 ml of 10% aluminum chloride (AlCl3), 0.1ml of 1M potassium acetate (CH3COOK) and 2.8 ml of distilled water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415 nm against distilled water as blank, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. Quercetin was chosen as a standard of flavonoids for making the standard curve (0–50mg/l). The concentrations of total flavonoids contents were expressed as milligram quercetin equivalent (QE)/g based on dry weight.

Analysis of polyphenols by HPLC technique

Phenolic compounds were determined by HPLC according to the method of Goupy *et al.,* (1999), as follow; 5 g of plant powder sample was mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2µm Millipore membtrane filter then 1-3 ml was collected in a vial for injection into HPLC Agilent 1200 series equipped with auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quarter HP pump (series 1050). The column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. Phenolic acid standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by the data analysis of HEWLLET packed software.

Analysis of flavonoids by HPLC technique

Flavonoid compounds were determined by HPLC according to the method of Mattila *et al.,* (2000), as follow; 5 g of sample was mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2µm Millipore membtrane filter then 1-3 ml was collected in a vial for injection into HPLC Agilent 1200 series equipped with auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 254 nm and quarter HP pump (series 1050). The column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. Flavonoid standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by the data analysis of HEWLLET packed software.

Determination of reducing power, (FRAP)

Reducing power of methanolic flowers extracts was determined according to the method of Oyaizu., (1986), Extract (0–100mg) from each sample in 0.20 mol phosphate buffer, pH 6.6 (2.5ml) was added to 2.5ml potassium ferricyanide (10mg/ml), mixture was incubated at 50ºC for 20min. Trichloroacetic acid (TCA) (2.5ml, 100mg/ml), was added to the mixture then centrifuged at 650g for 10 minutes. The supernatant (2.5ml) was mixed with distilled water (2.5ml) and 0.5ml ferric chloride solution (1mg/ml) was added and the absorbance of the resultant color was measured using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer at 700nm. Higher absorbance of the reaction mixture indicated greater reduce.

Determination of (DPPH) radical scavenging activity

The DPPH free radical scavenging activity of plant Flowers extracts at different concentrations were measured from bleaching of the purple color of (2.2 Diphenyl -1-picryl hydrazyl) was based on the method of Pratap *et al.,* (2013). Exactly 0.1 ml solution of different concentration of extract was added to 1.4 ml of DPPH 0.1mM and kept in dark for 30 min. The absorbance was measured at 517 nm, using a Spekol 11 (Carl Zeiss -Jena)

spectrophotometer. And the percentage inhibition was calculated by using the following Equation.

Percentage inhibition (%) = (A Blank – A Test) / (A Blank) × 100

Animals

Forty males Albino Wistar rats weighing about 150-200 g obtained from the animal house of Faculty of Pharmacy, Mansoura University, Egypt. All mice were housed in microlon boxes in a controlled environment (temperature 25±20ºC and 12 h dark/ light cycle) with standard laboratory diet and water ad libitum. Animals allowed to acclimatizing for two weeks before starting the study.

Experimental design

The rats were divided into four groups, each group comprised of 10 rats as follows:

Group 1 (**control negative**): was injected with (1 mL kg) liquid paraffin;

Group II (**control positive**): received intra peritoneal carbon tetrachloride (CCl_4) injections twice weekly (1 mL kg) bw. as a 30% solution in liquid paraffin);

Group III (**treated medium dose**): received 62.5 mg kg bw. extract daily and CCl_4;

Group IV (**treated high dose**): received 125 mg kg bw. extract daily and $CC1_4$. Extract was administered via a stomach tube as a suspension in 0.25% agar, after vacuum dessication at room temperature. Treatment was carried out simultaneously, for 3 consecutive weeks, on all groups. Rats were euthanized at the end of the experimental period. The rats from each group were fasted overnight before being euthanized by decapitation.

