IASJ المجلات الاكاديمية العراقية

Tikrit Journal of Pure Science

ISSN: 1813 – 1662 (Print) --- E-ISSN: 2415 – 1726 (Online)

Journal Homepage: http://tjps.tu.edu.iq/index.php/j

The role of *Rheum ribes* roots alcoholic extract in reducing the effect of oxidative stress on the reproductive system of male rats

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https://doi.org/10.25130/tjps.v25i5.286

ARTICLE INFO.

Article history:

-Received: 18 / 12 / 2017

-Accepted: 27 / 2 / 2018

-Available online: / / 2020

Keywords: Reproductive system, Rheum ribes, Oxidative stress, Alcohol extract, Rats.

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ABSTRACT

The current study investigated the effect of *Rheum ribes* extracts alone or synergistically with zinc in the oxidative-antioxidatve status in semen of male rats exposed to oxidative stress with hydrogen peroxide (0.5%) and compared these effects with the role of vitamin E. 40 Male rats were used aged (12-14) weeks and weighed (130_220) g and randomly divided into (8) groups, 5 animals for each as follows:

1-Control group, **2**-hydrogen peroxide group (H_2O_2 0.5%), **3**-Ethanolic root extract of Rheum root + zinc (100 mg / kg bw) + H_2O_2, **4**-ethanolic extract of Rheum root + vitamin E (500 mg / kg),+ H_2O_2,**5**-ethanolic extract of Rheum root (500 mg / kg bw) + H_2O_2,**6**-Zinc group (100 mg / kg body weight), **7**-vitamin E 500 (mg/kg body weight), **8**-ethanolic extract of Rheum roots. The results showed a significant increase ($P \leq 0.05$) in the level of malonadialdehyde (MDA) and a significant decrease in the levels of antioxidant enzymes, catalase enzyme (Cat), superoxide dismutase (SOD), reduced glutathione (GSH) and glutathione peroxidase (GPx) and total antioxidant level in semen. The treatment of rats exposed to hydrogen peroxide (0.5%) with the extract of the roots of the Rheum showed a significant decrease in the level of malonadialdehyde compared with the group of hydrogen peroxide and a significant increase in the levels of antioxidants enzymes in semen. The use of the extract synergistically with zinc sulphate (100 mg / kg bw) and vitamin E (500 mg / kg bw) showed similar results to the control group. Current results indicate the role of the alcoholic extract of the roots of the *Rheum ribes* plant in reducing the effects of oxidative stress and thus improve the function of the reproductive system in male rats.

Introduction

Since the earliest times, in all parts of the world and today, medicinal plants have been used in the traditional medicine system because of their wide therapeutic effects in many diseases. Interestingly, the demand for their use in industrialized countries as well as in developing countries. About 80% of the population in developing countries provide primary health care needs from traditional medicine and 25% of the drugs prescribed and disposed in the United States contain at least one active ingredient of plant origin and some are manufactured in a manner that mimics natural plant compounds. Medicinal plant extracts are made up of effective compounds such as non-food chemicals that have a protective or therapeutic effect for many pathological conditions and have little or no effect compared to laboratory-manufactured chemical drugs [1]. Oxidative stress expresses the state of cells characterized by abnormal production of active oxygen species or weakness in the antioxidant system. The organism combats the accumulation of reactive oxygen species through an antioxidant system that includes enzymatic ones such as catalase and not enzyme Such as glutathione. Despite these mechanisms, oxidative stress is possible with a lack of equilibrium between the antioxidant system and reactive oxygen species causing oxidative damage to many biomolecules such as proteins, nucleic acids and then oxidative stress in the organism are the beginnings of the development of most contemporary human diseases such as

cardiovascular disease, cancer, diabetes, geriatric diseases as well as reproductive diseases [2,3]. 40-90% of infertility in males can be attributed to the decline in sperm count. [4]. Research has indicated an increase in the level of reactive oxygen species with low levels of antioxidants in semen for this type of infertility, although the natural concentrations of reactive oxygen species in semen are necessary for sperm and play a vital role in their functions, mobility and capacitation [5]. Therefore, one of the mechanisms of male infertility is the increased production of reactive oxygen species, which causes oxidative stress [2]. Reducing oxidative stress and preventing it can reduce the incidence of many diseases. Many edible plants and natural antioxidants can be used to control the oxidative-antioxidants status and thus not reach the state of oxidative stress by possessing many effective chemicals such as phenols, riboflavones, terpenes and glycosides, which provide an important source of antioxidants as well as many trace elements such as zinc [6].

