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Assessment of *Urtica pilulifera* extracts on humeral immune response and hormones

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ABSTRACT

Urtica pilulifera L. (*U. pilulifera*) is an annual herb that is distributed widely in the Mediterranean region. This study is an attempt to clarify the further potential biological activity of petroleum ether and water methanol extract of different plant parts (herb, root and seeds) on the immunoglobulin G and M and hormones in rats. Study recorded increased levels of IgG and M in serum experimental groups with all extracts compared to controls. The most effective extract on all parameters studied was aqueous methanol extract (AME). The immunoglobulin G was increased by 36% and 33.9% using herb and roots AME respectively. The production of immunoglobulin M was increased by 22.1 and 20.3% using herb and roots AME respectively. Seed extract was less effective. Aqueous methanol extract of all plant parts record reduction in cortisol and increased in thyroid hormones. It showed different effects according to the plant parts and sex of the experimental animals. In conclusion, aqueous methanol extract of nettle has a positive effect on the immune system and hormones in addition to the effect of the extract as gonadotrophic hormones in rats.

KEYWORDS

Urtica pilulifera;
Methanol and petroleum ether extracts;
IgG and M;
Cortisol;
T_3;
T_4;
Gonadal hormones.

INTRODUCTION

Medicinal plants have been used in traditional health care systems since prehistoric times and are still the most important health care source for the vast majority of the population around the world[20].

According to the World Health Oganization in 2002, it is estimated that 70-80% of people worldwide rely on traditional herbal medicine to meet their primary health care needs. Globally, millions of people rely on medicinal plants not only for primary health care, but also for income generation and livelihood improvement.

Urtica pilulifera L. (*U. pilulifera*) is an annual herb of this family that is widely distributed in the Mediterranean region, where it is known as Qurrais and Nettle in Romman[3]. For decades, this plant is a very valuable herbal medicine because, it has been used in a number of areas of the world as traditional medicine for the treatment of various diseases including stemming internal bleeding, anemia, arthritis, rheumatism, hay fever.

In addition, this plant has demonstrated a wide range of biological activities such as antiasthmatic, antitumor, astringent, diuretic, antidandruff, and anti-hyperglycemic[1,9,19,35]. In addition, a tea made from the leaves of this plant has also been traditionally used as a stimulating tonic and blood purifier as well as hemostatic[9]. The efficacy and safety of this herb to blood, liver, kidney and thyroid gland in diabetic rat were studied by Irshaid and Mansi[17]. *Urtica* sp. was reported as one of the most effective medicinal plant to treat benign prostate hyperplasia Hirano *et al*.[16].

Many plants possess medicinal properties that can help our immune system. According to the National Institute of Allergy and Infectious Disease, our immune system comprises cells, tissues and organs that work in concert to ward off attacks by foreign invaders. Bacteria, parasites, fungi and viruses can all cause infections that challenge our immune system. The present study is therefore an effort to further elucidate the bioactivity potentials of *U. pilulifera* extracts on immune system and different hormones in mice.

MATERIAL& METHODS:

This experiment was carried out to study the effect of Petroleum ether and methanol extract of *U. pilulifera* different parts (herb, root and seeds) on immune system (IgG and IgM) and methanol extract on different hormones (cortisol, thyroid and sexual hormones) in male albino mice.

Plant extract

The plant extracts are performed according to Mahmoud et al.[22].

Animal design

42 male albino mice were classified into seven groups (six mice per each). Group1 received only vehicle and saved as control, Group2, 3 and 4,received petroleum ether extract of herb, root and seeds respectively. Group 5,6 and 7 received methanol extracts of herb, roots and seeds respectively. Animals of each group were injected intraperitonealy with 2.9mg/kg bwt for 10 days successively. At the end of period, bloods samples were retro-orbitally collected from the inner canthus of the eye under light ether anesthesia using capillary tubes (Micro Hematocrit Capillaries, Mucaps). Blood was collected in clean vials and serum was separated in cooling centrifuge at 3000 rpm for 10 min.

For studying the effect of different parts of methanol extract on serum sexual hormones four groups of female albino mice were treated as male groups.