Histological studies

After euthanizing, Specimens from liver were collected and fixed in formal saline 10% then washed, dehydrated, cleared and embedded in paraffin. The paraffin embedded blocks were sectioned at 4-5 micron thickness and stained with Hematoxylin and Eosin (Bancroft *et al.*, 2012).

Statistical analysis

Obtained data were analyzed using the statistical software package CoStat., (2005). All comparisons were first subjected to one way ANOVA and significant differences between treatment means were determined using Duncan's multiple rang test at $p<0.05$ as the level of the significance (Duncan, 1955).

RESULTS AND DISCUSSION

Total polyphenols and total flavonoids content

Total polyphenols include several classes of phenolic compounds that are secondary plant metabolites and integral part of human and animal diets. Flavonoids are large group of the phenolic compounds consisting mainly of flavonols, flavanols and anthocyanins. Phenolic compounds can play an important role in preventing body cells and organs from injuries by hydrogen peroxide, damaging by lipid peroxides and scavenging or neutralizing free radicals. Figure (1) showed the total polyphenols (mg GAE/g) and total flavonoids (mg QE/g) contents of *Chelidonium majus*, Data in figure (1) showed that, the total polyphenol and flavonoid contents of *Chelidonium majus* flowers were 87.5mgGAE/g and 9.45mgQE/g, respectively.

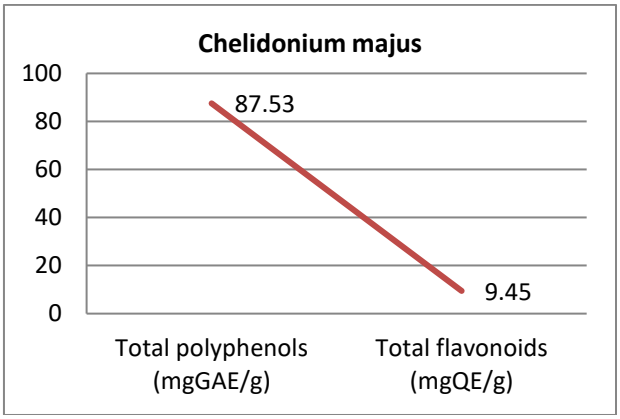

Fig. 1: Total polyphenols and total flavonoids content.

Our results were in the same line with those reported by Jakovljević *et al.*, (2013), who found that, the total phenolic and flavonoid contents of *Chelidonium majus* methanolic extract, which were 60.96mg GA/g and 96.84mg RU/g of rosette and flowers, respectively. Choi *et al.*, (2017), who established that, the total phenolic compound content, and flavonoid content of *Chelidonium majus* methanolic extracts, which were 57.8±1.7µg/ml and 30.0±1.4µg/ml, respectively.

Identification of polyphenolic compounds using HPLC technique

High performance liquid chromatography (HPLC) procedure was used for qualitative and quantitative analysis of polyphenolic compounds of *Chelidonium majus*. Eighteen polyphenolic compounds as authentic samples namely: Gallic, Pyrogallol, 4-Amino benzoic, Protocatechuic, Catechin, Chlorogenic, Catechol, E.picatechen, Caffien, P-OH Benzoic, Caffeic, Vanillic, Ferulic, Ellagic, Benzoic acid, Salicylic acid, Coumarin and Cinnamic acid. These standard samples were used to identify the corresponding components of plant polyphenols. From Table (1), it could be noticed that Gallic acid was the predominant identified component of *Chelidonium majus* in concentrations which was 76.84 ppm. The pyrogallol compound was not found in *Chelidonium majus*. The 4-Aminobenzoic content of *Chelidonium majus* was 10.00 ppm. While Protocatechuic content of the same line was 28.30 ppm. Also, the cataehein content was 15.73 ppm. From Table (1), it could be noticed that the chlorogenic content of *Chelidonium majus* was 88.59ppm. While Catechol component content of *Chelidonium majus* was 16.07ppm. While, E.picatechen content of *Chelidonium majus* was 2.55 ppm. From Table (1), it could be noticed that ellagic was the predominant identified component in *Chelidonium*

majus, in concentration of 43.99 ppm. Also, the highest content of Benzoic compound which was 173.91 ppm. Likewise, Salicylic which was the main component of polyphenols of the same plant in concentration of 103.69 ppm. The P.oH.benzoic and Ferulic compounds were not found in Chelidonium majus. While compounds (Caffien, Caffeic, Vanillic, Coumarin and Cinnamic), there were intermediate values.