Rheum ribes plant are spread in a number of countries around the world, especially Iraq, and are used as food. Fresh vegetables are eaten in the spring and described by folk medicine practitioners for prevention and treatment of many diseases such as ulcers, jaundice, nephritis and ureters. Plant roots are used to treat diabetes, hemorrhoids, atherosclerosis and many infections [7]. The present study was designed to investigate the protective role of *Rheum ribes* alone or synergistically with zinc in the reproductive system of male rats exposed to oxidative stress with hydrogen peroxide.

Materials and Methods

Animals used in the study

40 male rats aged 130- 220 grams were used, animals were placed in plastic cages covered with metal covers of dimensions (30 x 25 x 15 cm), with a wood-sawing floor and the cages sterilized with disinfectants.

Collection of plant and preparation of root alcoholic extract

The roots of the Rheum plant were obtained from the Mariwan area on the Iraqi-Iranian border north of Sulaymaniyah; the roots of the plant were taken and cleaned with special brushes of dust and impurities. The roots were then cut into small pieces using a sharp machine and then dried and grinded with an electric grinder to a fine powder, then stored in a sealed container until it was used in the extraction process.

Design of Experience

The adult experimental male rate (40) were randomly divided into 8 groups, each group included 5 animals.

Group1 G1: (control group) this group was given normal drinking water and daily food for 28 days.

Group2 G2: (H_2O_2) this group was given H2O2 at a concentration of 0.5% with drinking water.

Group3 G3: (*R. ribes* root group + H_2O_2) This group was treated with *R. ribes*root extract 500 mg / kg body weight as well as H_2O_2 at a concentration of 0.5% daily.

Group4 G4: (*R. ribe s*root group + zinc + H_2O_2) This group was treated with *R. ribes* root extract 500 mg / kg body weight as well as zinc 100 mg / kg body weight as well as H_2O_2 with 0.5% concentration with drinking water.

Group5 G5: (*R. ribes* root group + vitamin E + H_2O_2) This group was treated with *R. ribes*root extract 500 mg / kg body weight as well as vitamin E 500 mg / kg body weight as well as H_2O_2 with 0.5% concentration with drinking water.

Group6 G6: (Zinc group) this group was treated with Zinc Sulphate 100 mg / kg body weight orally by tube feeding.

Group7 G7: Vitamin E Group This group was treated with vitamin E 500 mg / kg bw / day by tube feeding daily.

Group 8 G8: (*R. ribes*roots Group) This group was treated with a *R. ribes*root extract 500 mg / kg oral weight through tube feeding.

Hydrogen peroxide is present at 0.5% concentration with drinking water and is changed daily. The dose of ethanolic extract of *R. ribes* root (500 mg / kg) of body weight based to [8]. Vitamin E (0.5 g / kg body weight) was prepared by dissolving 500 mg of this vitamin in 2.5 ml of olive oil and giving each animal 1/2 ml bearing the weight of the animal [9]. Zinc sulfate Prepared with a concentration of 100 mg / kg body weight.

collection of semen

The epididymis was opened using an autopsy kit. The ejaculation of the epididymis was removed. The epididymis was then separated from the testis. After removal of the lipid material, they were placed in a glass petri dish. Using a sharp scalpel, the epididymis was cut into small pieces and a normal saline solution was added [10].

Chemical tests

Determination of glutathione concentration in semen

The concentration of glutathione in semen was estimated using the Ellmans detector method [11]. By mixing an equal volume of 150 microliters of serum or semen and Sulfosalicylic acid solution at a concentration of 4%.

Determination of the concentration of MDA in semen

TBA (0.375gm) Thiobarbituric Acid and Hydrochloric Acid 0.25m were used with Thiobarburic acid (15%) [12].

Determination of the Effect of Superoxide Enzyme in semen

This method is based on the ability of Superoxide Dismutase (SOD) to inhibit adrenaline oxidation to adrenochrome and the reaction is done at 37 ° C [13].

Determination of catalase activity in semen

The efficacy of the catalase enzyme was estimated using the special analysis kit [14].

Determination of total antioxidant content in semen.

(Fe3 +) to Fe2 + and Fe2 + tripyridyltriazine by giving an electron, as Fe3 + (Fe3 +) works to limit Antioxidants in the form prepare the FRAP solution.

Determination of the concentration of glutathione peroxidase in semen.

The concentration of the enzyme was estimated based on the method of [15].

