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Effect of *Anastatica Hierochuntica* on Balancing Fertility Hormones Of Albino Male Mice

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Abstract

Crude extract of *Anastatica hierochuntica* L was analyzed for active compounds and tested for its effect on the body of laboratory mice and their fertility. Data obtained showed that the extract contained saponins, flavonoids, alkaloids and terpens. Test period lasted for a year included 60 mice each time that were divided into 6 groups. Each group was administered orally with 100, 200, and 300 mg/kg of the plant extract, control group positive (vitamin C and E) and control group negative (water and DMSO). Results of histology examination showed no toxic effect on liver and kidney of treated animal even with prolonged administration, while there is an elevated level of testosterone hormone and an increase in sperm count with increased body mass index of 2%.

Key words: *Kaff Maryam (Anastatica hierochuntica L.), active compounds, fertility, antioxidant activity, free radical-scavenging activity*

Introduction

Anastatica hierochuntica L. is an annual, small, and grey herb blossom in winter to a final high of 6 inches with white tiny flowers. It is a part of plants in multiple countries like Jordan, Iraq, Saudi Arabia, UAE, and Kuwait with high indurance to draught [1]. It is known in Arabian countries as kaff Maryam and consumed as beverage to treat some clinical conditions like difficulty of pregnancy [2]. In addition, some reports said it treats gastrointestinal problems, asthma, depression, and antioxidant and have hypoglycemic properties [3]. Herbal medicine is still main remedy in many developed countries because of their phytochemical component that in most cases are more suitable for human physiology with less side effect [4]. These compounds include alkaloids, tannins, flavonoids, and phenolic compounds that may play role to inhibit lipid

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oxidation, reducing free radicals, and activating body immune system against pathogenic infections [5]. The main use *Anastatica hierochuntica* L was to increase fertility and help couples to have children by continuous use of this plant, especially for women. Such prolonged consumption requires the study the impact of this plant on the body including increase seminal fluid production, effect on liver, and kidney on which this study was designed to be tested on mice.

Material and Method

Plant Samples: Were obtained from native herbariums and used to obtain active compounds used in this study.

Extraction of plant crude compounds: About 50 gm of dried flowers were grind to powder and mixed with 250 ml of sterile D.W, left in arotary mixer at 50^{o}C for 24 hours, filtred through Watman NO. 1 filter paper and concentrated by rotatry evaporater at 40^{o}C [6].

Detection of Plant Active Compounds

Detection of Saponins: A five ml of extracted plants component was mixed with 3 ml of (HgCl3) solution. The appearance of white precipitate indicates the presence of saponins and also the formation of foam for a long time as a result of stirring the aqueous solution of plant powder in test tube indicates saponins existence [7].

Detection of Alkaloids: Five ml of plant extract and 2 ml of marquis reagent were added in test tube. Shaking the tube caused the appearance of gray precipitate of Alkaloid [7].

Detection of Flavonoids: Ten ml of 50% ethyl alcohol were mixed within 10 ml of 50% potassium hydroxide solution and added to equal volume of plant extract. The appearance of yellow colour indicates the presence of Flavonoids [7].

Detection of Terpenes and Steroid: One gram of plant extract was dissolved in 1-2 ml of chloroform and then a drop of acetic anhydride and drop of concentrated sulfuric acid were added. Then the appearance of brown color represents the presence of terpenes. After some time, if a dark blue color was appeared it indicates the presence of steroid [7].

Fourier-transform infrared spectroscopy (FTIR) to identify chemical compounds.

The fourier-transform infrared spectroscopy (FTIR) based on the mathematical process (Fourier transform) to interpretate the raw data (interferogram) into the real spectrum. FTIR

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method is used to obtain the infrared spectrum of transmission or absorption of active compounds present in the plant extract. Spectrum measurement and analysis of data obtained was done in College of Pharmacy, Al- Nahrain University using SHIMADZU/ IS FTIR device.

