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The Effect of Chelidonium majus Extract on the Lipid Profile and Activity of Pituitary-Gonadal Axis in Hypercholesterolemic Rats

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Abstract

***Background*:** Over the past centuries, global population has increased at different rates and so has been the case with cardiovascular diseases. Therefore, given the importance of population and cholesterol control, the purpose of this study is to investigate the effect of *Chelidonium majus* (*C. majus*) aerial parts extract on the lipid profile and prolactin levels and the activity of pituitary-gonadal axis in hypercholesterolemic rats.

***Materials and Methods*:** In this experimental study, 35 Wistar rats were selected and categorized into 5 groups. The control group had ordinary diet, the model group had high-fat diet, and experimental groups consisted of hypercholesterolemic rats that respectively received minimal dosages of 100, 200 and 300 mg/kg of *C. majus* extract. After 21 days, blood samples were taken and the factors of interest were measured. Then, the gathered data were analyzed using SPSS-11.5.

***Results*:** The amount of triglyceride and cholesterol were increased in the model group compared to the control group whereas the same items were decreased in the experimental group. *C. majus* extract also decreased testosterone and increased prolactin and gonadotropins.

***Conclusion*:** In this study, *C. majus* extract resulted in decreased fat and testosterone levels as well as increased prolactin level; however, since many sources have informed of the toxicity of this plant, cautious use of the plant is advised.

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Introduction

Over the past centuries, global population has increased at different rates and so has been the case with cardiovascular diseases. The prevalence of such diseases is attributed particularly to lipid metabolisms and primarily cholesterol. High serum levels of low-density lipoprotein (LDL) and low levels of high-density lipoprotein (HDL) have shown a clear contribution to atherosclerosis formation in many studies. As a result, if the population growth surpasses normal limits, it can lead to a complicated economic and social problem [1, 2].

Testes are divided into spermatogenic (seminiferous tubules) and steroidogenic (interstitial cells) parts. Compounds affecting spermatogenesis and halting sperm formation act differently. Some inhibit synthesis or gonadotropin release and some other have anti-androgenic impacts, inhibiting spermatogonic [3]. Prolactin (PRL) is another factor that can adjust gonadotropins [4]. PRL itself is adjusted via dopamine. Dopamine is a neural transmitter which has an inhibitory effect on hypothalamic-pituitary-gonadal axis [5].

Given the abundance of information on men's reproductive organs, expansive research has been conducted to explore and study male infertility [6]. Taking into account various side effects associated with fertility medicines in the market and the inclination of a most people towards herbal medicine due to lower complications, study of infertility treatment drugs is of value [7]. Today, the use of herbal medicine has become a common practice. Due to the presence of various chemical compounds in *Chelidonium majus* (*C. majus*), this plant, which belongs to the papaveracea family, is a medicinal herb with different uses both in traditional and modern medicine [8]. The plant is highly bitter, with a smell that stimulates the respiratory tract and causes repetitive coughing. It is found all around the world and mainly grows in nitrogen-rich soil [8]. Chemical compounds in this plant include some alkaloids (benzophenanthridines: chelerythrine, chelidonine, sanguinarine, isochelidonine protoberberines berberine, coptisine, dihydrocoptisine, stylopine protopine) and acids (chelidonic, malic, citric, caffeic (0.4%) ferulic (0.02%), P-coumaric (0.06%), gentisic and P-hydroxybenzoic acids). There is also some calcium, aluminum and magnesium salts as well as resin and mucilaginous substance in the plant. *C. majus* extract also acts as a rich source of biological substances, including alkaloids, flavonoids, and phenolic acids, with strong antitumor properties [9-11]. These compounds can also explain the hypoglycemic and hypolipidemic properties of some plants that are effective in treating diabetes and hyperlipidemia; however, given the strong antimitotic

properties of alkaloids, it can have shrinking effects on testes' activity [2, 12]. Therefore, the present study investigates the effect of *C. majus* aerial parts extract on the lipid profile and PRL levels and the activity of pituitary-gonadal axis in hypercholesterolemic rats.