The levels of IgG and IgM in sera of different experimental groups were determined according to the Hillyer *et al*.[15]. The sexual hormones were determined by radio immuno assay according to Yollow and Berson[36] where serum tri-iodothyronine (T_3) and tetra-iodothyronine (T_4) levels were determined by an enzyme-linked immunosorbent assay kit[8]. Serum cortisol level was estimated using Elisa Kits[5].

STATISTICAL ANALYSIS

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean ± S.E. The significant differences among values were analyzed using analysis of variance (one-way Anova) coupled with post-hoc (LSD). Results were considered significant at $P< 0.05$.

RESULTS AND DISCUSSION

Application of plant extracts in paste form is common ethnobotanical practice for the treatment of different diseases. In previous study, extract of *U. pilulifera* had proved to have antioxidant activity[22,30]. In the present study, this ethnobotanical practice was examined to investigate its possible role in humeral immune response, thyroid and gonadal hormones.

Immunoglobulin G and M productions were increased using all extracts (2.94mg/ kg b.wt.) as compared to control group TABEL 1. The most effective extracts on IgG and IgM production were the aqueous methanolic extracts of herb and roots; where IgG enhanced by 36 % and 33.9%, respectively and IgM production by 22.1% and 20.3%. The seeds extracts showed smaller effect than the other parts of the plant. From the phytochemical investigation of the plants, it was shown the presence of flavonoids, coumarins, some sterols, alkaloids and hydrocarbons by aqueous menthanol extract meanwhile sterols, fatty acids and terpenes were extracted by petroleum ether (unpublished data). This can explain why methanol extract is more potent than petroleum ether extract. With regard

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to different parts of plants, Leaves contain large amount of phenolic compound than roots which may explain the difference in percent of activation while roots contain β-Sitosterol and scopoletin[32]. The lowest effect of seed extracts on antibody production due to absence or less quantity of phytochemical metabolites in seeds[18].

Urtica pilulifera aqueous methanolic extracts and petroleum ether extracts contain many bioactive compounds that activate immune system therefore they activate the antibody production[12]. The activity of herb methanol extracts is increased due to presence of phenolic compounds, caffeic acid derivatives and polysaccharides in high amount as compared to petroleum ether extracts[1,2,5,13]. These compounds make an additive effect in methanol extract while petroleum ether extract partition show less effect due to presence of less amount of these compounds as well as fatty acids and β-sitosterol. These compound stimulate lymphocyte transformation IgG, IgM and induce T-helper-type 1-cytokines and IL-10, T-helper type-2 cytokines which are involved in IgG production[21,25]. On the other hand, some flavonoids, tyrosine kinase inhibitor, such as genstein, which is found in *U.pilulifera* methanol extract, up regulate IgG receptors on T-cells during a primary response to antigen[34].

TABLE 1 : Effect of *Urtica pilulifera* extracts (2.94mg/ kg b.wt./ Day) on humoral immune response .

Extracts	Groups	Antibody production	
		Immunoglobulin G(mg/ml)	Immunoglobulin M(mg/ml)
Control	1-Control	1233.41 ± 1.495	342.49 ± 3.434
	%	---	---
	LSD	(2, 3, 4, 5, 6, 7)	(2, 3, 4, 5, 6, 7)
Petroleum Ether extracts	2- Herb	1529.34 ± 7.38	392.56 ± 7.49
	%	24	14.6
	LSD	(1, 3, 4, 5, 6, 7)	(1, 5, 6)
	3- Root	1460.91 ± 7.05	391.59 ± 8.1
	%	18.4	14.3
	LSD	(1, 2, 4, 5, 6, 7)	(1)
	4- Seed	1338.061 ± 6.2	371.86 ± 3.8
	%	8.5	2.9
	LSD	(1, 2, 3, 5, 6, 7)	(1)
Aqueous Methanolic extract	5- Herb	1677.56 ± 1.901	418.31 ± 2.64
	%	36.0	22.1
	LSD	(1, 2, 3, 4, 6, 7)	(1, 2)
	6- Root	1651.4 ± 5.37	412 ± 0.7
	%	33.9	20.3
	LSD	(1, 2, 3, 4, 5, 7)	(1, 2)
	7- Seed	1572.92 ± 1.71	404.2 ± 1.274
	%	27.5	18.0
	LSD	(1, 2, 3, 4, 5, 6)	(1)
ANOVA			
F	P	145.57 0.00	10.17 0.01

Values represent the mean ± S.E of six animals for each group.
LSD: Least significant Difference
P<0.05: statistically significant .