Animals used for experimentation: Male albino mice (22–29 g) were obtained from the animal house of Research Biotechnology Center, Al- Nahrain University. Sixty Males albino mice were divided into six groups of ten animals each and they were administered orally with *Anastatica hierochuntica* L. extract at different doses for 10 days. Group A: Normal, Group (untreated), group B treated with 100mg/kg, group C treated with 200 mg/kg and group D treated with 300 mg/kg: *Anastatica hierochuntica* L. extract. Group E: Positive control treated with vitamin C and E and Group F: Negative control (water and DMSO).

Fertility Hormone Measurement: Mice testosterone levels were measured by the Bio – Merieux kit Sa. 69230 marcy I' Etoile – France, as instructed by the manufacturer.

Histopathology: This was performed by using method of [8].

Determination of antioxidant properties: The ant oxidation property of plant aqueous extract was determined based on the radical scavenging ability reacting with a stable DPPH free radical [9].

Results

Identifying fractions of active compounds in *Anastatica hierochuntica extract plant.*

The used of specific chemical reagent revealed the presence of various active components *Anastatica hierochuntica* illustrated in table (1).

Table (1). Detection of some active compounds in *Anastatica hierochuntica* of plant extract.

No.	Chemical compounds	Result of detection
1.	Alkaloids	+ ve
2.	Flavonoids	+ ve
3.	Saponins	-ve
4.	Steroids	-ve
5.	Tannins	+ ve
6.	Terpense	-ve

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Results obtained by chemical detection indicated the presence of alkaloid, flavonoids, and tannins in the *Anastatica hierochuntica.*

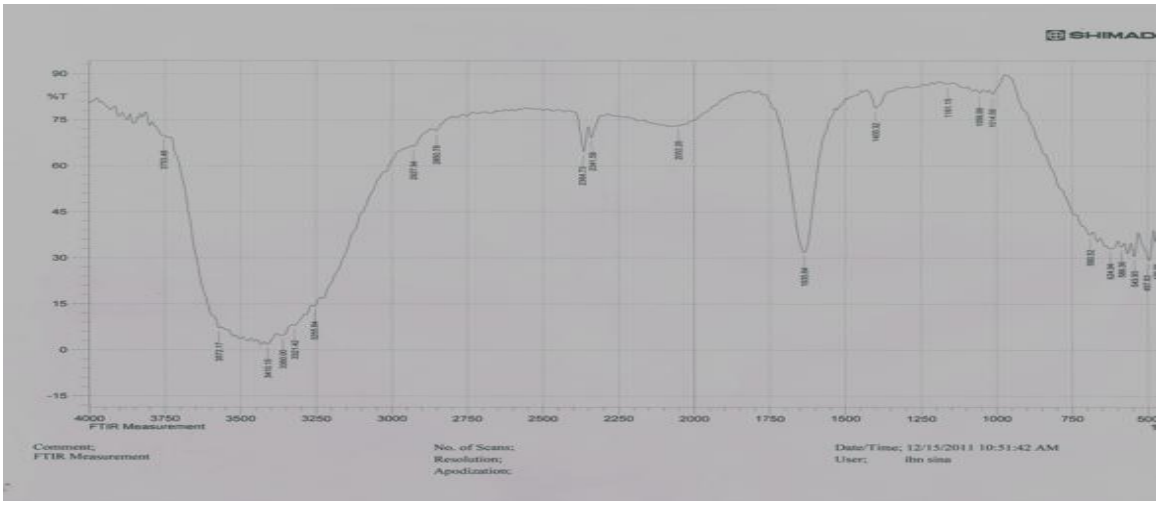

Figure (1). FTIR spectroscopy of the in *Anastatica hierochuntica* extract plant.

Identification of function groups

The infrared spectroscopy is a powerful in identifying active groups of the active components present in chemical compound based on the peaks values in the region of IR radiation. When the plant extract examined by the FTIR, the active groups of the compound are separated according to its peaks ratio. The results of FTIR analysis showed the presence of carboxylic acid, alcohol, phenol, alkanes, phenyl ring, amin, ether carboxylic acid, and ester shown in (figure 1 and table 2).

Table (2): Analysis active compound by FTIR.