Materials and Methods

The present study is an experimental research. All the animal species to be studied were obtained from the reproduction and breeding center of Razi Institute, Fars province, and were kept at standard temperature and light condition. The study was conducted in conformance with all codes of ethics in the use of laboratory animals developed by the Iranian Ministry of Health and Medical Education. Before the study began, the rats were weighted so that they could be placed in a specific weight range. The average weight of the male rats used in this study was 170±5 g. The 35 rats included in the study were randomly divided into 5 groups of 7 rats as follows:

1. Control group: During the experiment, the rats within this group received no solvent or drugs and were under ordinary diet.
2. Injection model group: The hypercholesterolemic rats (2% of cholesterol was added to the rats' food to hypercholesterolize them) were injected with 0.2 mL of medicine solution (normal saline) on a daily basis over 21 days [2, 12].
3. Experimental group 1: The hypercholesterolemic rats were forced-fed (gavaged with) 100 mg/kg per day (minimum dosage) of alcohol extracts of *C. majus* over 21 days.
4. Experimental group 2: The hypercholesterolemic rats were forced-fed 200 mg/kg per day (minimum dosage) of alcohol extracts of *C. majus* over 21 days.
5. Experimental group 3: The hypercholesterolemic rats were forced-fed 300 mg/kg per day (minimum dosage) of alcohol extracts of *C. majus* over 21 days.

Preparation of high-fat food (nutriment): An amount of 20 g of pure fluke Chemika powder was solved in 5 mL of warmed olive oil and was thoroughly mixed with 1 kg of rat food [2].

Extraction: To prepare the alcohol extract of *C. majus*, after obtaining aerial parts of the plant from the northern gardens and removing the impurities, an amount of 600 g of the plant was ground and mixed with ethylic alcohol 98% with a 1 to 5 ratio. The content was then kept in vitro for 48 hours in a capped container. Then filtration was performed thoroughly using small and big filter papers. The filtrated liquid was poured in bain-marie container for condensation purposes. Finally, different concentrates were obtained from the extract (about 12 g for each 100 g of the ground plant) using normal saline as mg/kg of the rats' weight.

All experimental groups were maintained on high-fat diet treatment during the experiment period. The experiment period was 21 days. Every day at 9 am, the medicine was gavaged to the rats. After the mentioned period, to measure the concentrates of the biochemical factors of plasma, light ether anesthesia was used and then blood sampling from the heart was performed. After centrifuging the blood at 3000 rpm, serums were separated and transferred to laboratory to measure the factors of interest.

In order to measure lipid, PRL, testosterone, and gonadotropin profiles, radioimmunoassay (RIA) technique, Pars Azmoon Kit, RIA1000 device (made in the USA) were used. The mean scores (Mean±SEM) obtained from the mentioned factor in various groups were analyzed using one-way ANOVA and Tukey test. All the statistical analyses were performed using SPSS-11.5 with $p<0.05$.

Figure 1. Chelidonium majus

Results

Comparison of the results of the statistical tests reveals that the increase in the amount of cholesterol in the model group compared to the control group was significant, and the group that received minimum *C. majus* dosage showed a significant decrease in cholesterol compared to the model group ($p=0.007$). The differences between experimental groups were also insignificant.

The increase in the amount of triglyceride (TG) in the model group compared to the control group was significant, and the group that received maximum *C. majus* dosage showed a significant decrease in TG compared to the model group. Moreover, the groups receiving moderate and maximum *C. majus* dosages also showed a significant decrease in TG compared to the group receiving the minimum dosage of *C. majus* ($p=0.001$). Variations in LDL and HDL were insignificant among all groups. The amount of Follicle-stimulating hormone **(**FSH**)** for the model group showed a significant decrease compared to the control group, and the group that received minimum *C. majus* dosage showed a significant increase in FSH compared to the model group ($p=0.001$). The differences between experimental groups were also insignificant.