Table 1: Identification of polyphenols compounds using HPLC technique.

Phenolic Compounds	R_{time}	*Chelidonium majus* (ppm)
Gallic	7.603	53.46
Pyrogallol	--	--
4-Aminobenzoic	8.055	10.00
Protocatechuic	8.341	28.30
Catechin	8.590	15.73
Chlorogenic	9.200	88.59
Catechol	9.411	16.07
E.picatechen	9.690	2.55
Caffien	9.911	11.71
p-Hydroxy benzoic acid	--	--
Caffeic	10.388	15.33
Vanillic	10.632	26.09
Ferulic	12.014	4.55
Ellagic	13.260	43.99
Benzoic	13.787	173.91
Salicylic	14.300	103.69
Coumarin	14.480	4.87
Cinnamic	15.801	2.43

Wojdyło *et al.* (2007) showed that, the quantitative analysis of major phenolic compounds identified in *Chelidonium majus*, were 186, 167 and 71.7mg/100 gm, of caffeic acid, neochlorogenic acid and p-coumaric acid, respectively. Gîrd *et al.*, (2017) showed that, the HPLC results of *Chelidonium majus* dry extract has a higher content of caffeic acid (0.2 g%). The results were highest compared with that obtained by Parvu *et al.*, (2013), Identification and quantitative determinations of the polyphenols, such as Caftaric acid, Gentisic acid, Caffeic acid, Chlorogenic acid, p-coumaric acid, Ferulic acid, Sinapic acid and Hyperoside which were 2.10, 2.15, 5.60, 8.7, 12.2, 14.3 and 18.60µg/mL^{-1}, respectively.

Identification of flavonoid compounds using HPLC technique:

High performance liquid chromatography (HPLC) procedure was used for qualitative and quantitative analysis of flavonoids compounds of *Chelidonium majus*. Eleven Flavonoids fractions as authentic samples namely: Narengin, Rutin, Hisperdin, Romarinic, Quereitrin, Quereetrin, Narenginin, Kampferol, Luteolin, Hispertin, and 7-Hydoxyflavon were used different concentrations comparing with standard compounds. Obtained data revealed that ten compounds with different retention times were recognized in HPLC chromatogram of *Chelidonium majus* flowers. From Table (2), it is clear that all investigated Flowers samples contained, Rutin, Hisperdin, Rosmarinic, Quereitrin, Quereetrin, Narenginin, Kampferol, Hispertin, and 7-Hydoxyflavon with different concentrations comparing with standard compounds. From Table (2), it could be noticed that Narengin was noticed in *Chelidonium majus* in a concentration of 308.55 ppm. While, the rutin was the main component of flavonoids in *Chelidonium majus* in concentration of 57.61 ppm. Though, the highest value of hesperidin was 2690.72 ppm. From Table (2), it could be noticed that highest value for Romarinic, of *Chelidonium majus*, was 74.04 ppm. The content, Quereitrin and Quereetrin, were 132.13 and 58.06 ppm, of *Chelidonium majus*. Where the values Quereitrin, higher compared with Quereetrin. From Table (2), it could be noticed that, the low values of Narenginin compound, which was 16.65 ppm of the same plant. Moreover, The Kampferol content of *Chelidonium majus*, which was 154.91 ppm, while, Luteolin compound not found of the same plant. The value of Hispertin compound, was 7.98 ppm of *Chelidonium majus*. While the lowest value of 7-Hydoxyflavon, which were (6.00 and 4.76 ppm), of While the value for 7-Hydoxyflavon in *Chelidonium majus was* 4.76 ppm.