No.	IR wavelength	Active compound measured
1.	3572.17	Alcohol ,phenol ,carboxylic acid
2.	3255.84	Alcohol ,phenol ,carboxylic acid
3.	2927.94	Alkanes
4.	2850.79	Alkanes
5.	2052.26	Phenyl ring (finger print region)
6.	1635.64	Amin
7.	14000.32	Phenyl ring substituation(finger print region)

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8.	1161.15	Alcohol ,ether,carboxylic acid ,esters
9.	1056.99	Alcohol ,ether,carboxylic acid ,esters
10.	1014.56	Alcohol ,ether,carboxylic acid ,esters
11.	690.52	Phenyl ring(bend)
12.	624.94	Phenyl ring(bend)

Difference in spectrum reflects active compound. Thus by using FTIR sepectroscopy it is possible identify the functional group content present in the examined parts and extract, identify the main components from the adulterate and even evaluate the qualities of medicinal drug [10].

The results obtaind in this study showed a spectrum identifying which are the functional constituents present in the aqueous extract of *Anastatica hierochuntica*.

Effect of plant extract on fertility.

Hormone, by their very nature, is potent bioactive compounds. The importance of fertility hormone for a wide processes of reproduction became common knowledge; which include development, puberty, behavior, gametogenesis, and integrated sexual function. In general, mice levels of testosterone compared to control group examined in this part of study are listed in table (3).

Table (3). Effect of *Anastatica hierochuntica* extract on elevation of serum testosterone in mice.

Mice groups according to treatment		Testosterone concentration ng/ml (Mean ± SE)*
Plant extract doses	100 mg/Kg	0.462 ± 0.018A
	200 mg/Kg	0.657 ±0.037 B
	300 mg/Kg	0.205 ± 0.015 C
Negative controls	Water	0.300 ± 0.015 C
	DMSO	0.182 ± 0.02 D
Positive controls	vitamin C and E	0.432 ± 0.05 A

*Different letters (A, B, C, D): Significant difference (p = 0.06) between means of column

The indication elaborated from the table showed significant increase (P= 0.06) in testosterone level within 10 days of treatment with *Anastatica hierochuntica* extract compared with negative and positive controls mice. Serum testosterone in mice treated with *Anastatica hierochuntica* with 100, 200 and 300 mg/kg were elevated to (0.462 ± 0.018, 0.657 ±0.037 and 0.205 ± 0.015 ng/ml) while in negative controls mice (water and DMSO) treated mice were

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(0.300 ± 0.015, 0.182 ± 0.02 ng/ml) and in positive control (vitamin C and E) treated mice was (0.432 ± 0.05 ng/ml).

Table (2) gives a clear idea about the relation between treatment and fertility concerning seminal fluid production. In all cases, the total count showed a significant difference in mice treatment from that in normal cases. In all cases studied, it showed increased total count of semin in treated mice compared to normal. Therefore, crude extracts from *Anastatica hierochuntica* can enhance fertility in mice and increase testosterone levels,

Impact of plant extract on liver and kidney.

Different changes in tissue histopathology observed in liver and kidney for the eight groups of mice. Liver section of water and DMSO treated mice and negative controls showed normal appearance of hepatocytes (figures 2 A). The same result was obtained in mice treated with C and E vitamin, as positive control (figure 2 B, C, D, and E). What is important that mice treated with plant extract have the same result of normal hepatocytes as show in (figure 2).