Table 1. The impact of the extract of various parts of Chelidonium majus on lipid profile, pituitary-gonadal and prolactin hormones

	Control	Sham	100 mg/kg	200 mg/kg	300 mg/kg
Cholesterol	2.9±69.9	1.9±86.57 *	2.6±64.3 β	4.4±72.6	6.3±72
TG	109.3±4.9	157.3±13.3 *	164.7±11.9	118.7±8.6 α	116.7±9.8 αβ
LDL	27±1.9	32±2.1	35.3±1.5	30.3±2.2	29.2±2.2
HDL	19.8±1.1	21.5±1	24.3±1.2	22.2±1.3	19.5±1.3
LH	1.1±0.2	0.71±0.03	0.95±0.05 α	1.1±0.07	1.53±0.2 β
FSH	2.8±0.4	1.03±0.1 *	2.6±0.2 β	1.7±0.2	1.9±0.3
Testosterone	6.7±1.6	5.9±1.5	2.8±0.1	2.9±0.5	1.01±0.1 β
Prolactin	3.45±0.3	5.5±0.6	12.7±1.3 β	8.2±0.8	10.3±2 β

* is indicative of significant changes in compared with the control group; β is indicative of significant changes of each group compared with the model group; and α is indicative of significant changes of various dosages of Chelidonium majus extract compared to each other

The amount of LH for the model group did not show any significant change compared to the control group; however, increase in Luteinizing hormone **(**LH**)** level in the group receiving maximum *C. majus* dosage was significant compared to the model group and the experimental group that received minimum *C. majus* dosage (p=0.005).

The amount of testosterone for the model group did not show any significant change compared to the control group; however, decrease in testosterone level in the group receiving maximum *C. majus* dosage was significant compared to the model group (p=0.003). The differences between experimental groups were also insignificant.

In terms of PRL, its amount in the model group was increased, though not significantly. Increase in PRL level in the group receiving minimum and maximum *C. majus* dosage was significant compared to the model group (p=0.001). The differences between experimental groups were also insignificant.

Discussion

The results of this study show that *C. majus* extract reduces cholesterol, triglyceride and testosterone levels, but increases PRL and gonadotropin amounts.

Testosterone negative feedback is one of the ways to regulate gonadotropins LH and FSH levels; that is, when the amounts of these hormones increase, gonadotropin level decreases and vice versa [13]. In the present study, too, observing an increase in gonadotropin level following a decrease in testosterone level seems logical.

PRL is a polypeptide hormone which is produced by lactotroph cells in the pituitary gland. The reference range for this hormone is 1-25 µg/mL for women and 1-20 µg/mL for men. Derivatives of opium, heroin, morphine and methadone are some of the factors that increase PRL levels. Two other factors contributing to PRL increase are chronic kidney failures and cirrhosis of the liver [14]. On the other hand, *C. majus* belongs to the papaveracea family. The plants within this family contain morphine, opioid, papaverine, and codeine and other compounds and their impact mechanism takes effect through opioid, dopamine, benzodiazepines and cholinergic neurotransmitters [8]. The effect of *C. majus* extract and also the effect of plants with alkaloid on hepatic toxicity, e.g. hepatisis and fibrosisis, have been verified in many sources [8, 15-17], resulting in significant increase in ALT and AST [18].

Arcuate nucleuses are active centers for the release of gonadotropin-releasing hormone (G_nRH) into blood stream. On the other hand, dopamine generative cells are found in arcuate and periventricular nucleus. Intravenous injection of dopamine prevents the flow of gonadotropins and PRL [5, 19].

Since *C. majus* extract leads to an increase in PRL, LH and FSH, and also taking into account alkaloid compounds (including papaverine, morphine, codeine, opioids, etc.) of this family, it is likely that this plant reduces dopamine levels. As it is observed in this study, increase in PRL hormone in male species leads to decreased sexual instinct through decreasing sex hormones, including testosterone [20].

Luteinizing hormone-releasing hormone (LHRH) hormone can also be another potential factor contributing to testosterone decline. Increase of this hormone increases secretion of LH and FSH, on the one hand, and prevents synthesis and secretion of testosterone through reducing LH receptors found in testes [21, 22]. As a result, despite an increase in LH and FSH levels in this study, the decrease in testosterone levels seems to be logical, and the results of the present study are in line with those of previous research on the skin of cinnamon stem extract [21].