Table 2: Identification of Flavonoid compounds using HPLC technique.

Flavonoid compounds	R_{time}	*Chelidonium majus* (ppm)
Narengin	11.902	308.55
Rutin	12.465	57.61
Hisperdin	12.712	2690.72
Rosmarinic	12.900	74.04
Quereitrin	13.520	132.13
Quercetin	15.101	58.06
Narenginin	15.398	16.65
Kampferol	15.559	154.91
Luteolin	--	--
Hispertin	15.860	7.98
7-Hydoxyflavon	17.564	4.76

Wojdyło *et al*. (2007), who showed that, the quantitative analysis of major flavonoid compounds identified in *Chelidonium majus*, were 759, 11.65 and 20.0mg/100 gm, of quercetin kaempferol and apigenin, respectively. Parvu *et al.*, (2013), Identification and quantitative determinations of the polyphenols (flavonoid compounds), such as Isoquercitrin, Quercitrin, Patuletin, Luteolin, Kaempferol and Apigenin, which were 19.60, 23.00, 28.70, 29.10, 31.60 and 33.10µg/mL^{-1}, respectively.

Reducing power of investigated plant extracts

Efficiency of methanolic and aqueous leave extracts to reduce Fe^{+++} to Fe^{++} was determined according to the method described by Oyaizu (1986). Optical density of reaction mixture was measured at wave length 700nm using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. The obtained data are presented in Table (3), the absorbance showed the reducing power for different concentrations of crude methanolic and aqueous extracts of *Chelidonium majus* flowers. Data expressed as absorbance at 700nm for producing color as a result for using three concentrations (20, 40 and 80 mg/ml) for each sample.

The reducing power capacity increased with increasing the methanolic extract concentrations of all samples. *Chelidonium majus* extract has reducing power from 0.508 to 1.467 at the concentrations of 20 and 80 mg/ml. High levels of reducing power indicated the presence of some compounds which could be considered electron donors and could react with free radicals to convert them into more stable products, (Arabshahi and Urooj (2007). Different studies

indicated that, the electron donation capacity which reflecting the reducing power of bioactive compounds was associated with high antioxidant activity, (Siddhuraju *et al.,* (2002).

The results were in the same line with those reported by Oarcea *et al.,* (2016), who found that, the antioxidant capacity using FRAP of *Chelidonium majus*, which were (2.555mM Trolox/L plant extract). Khodabande *et al.,* (2017), showed the ferric-reducing antioxidant power of *Chelidonium majus* flower and fruiting stage extracts which were (0.50 and 1.75 mg/g FW).

Table 3: Reducing power of crude methanolic extracts

Plants extracts	Concentration mg/ml	O-Density at 700nm
	20	0.508
Chelidonium majus	40	1.094
	80	1.467

Antioxidant activity using ($DPPH^+$) radical

The antioxidant activity of the methanolic extract prepared from the *Chelidonium majus* plant species was reported in table (4). The concentration of an antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely adapted to measure the antioxidant activity according to Sanchez *et al*., (1998). The lower EC_{50} pointed to the higher antioxidant activity. The antioxidant activity of the tested extract was measured using DPPH radical scavenging activity. The antioxidants scavenging activities of DPPH are attributed to their hydrogen - donating abilities. Biswas *et al*., (2010). Vitamin C was used as the reference compound.

