

Effect of *Cucurbita pepo* L Extract on Histology Changes of Some Entry Organs Anatomy in the Males of Rats Exposed to Oxidative Stress

Shurooq Hameed, Ayyub J Abdlrhmaan* and Iktefaa Abdul Hameed

Department of Education, Tikrit Univeresity, Tikrit, Iraq

Abstract

The aim of this study to know the effect cold and hot aqueous extract of Pumpkin fruits on some anatomical characteristics on male rates exposed to oxidative stress by H_2O_2 0.5% on drinking water for 30 days. Fourty white albino rates used at ages 10-12 weeks and 200-250 gm weight divided randomly into 8 groups include 5 rates in each as following group: Without treatment (control), H_2O_2 , cold extract (250 ml/kg), hot extract (250 ml/kg), cold+hot extract, hot extract+ H_2O_2 , cold extract+ H_2O_2 , hot+cold extract+ H_2O_2 . The extracts given by mouth by tube feeding and results showed: Rates given natural water had normal sexual cells and normal seminiferous tubules while the group which given H_2O_2 showed bloody congestion, necrosis epithelial cells, and hyperpigmentation of connective tissues between tubulets. Rates given (hot+cold extract+ H_2O_2) showed increase of seminiferous tubules count also in super fat sperms.

Keywords: Plant extract • Tubulets • Sperms • Bloody congestion • Hyperpigmentation

Introduction

Food of all kinds is a primary source for body building, and also important energy source to do vital functions that includes reproduction and growth. Therefore eating a variety of foods with suitable concentrations has a major role in maintaining these jobs perfectly. Medicinal plants used since ancient times [1] as a cure for many diseases that was affecting humans, despite the development of the pharmaceutical industry it is still 80% the population of developed countries depends on herbs and medicinal plant as medicine because these natural products are free from side effects caused by the manufactured chemical drugs in addition to its cheap and used as a natural source for the drug industry through its possession of some materials attributed to the medical effect [2-5].

Cucurbita pepo vegetable crop belonging to the *Cucurbitaceae* family [6], It is grown in the Mediterranean countries, Turkey, Italy and Egypt and its cultivation is spread in Korea and Spain [7,8]. It contains special compounds and components of vitamins and minerals that help get rid of free radicals in the body, its rich source for proteins, fatty acids also contains vitamin C, which helps to fight free radicals and vitamin A which enhances the body's immunity in addition to containing pectin and salts also contains active compounds as saponins, tri-terpinins, fibers and poly saccharides [9,10].

Oxidative stress is result of disturbance in the balance between the body's defenses and the free radicals in the tissue, including the increase in Reactive Oxygen Species (ROS) like negative super oxide O and hydrogen peroxide H_2O_2 they are among the most dangerous and most toxic types so that it becomes more capable of antioxidants and the body becomes unable to remove the free radicals [11,12], and oxidative stress causes the breakdown of cellular tissues and oxidative stress occurs when exposed to toxic substances, wounds and food poisoning cases [13,14]. The oxidative stress caused by free radicals plays an important role in fertility and infertility. Recent studies have confirmed the role of oxidative stress in heart disease, hypertension, and diabetes [15]. Many plants have been used to treat oxidative stress, including the use of *Nigella sativa* L. plant

oil to treat stress in the reproductive system of white mice and results of studies appeared Ginseng plant enhances sperm greatly in terms of fertility and mobility and Abdurrahman and Tayawi indicated that exposure of male white rats to oxidative stress by H_2O_2 Abdurrahman and Tayawi indicated that exposing male white rats to oxidative stress resulted in a significant increase in the percentages of dead sperm and deformed sperm and a decrease in the number of total sperm. The present study aimed to know the effect of pumpkin plant extract on tests histological sections of rates subjected to oxidative stress [16,17].

Materials and Methods

White male rates were used in experiments (40) at age 10-12 weeks by weight 200-250 gm on plastic cages it is furnished with sawdust from the period (2nd December 2018 to 2nd January 2019). The animals were fed with the diet consisting of 35% Wheat, 35% maize, 20% soy bean and 10% concentrated animal protein with vitamins and antifungal additives, while providing an appropriate temperature 25°C and suitable lighting consist of 10 hours light and 14 hours darkness the concentration of given extract were 250 mg/gm weight and H_2O_2 were 0.5% (NRC,1995). Note that drinking water is exchanged daily and hydrogen peroxide is replaced every 48 hours, so it is effective.

Experiment design

The animals divided to 8 groups each group consist of 5 rates with close weights as follows:

Group 1: Control (natural water).

Group 2: Cold extract.

Group 3: Cold extract+ H_2O_2 .

Group 4: Hot extract.

Group 5: H_2O_2 .

*Corresponding Author: Ayyub J Abdlrhmaan, Department of Education, Tikrit Univeresity, Tikrit, Iraq; Email: mayyubayyup@gmail.com

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Group 6: Hot extract+H₂O₂.