The aqueous methanol extract of *U. pilulifera* contains water soluble polysaccharides[1] which have the ability to increase the level of immune potential activity[29]. The present results are in accordance with different authors; Musette *et al.*[26], found that aqueous extract of *U. dioica* induced antibody production, Nagata *et al.*[27] found that polysaccharides (arabinose, galactose, xylose, glucose and rhamnose) have heterogeneous structure which significantly stimulate humeral immune response, Liou *et al.*[21] showed that fraction containing polysaccharides stimulates antibody production IgG and IgM through the augmentation of splenocytes proliferation; the polysaccharides induce erythrocyte-antibody complement cells and erythrocyte-rosette-forming cells as well as induction of interleukin secretion and interferon-gamma mRNA.

Kan *et al.*[18] investigated the fatty acid composition of the seed oils obtained from *U. pilulifera* and found that linoleic acid was found to be the main fatty acid in *U.pilulifera* (62.99 %), followed by oleic acid (21.91 %) and trace amount of linolenic acid (0.55 %). The presence of linoleic acid, ω- 6 and ω- 3 fatty acids in *U. pilulifera* seeds methanol extract have activate IgM production through the protection of B cell from depletion and enhance antibody production[6,7].

Hormones, better known as the "chemical messengers of the body", are released by specialized organs (called glands) during certain events. One of the chief hormones released under stressful conditions is cortisol. The daily dose of *U. pilulifera* extract for 10 days decreased cortisol concentration TABEL 2. It is released in response to stress and a low level of blood glucocorticoids. Among the primary functions are increasing blood sugar through gluconeogenesis and suppress the immune system[33]. Treatment of mice with the current plant extract decrease cortisol level when compared to control. The decrease in cortisol level is coincided with Irshaid

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TABLE 2 : Effect of *Urtica pilulifera* aqueous methanolic extracts (2.94mg/ kg b.wt./ Day) on hormones.

Groups	cortisol (mg/ml)	Thyroid hormones	
		T_3 (µg/ml)	T_4 (µg/ml)
1- Control	32.99±1.88	67.99±3.81	4.17±0.23
%	—	—	—
LSD	(2,3,4)	(2,3,4)	(2,3,4)
2- Herb	24.43±1.3	80.62±4.29	4.94±0.26
%	-25.95	18.58	18.46
LSD	(1)	(1)	(1)
3- Root	25.57±0.81	84.37±2.66	5.17±0.16
%	-22.5	24.09	23.98
LSD	(1)	(1)	(1)
4- Seed	26.20±0.95	86.77±3.14	5.31±0.19
%	-20.58	27.62	27.34
LSD	(1)	(1)	(1)
ANOVA F P	8.82 0.002	5.60 0.012	5.53 0.013

Values represent the mean ± S.E of six animals for each group.
LSD: Least significant Difference
P<0.05: statistically significant .

and Mansi[17] who found that *U. pilulifera* extract lowered blood glucose level in diabetic rats.

It has been noticed that thyroid hormones regulate the basal metabolic rate and appropriate indicators of thyroid function[14]. The results obtained herein showed that both T3 and T4 level were increase in mice serum.

treated with *U.P.* This is due to the antioxidant activity against various oxidative stress in vivo[22,30]. The antioxidant activity reduces lipid peroxidation in folleculer cells of thyroid gland and improve thyroid function.