Table 4: ($DPPH^+$) radical of Plant extracts

Plants Extracts	$DPPH^+$ IC_{50}
Chelidonium majus	0.41

The results were in the same line with those reported by Hădărugă and Hădărugă (2009), who found that, the Mean reaction rate of DPPH in the case of *Chelidonium majus* L. hot extracts, were 1.3, 0.6, 2.1, 2.0, 1.5, 3.0, 2.9 and 2.5µM/s, of *C. .majus* hot extract (EtOH96%), undiluted, *C. majus* hot extract (EtOH96%), diluted, *C. majus* hot extract (EtOH60%), undiluted, *C, majus* hot extract (EtOH60%), diluted, *C. majus* hot extract (EtOH20%), undiluted, *C. majus* hot extract (EtOH20%), diluted, *C. majus* hot extract (water), undiluted and *C. majus* hot extract (water), diluted, respectively. Oarcea *et al.,* (2016) found that, the antioxidant capacity using of *Chelidonium majus* was (2.399mM Trolox/L plant extract). Huh *et al.,* (2016) found that, the DPPH scavenging activity of C. majus stem, leaves and root extracts, were 55.8, 23.3 and 19.6% at concentration 4.0 mg/ml, respectively.

Histopathological examination

Microscopic examination of H&E stained hepatic tissue at the end of the experiment from group I (Control negative normal rats) revealed normal hepatic parenchyma with normal hepatic cords, blood sinusoids, and portal tract (Plate 1.a). While group II (control positive intoxicated group) showed severe hepatic toxicity induced by CCl_4 in the form of massive and diffuse degenerated and necrosed hepatocytes, Moreover, there were multifocal hemorrhagic areas (Plate 1.b). Group III (Medium dose *Chelidonium majus extract* protected rats) reported moderate toxic effect in hepatic tissues with focally scattered fatty degeneration with circumscribed hepatocytic vacuolation with no areas of hemorrhage which attributed to the protective effect of the medium dose of *Chelidonium majus* flowers extract (Plate 1.c). Group IV (Large dose *Chelidonium majus extract* protected rats) recorded very mild toxic effect in hepatic tissues with randomly scattered vacuolated hepatocytes among the healthy hepatic parenchyma which indicated the valuable protective effect of the high dose of *Chelidonium majus* flowers extract (Plate 1d).

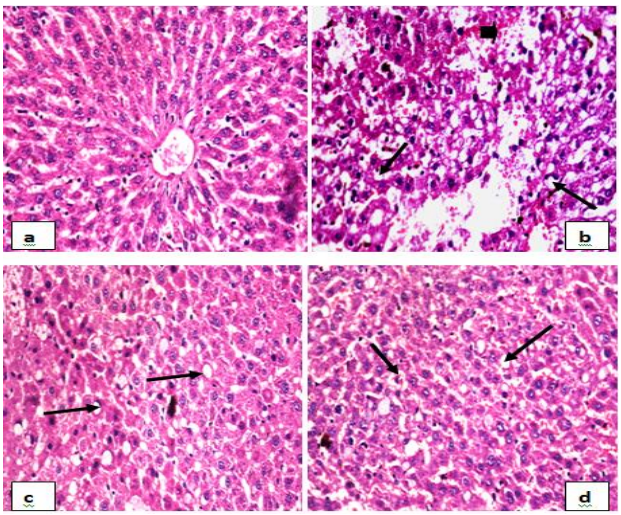

Plate 1: Microscopic examination of rats Hepatic tissues from all experimental groups, (H&E X 400); a) Group (I) showing normal hepatic parenchyma with normal hepatic cords, blood sinusoids, and portal tract. b) Group (II) showing severe hepatic toxicity in the form of massive and diffuse degenerated and necrosed hepatocytes (arrows), Moreover, there were multifocal hemorrhagic areas (arrow head). c) Group (III) showing moderate toxic effect in hepatic tissues with focally scattered fatty degeneration with circumscribed hepatocytic vacuolation (arrows) with no areas of hemorrhage. d) Group (IV) showing very mild toxic effect in hepatic tissues with randomly scattered vacuolated hepatocytes (arrows) among the healthy hepatic parenchyma.

Conclusions

Chelidonium majus flowers extract contain a high percentage of active compounds such as polyphenols and flavonoids compounds. The effect of natural extract as antioxidant showed high ability of plant flowers extract to scavenging the free radicals in laboratory. Moreover, *Chelidonium majus* flowers extract possesses a measurable protective effect against Hepatotoxic Activity, However, further clinical trials in toxicity are recommended.

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