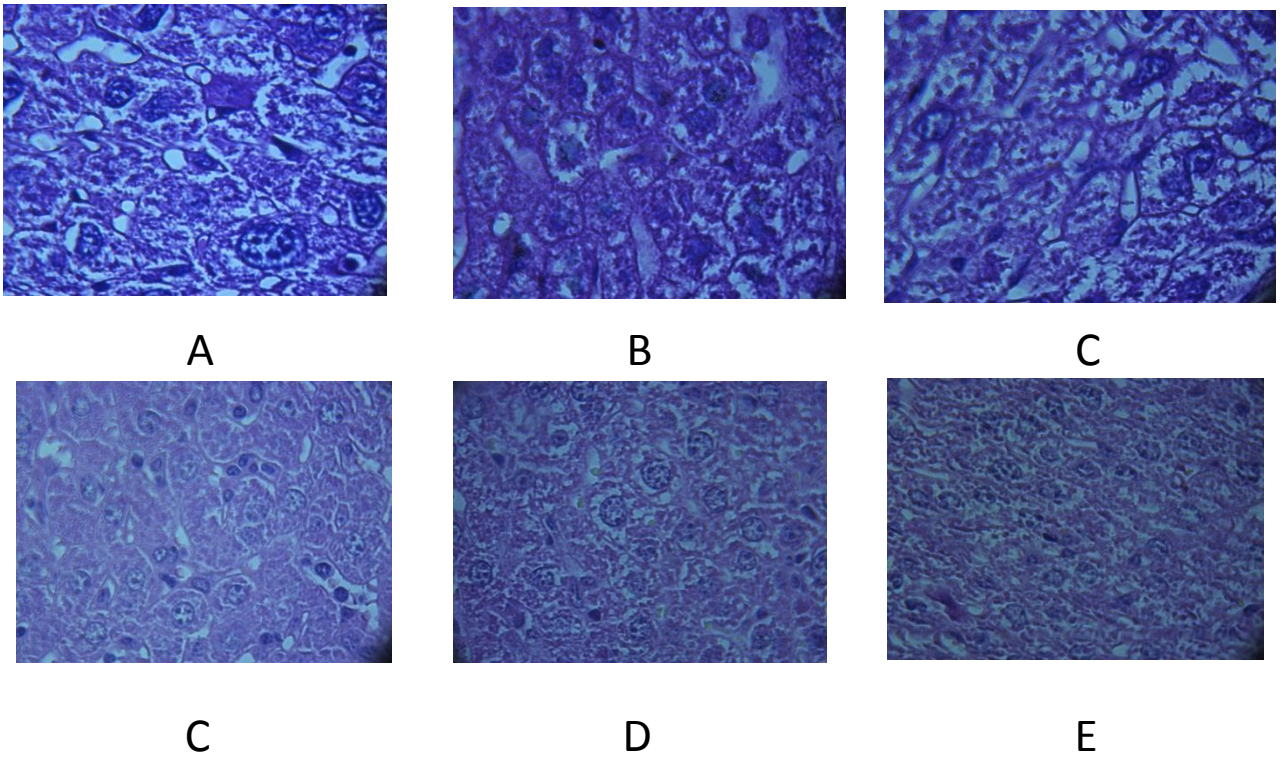

Figure (2): Histological Liver section of mal albino mice. Group A, water and DMSO, negative controls ; group B, vitamin C and E, positive control; group C, *A. hierochuntica* extract (100 mg /kg for 10 days); group D *A. hierochuntica* extract (200 mg /kg for 10 days); group D *A. hierochuntica* extract (300 mg /kg for 10 days).

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Further results were obtained from kidney examination that showed water and DMSO treated mice retained normal kidney tissue (epithelial, glomerulus, distal and proximal tubule) as in figure (3 A). The same result was obtained with mice treated with C and E vitamins and positive control figure (2 B). Mice treated with plant extract 100,200 and 300 mg/kg preserved normal tissue in all aspects figure (3 C, D, E).