Results

Study of the balance of oxidation_ antioxidants in semen

The following table shows that treatment of male rats with H_2O_2 resulted in significant decrease ($P \leq 0.05$) in GSH, TAC, CAT, SOD, GPx, and significant increase in MDA level in semen compared with control group, While the treatment of rats with H_2O_2 with the ethanolic extract of the roots of the Rheum resulted in significant increase in the superoxide dismutase, catalase enzyme, in addition to decreased glutathione peroxidase, reduced glutathione, total antioxidant content in the semen and a significant decrease in the level of MDA in semen compared to the treatment group H_2O_2, and the administration of rats with H_2O_2 and Rheum roots with zinc (100 mg / kg bw) resulted in close results with a slight decrease compared to the control group. Also, the results of vitamin E (500 mg / kg bw) were comparable with the group of rats that were injected with ethanolic extract of the Rheum root (500 mg/ kg bw) compared to the control.

Values of Oxidation - Antioxidants status parameters in semen

Groups	GPx U/L	SOD U/mL	CAT IU/ml	TAC mmol/L	GSH micromol/L	MDA Micromol/L
control	11.930± 0.025 a	27.547± 0.029 a	31.517± 0.033 a	0.482± 0.003 a	5.762± 0.043 a	0.457± 0.039 bc
controlH_2O_2	8.455± 0.044 e	21.187± 0.026 f	9.912± 0.058 b	0.365± 0.003 c	4.082± 0.031 c	0.627± 0.340 a
Rheum +H_2O_2+Zinc 100 mg	10.170± 0.031 d	25.152± 0.037 e	30.935± 0.037 a	0.473±0.002 a	5.447± 0.029 b	0.435± 0.025 c
Rheum H_2O_2+Vit E	10.872± 0.049 c	26.305± 0.020 c	30.247± 0.031 a	0.423± 0.003 b	5.696± 0.029 ab	0.362±0.022 d
Rheum +H_2O_2	10.147 ±0.029 d	25.642± 0.029 d	30.332± 0.033 a	0.435± 0.003 b	5.442± 0.034 b	0.437± 0.034 c
Zinc 100 mg	11.642 ± 0.33 ab	27.450 ± 0.37 a	31.147 ± 0.041 a	0.483± 0.003 a	5.595± 0.034 ab	0.470 ± 0.040 b
Vit E 500 MG	11.240 ±0.036 b	27.180 ±0.043 a	30.850 ±0.033 b	0.464±0.004 a	5.647±0.033 ab	0.420 ± 0.018 c
Rheum 500 mg	11.322 ±0.029 b	27.645 ± 0.038 a	31.085 ± 0.026 a	0.431±0.002 b	5.680±0.054 ab	0.450 ±0.028 bc

- The difference in letters arranged vertically mean a significant difference at a significant level ($P \leq 0.05$).

Discussion

The results showed that there was a significant increase ($p \leq 0.05$) in the antioxidants parameters in the semen and a significant decrease in the level of MDA in the group of animals that were injected with the ethanolic extract of the Rheum roots with the hydrogen peroxide and the Rheum roots with the zinc sulphate (100 mg / kg bw) with hydrogen peroxide as well as the roots of the Rheum with vitamin E with hydrogen peroxide and vitamin E 500 (mg / kg body weight) and the ethanolic extract of the Rheum roots (500 mg / kg body weight) compared with the control group where these groups without the use of hydrogen peroxide with water Drinking, while the treatment of animals with hydrogen peroxide to a significant reduction of antioxidants and a significant increase in the level of MDA compared with the control group. Hydrogen peroxide resulted in increased lipid peroxidation, accompanied by a decrease in most antioxidants, superoxide dismutase, catalase enzyme, as well as decreased glutathione peroxidase, reduced glutathione and total antioxidant content in semen [16,17].

The reduction of these antioxidants increases MDA, which is the final product of lipid oxidation, and MDA inhibits the manufacture of nucleic acids, inhibits the effectiveness of amino acids and disrupts enzymes and proteins [18,19].The increase in the rate of consumption of glutathione in rats treated with hydrogen peroxide has led to a decrease in its level compared with the control group, which is one of the most important non-enzymatic antioxidants in the removal of free radicals. The sulfur group in the synthesis of glutathione is an important limiting factor as the hydrogen atom is easily released due to the strength of the carbon- (CH) in free radicals, so they protect cellular membranes from damage to free radicals [20].