The sex hormones, estrogen, progesterone and testosterone, were statistically analyzed and tabulated in TABLE 3. The obtained data show that *Urtica pilulifera* aqueous methanolic extracts have different effects according to the plant parts and sex type of experimental animals. In case of male albino mice the herb extract increased testosterone level by 54.62% in mice treated with herb extract, this mainly due to the presence of linoleic acid (18: 2) which is consumed in arachidonic acid biosynthesis in mammals[28]. Arachidonic acid activates cAMP which increases steroid production significantly[4] but the presence of genistein in *Urtica pilulifera* herb, tyrosine kinase inhibitor, limited its activation followed by reduction of its effect on steroidogenesis[11].

The testosterone level was increased by 150.4% using root extract while estrogen was decreased by 15.43% as compared to control, this may be due to the absence of tyrosine kinase inhibitor (genistein) as well as the extract contains lipoidal matter, i.e. octadecadienoic acid (18: 2) which has an aromatase inhibitory effect. Aromatase is a key enzyme in steroid hormone metabolism that mediates the conversion of androgens to estrogens. As a result of an aromatase inhibition, estrogen levels was decreased[10]. According to the above

TABLE 3 : Effect of *Urtica pilulifera* aqueous methanolic extracts (2.94mg/ kg b.wt./ Day) on sexual hormones in mice serum:

Groups	Female mice		Male mice	
	Estrogen (pg/ml)	Progesterone (ng/ml)	Estrogen (pg/ml)	Testosterone (ng/ml)
1-Control	16.41± 0.7	0.763± 0.08	11.86± 0.34	1.19± 0.008
%	-	-	-	-
LSD	(3, 4)	(2, 4)	(2)	(2, 3, 4)
2- Herb	15.74± 0.59	0.49± 0.07	12.75± 0.2	1.84± 0.07
%	-4.083	-35.8	7.5	54.62
LSD	(3, 4)	(1, 3, 4)	(1, 3, 4)	(1, 3, 4)
3- Root	24.14± 0.59	0.76± 0.06	10.03± 0.27	2.98± 0.04
%	47.11	-0.393	-15.43	150.42
LSD	(1, 2)	(2, 4)	(2)	(1, 2, 4)
4- Seed	20.84± 0.61	1.383± 0.06	11.093± 0.14	0.74± 0.12
%	26.996	81.3	-6.47	-37.82
LSD	(1, 2)	(1, 2, 3)	(2)	(1, 2, 3)
ANOVA F P	1.77 0.02	0.75 0.00	1.7 0.00	1.54 0.00

Values represent the mean ± S.E of six animals for each group.
LSD: Least significant Difference
P<0.05: statistically significant .

theory this extract would improve the patients' prostatic disorder.

The seeds extract inhibited testosterone level by 37.82% in male mice that may due to the presence of non-esterified fatty acids (NEFA) which modulating testosterone synthesis stimulated by leutinizing hormone[23], the inhibition produced by NEFA was dependent on extracellular Ca^{2+}. Oleic acid constituted in *Urtica* seeds, 18.75%, is a more potent inhibitor than is linoleic, stearic or palmitic. The NEFA inhibit steroidogenesis at one of the steps preceding conversion of cholesterol to pregnenolone[24].

In case of female mice, the aqueous methanolic extract of seeds significantly increased progesterone levels by 81.3%, this due to the presence of linoleic

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acid used in arachidonic acid biosynthesis which enhances steroid hormone production such as progesterone and estrogen[36]. The root extract possesses the same effect on estrogen production 47.11% but the level of progesterone still at the control level, these results are in accordance with[31].

aqueous methanolic extract of herb had no significant decreased in estrogen level (-4.1%) and significantly decreased progesterone by 35.8%, this result may due to the presence of tyrosine kinase inhibitor (genistein) which inhibits progesterone through the cAMP inhibition while estrogen level was induced by roots and seeds extracts that may be attributed to the presence of sterols and fatty acids at higher amount than those in herb which contains genistein at high amount.

The above mentioned results show that, aqueous methanolic extracts of *Urtica pilulifera* roots may have guanadotrophic effect therefore it induce synthesis of testosterone in male mice and synthesis of estrogen in female mice without affecting progesterone biosynthesis The herb extract decreased the progesterone level (35.8%) that meaning it reduces the risk factor of breast cancer.

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