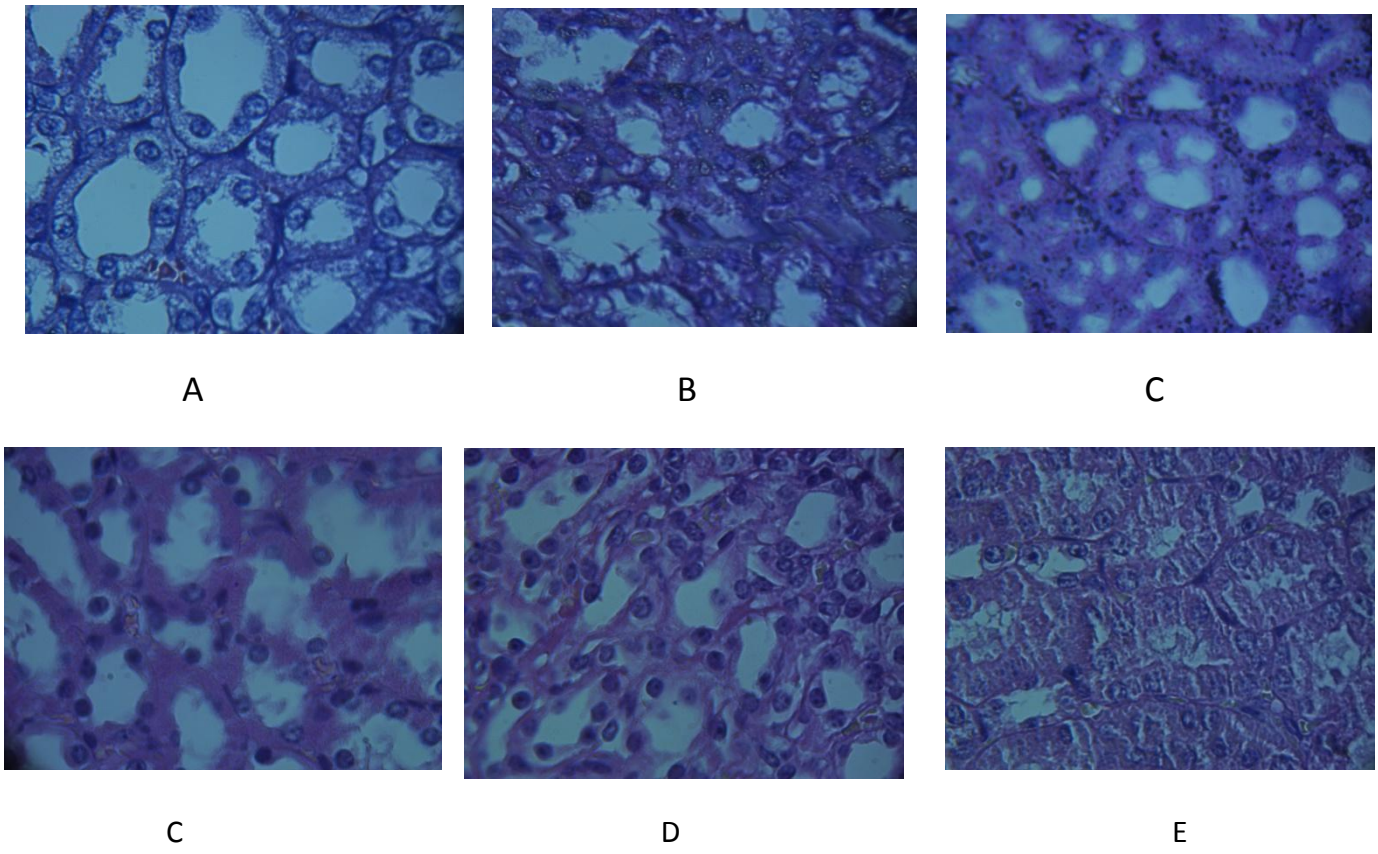

Figure (3): Histological Kidney section of mal albino mice. Group A, water and DMSO, negative controls ; group B, vitamin C and E, positive control; group C, *A. hierochuntica* extract (100 mg /kg for 10 days); group D *A. hierochuntica* extract (200 mg /kg for 10 days); group D *A. hierochuntica* extract (300 mg /kg for 10 days).

Testing liver and kidney is vital to any drug administration since they are the first organs that may affected if there is it showed any toxicity [11].

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Antioxidant activity

The scavenging of free protons is an important process of antioxidation. The widely used method is the stable DPPH scavenging to minimize time required to measure if the plant extract shows antioxidant activity. Figure (4) shows *Anastatica hierochuntica* antioxidant activity depending on DPPH increased scavenging. Such increment scavenging activity is associated with increased plant extract concentration.