The study showed that the treatment of rats exposed to oxidative stress with the extract of the roots of the Rheum has led to a significant increase in the level of glutathione in semen. This may be due to the containment of Rheum roots on effective antioxidant compounds such as carotenoids and lycopene as well as amino acids containing sulfur, which enters the synthesis of glutathione [21]. The results of the present study indicate that oxidative stress induced by hydroxide peroxide (0.5%) with drinking water produced in a significant increase in the level of malondialdehyde (MDA) in semen for male rats. In contrast, reduced glutathione, superoxide dismutase and catalase enzyme in addition to decreased

glutathione peroxidase and total antioxidant content compared to the control group and these results are consistent with the study of both [22,23]. It is also consistent with both [24]. Malondialdehyde is produced from oxidation of fatty acids through free radical reactions in lipid peroxidation [25,26]. At the treatment of animals exposed to oxidative stress with the alcoholic extract of the roots of the Rheum, a significant decrease was observed in melonadialdehyde, high level of catalase (CAT), superoxide dismutase (SOD), glutathione peroxide (GSH), and total content of antioxidants (TCA in semen).This means that these extracts have reduced the process of lipid peroxidation resulting from the presence of H_2O_2. This is due in turn to the active substances found in the extract of the root of the Rheum, for example, alkaloids, lycopene, flavonoids and phenols [21].

The role of vitamin E as an antioxidant reduces the harmful effect of hydrogen peroxide by stimulating the catalase enzyme (CAT), which plays an important role by removing the hydrogen atom from hydrogen peroxide and converting it into H_2O water [27], suggesting a reduction in the consumption of glutathione. Vitamin E also prevents oxidation by reducing the free radicals by merging with it. This reaction produces the tocopherol root, which is transferred to the cell surface and is reduced from the enzyme glutathione peroxide (GSH-px) back to the tocopherol again [28].

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دور المستخلص الكحولي لجذور نبات الريواز *Rheum ribes* في خفض تأثيرالاجهاد التأكسدي في الجهاز التناسلي لذكور الجرذان

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الملخص

تضمنت الدراسة الحالية البحث في تأثير المستخلص الكحولي لجذور الريواز *Rheum ribes* لوحده او بالتآزر مع الخارصينفي ميزان الاكسدة–مضادات الاكسدة في السائل المنوي لذكور الجرذان البيض المعرضة للإجهاد التأكسدي المستحدث ببيروكسيد الهايدروجين (0.5%)، ومقارنة هذه التأثيرات مع دور فيتامين E.استخدمت في هذه الدراسة (40) حيواناً من ذكور الجرذان بأعمار (12_14) أسبوع وأوزان(130_220) غم وقسمت عشوائياً إلى(8) مجاميع تضمنت كل مجموعة (5) جرذان وكالأتي:

مجموعة السيطرة، مجموعة بيروكسيد الهيدروجين(H_2O_2 0.5 %) ،مجموعة المستخلص الإيثانولي لجذور الريواز (500 ملغم/كغم من وزن الجسم)+ H_2O_2، مجموعة المستخلص الإيثانولي لجذور الريواز+ الخارصين (100 ملغم/كغم من وزن الجسم) + H_2O_2، مجموعة المستخلص الإيثانولي لجذور الريواز + فيتامين E(500 ملغم /كغم من وزن الجسم)+H_2O ، مجموعة كبريتات الخارصين(100 ملغم/كغم من وزن الجسم)، مجموعة فيتامين E 500 (ملغم/كغم من وزن الجسم) ومجموعة المستخلصالإيثانولي لجذور الريواز. أدى الاجهاد التأكسديإلى ارتفاع معنوي ($P\leq0.05$) في مستوى المالونداياليديهايد وانخفاض معنوي في مستويات مجمل مضادات الأكسدة الأنزيمية, أنزيم الكتاليز , سوبر أوكسيد ديسميوتيز , الكلوتاثيون المختزلوالكلوتاثيونبيروكسيديز والمحتوى الكلي لمضادات الأكسدة في السائل المنوي، أن معاملة الجرذان المعرضة للإجهاد التأكسدي بالمستخلص الكحولي لجذور الريواز أظهر انخفاض معنوي في مستوى المالونداياليديهايد بالمقارنة مع مجموعة بيروكسيد الهايدروجينوإلى زيادة معنوية في مستويات مضادات الأكسدة الأنزيمية في السائل المنوي. أما استخدام المستخلص بالتآزر مع كبريتات الخارصين (100 ملغم/كغم من وزن الجسم) واستخدام فيتامين E (500 ملغم/كغم من وزن الجسم) فأظهرت نتائج مشابهة لمجموعة السيطرة السليمة. وتشير النتائج الحالية الى دور المستخلص الكحولي لجذور نبات الريواز في الحد من تأثيرات الاجهاد التأكسدي وبالتالي تحسين وظيفة الجهاز التناسلي في ذكور الجرذان.