Leydig cells are the main source of testosterone and they have receptors for PRL. Prolactin at normal levels increases testosterone secretion. These studies indicate some coordination of PRL with LH and testosterone; however, high level of PRL decreases testosterone level [19].

Cholesterol is a precursor to steroid hormones and conversion of cholesterol to pregnenolone is done through P_{450} within mitochondria [19, 20]. The study conducted on the effect of alkaloids of *C. majus* on human hepatoma cells, it was revealed that these alkaloids have no impact on P_{450} expression; however, in the study by Habermehl et al., proapoptotic activity of ukraine (an antitumor drug produced based on *C. majus* alkaloids) was investigated and it was shown that this activity was mediated by mitochondria death path. The main composition of the said drug is a triphosphor triamid which is surrounded by three molecules of chelidonine through covalent bonds. Chelidonine is one of the most important alkaloids in *C. majus* plant [8, 23]. The extract

of this plant also decreases testosterone levels through decreasing cholesterol, on the one hand, and through reducing the activity and number of mitochondries.

Researchers have investigated the impact of *C. majus* extract on Wistar rats and found that high dosages of this plant reduces glutathione peroxidase (GPx) levels and superoxide dismutase (SOD) activity [8]. Glutathione peroxidase enzyme, as an antioxidant, plays an important role in protecting sperms within testes tissue and a shortage of it results in infertility. This enzyme inhibits destructive effects of DNA break in spermatozoa and sperm-producing cells [24]. Moreover, inscription of sanguinarin (an alkaloid derived from *C. Majus*) results in DNA damage in some cells, including bone marrow cells. Therefore, given the mentioned studies, reduction of testosterone levels appear to be reasonable. *C. majus* extract also increases nitrous oxide (NO) formation and TNF-α production [11]. NO increases secretion of gonadotropins and sperm motility and induces erection [20].

Through reducing androgen levels, alkaloids lead to epithelial cells atrophy and prevent androgen from affecting tissues and this way cancer is treated [15]. Moreover, alkaloids easily pass through cell membranes and can react with some intracellular elements such as protein tubulin in cells cytoplasm, and thus can damage cytoskeletons, release of free radicals and eventually destructive changes in cell structure. Moreover, this can result in overreaction of white blood cells (anti-inflammatory effects). Meanwhile, some studies indicate that they also have antioxidant effects as well [15, 25, 26].

Blood cholesterol increase is also associated with coronary artery diseases. Cholesterol level can be reduced through proper diet or medicine. Numerous herbs and compounds can decrease cholesterol level. Studies on barberry plant also show that it reduces blood lipid. Barberry mostly contains berberine [27, 28]. One of the compounds found in *C. majus* is berberine, and as it was observed in this study, *C. majus* also reduces fat levels a result which is in line with the results of previous studies. Studies show that berberine can be effective in preventing coronary artery disorders and is likely to control total cholesterol level [29, 30]. The major impact of berberine is blocking of calcium channels. In experiments on lipid peroxidation of erythrocytes, it is shown that alkaloids can have anti-ischemic and anti-arrhythmic effects [31]. Recently, it has been reported that berberine reduces cholesterol through a mechanism different than statin drugs, and that if statin and berberine are used together, they can more readily control cholesterol level. Berberine increases formation of some receptor in liver which is bonded to cholesterol and enables its excretion [32].

Previous research shows that polysaccharides, flavonoids, lipoproteins, poly-lipids, steroids, and alkaloids in herbal remedies can well explain the blood sugar and blood lipid reduction properties of some plants in treating diabetes in terms of preventing blood biochemical variations.

Alkaloids also inhibit cholesterol synthesis [12, 26, 32, 33]. Therefore, given the mentioned findings, reduction in cholesterol and triglycerides was predictable.

Although this study shows that *C. majus* extract reduces lipids and testosterone, and also increases PRL, it is not prescribed now as an effective substance for reducing fertility in men and also for reducing fat and increasing PRL, because many sources refer to its toxic effects. Therefore, it should be used with caution. Moreover, it is recommended that the impacts of *C. majus* on some body tissues (such as liver, testes, thyroids, etc.) should be investigated in more comprehensive studies so that the positive and negative effects of the extract can well be verified.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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