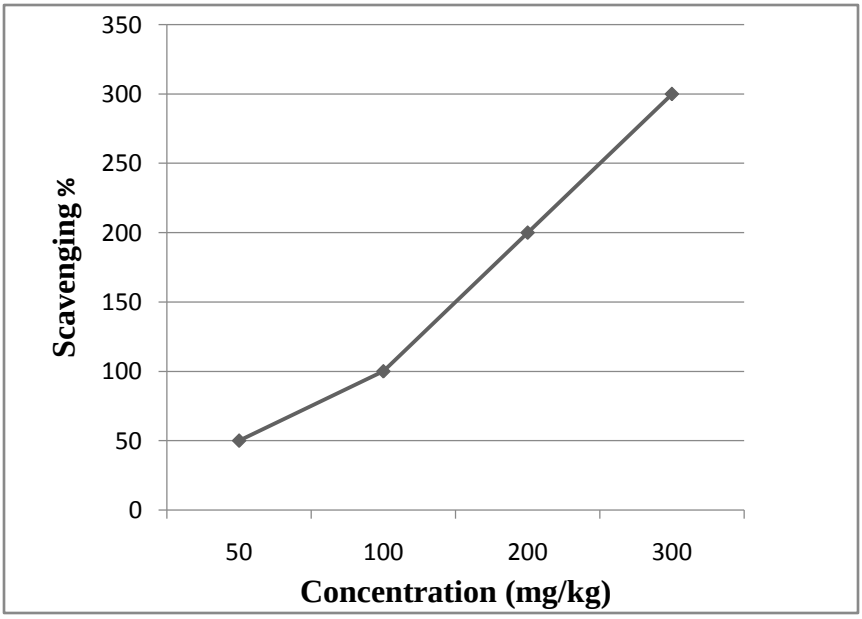

Figure (4): Free radical scavenging activity of different concentrations of *Anastatica hierochuntica* extract by DPPH radicals.

Discussion

Herbal as remedy were used in about 80% of the world countries as a continuous use of such herbs may preserve health of individuals and reduce subjection to illness [12]. *Anastatica hierochuntica* as a medicinal plant contain mostly luteolin-7- glucoside, isovitexin, 7-glucoside, kaempferol 3-rhamnoglucoside, flavonoids, quercetin, kaempferol and lucitin. It also contains glucosinolates: glucoiberin and glucocheirolin. The fruits contain glucose, galactose, fructose, sucrose, raffinose and stachyose [12]. Most of medicinal herb extracts show no toxicity but some reports showed they are not free of toxic effect [13]. For large numbers of people without children, lack of fertility is considered a personal, social and psychological problem, which evenly distributed between males and females. Thus, since centuries ago, herbal medication are

been one of the most widely used methods in the treatment of this disorder [14]. Even now, various compounds of natural sources like, tea, syrup, or total plants extracts are widely used as remedy for impotency low fertility in males such as low sperm accounts, the absence of libido [15]. In addition, the fertility in human may be a result of many factors, like various physiological diseases and disorders like hormonal imbalance, poor nutrition, and organ malfunction [16].

It is important to consume *Anastatica hierochuntica* by adults, since some reports indicated it may bear a toxic effect on small rats [17]. However, it can also cause certain toxicities if it is used for a long period of time and even may become a male contraceptive agent [18].

In addition to what previously found and results in this study, the plant extract has a potential anti oxidation activity which was found determining DPPH scavenging. Such property is an important criterion for this plant to protect cells from damaging and developing malignant tumors.

Conclusions

Anastatica hierochuntica is an important medical plant. It contains active compounds that may reduce or cure a number of diseases that may be observed in human. Using this plant for hundreds of years without any unwanted side especially for treating infertility proved to be considered a reliable source for medication. Spreading of this plant over wide area and flourishing in low demand soils makes it an economically candidate for drug industry.

Data and material availability

Data, samples, and materials used in this study are available and stored at AL-Esraa University College, and Biotechnology Research center, Al-Nahrain University.

Conflict of interests

This work was performed with no conflict of interest among authors or with any other research group in others institutes.

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