Group 7: Hot extract+cold extract+H₂O₂.

Group 8: Hot extract+cold extract.

At the end of the experiment, food and water were banned for 12 hours, then the animals were anesthetized, the testicles were extracted and placed in the formalin solution for 10 minutes then put on neutral formalin buffer solution for the purpose of preparing for histological examination, the testes were taken and then passed in a series of alcoholic concentrations (75%, 95%, 100%) to remove water from it then xylene for the purpose of clarification and then paraffin embedded in preparation for cutting it, and it was dyed using the double pigmentation method. using the Hematoxylin-Eocene (H&E).

Results

The cross section of the histological section on the control treatment showed the most important criterion, one of the changes one expressed in several parts for example the testes contain of somniferous tubules that consist of sperms in the inside, and the somniferous tubules segregates in them by Connective Tissue (CT) (Figure 1A).

Figure 1B appeared cross section of histological section of tests on rates given cold extract which showed normal semiferous tubules that content on normal sexual cells and sperms in cavities and the cross section of histological section of rates given (cold extract+ H₂O₂) Figure 2A showed simple extending in seminiferous tubules sinus also found little of sperms. Treatment rates with (hot extract+H₂O₂) verily histological section by cross section showed the extent change in this group represented with simple inhibition of spermatogenesis and Congestion (Co) in the tissues and extending in the semiferous tubules with found of a little sperms in itself (Figure 2B). The histological cross of testes by cross section in rates give only hot *cucurbit* extract while Figure 3A observed happening dissociate of cell layer that made up the sperm in the tissue and lack of thickness with extending the middle cavity and lack of sperms count, and from Figure 3B the cross section of histological section for rates given (hot+cold extract+H₂O₂) tests showed the most criterion on of these changes representing with increase of semiferous tubules count and distance of semiferous tubules and super fat in spermatogenesis cells with profusion of sperms in middle cavity of semiferous tubules.

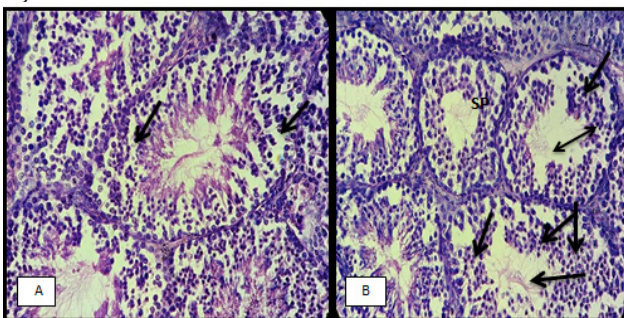


Figure 1. A-Normal sexual cell and sperm in cavity and Normal seminiferous tubules and Extended of seminiferous tubules (H&E400X). B-Inhibition of spermatogenesis cavity (H&E400). Abbrevtaion: SP: Seminiferous tubules

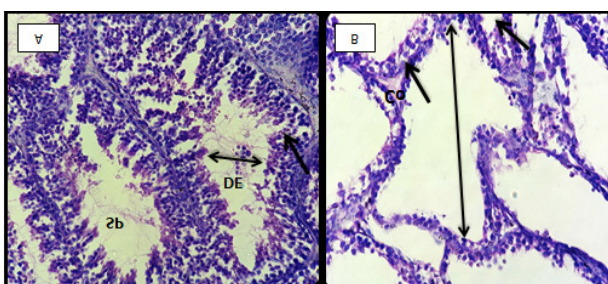


Figure 2. A-Simple inhibition of spermatogenesis extended tubules and

Degradation the germ cell H&E400X). B-Increase spermatogenesis inhibition, extending semniferous cavites congestion interstitial connective tissues (H&E400X). Abbrevtaion: DE: Degradation

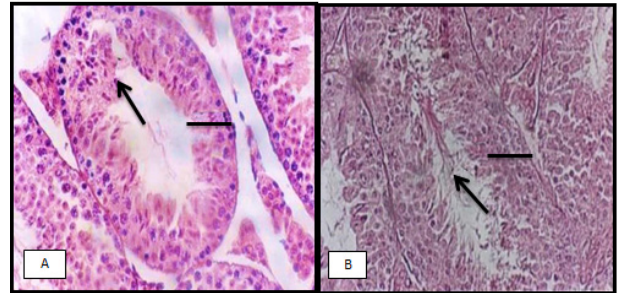


Figure 3. A-Cell layer dissociate that made up sperms lack of thickness with extending the middle cavity (H&E400X). B-Increase of somniferous tubules count and distance of itself super fat in sperms in middle cavity (H&E400X).

In this histological cross section (Figure 4A) appear the semniferous in testes of rates given only (hot+cold extract) a magician on sperms and spermate then the connective tissue and the last treatment (H₂O₂) of rates showed in histological section by cross section found bloody congestion and necrosis of epithelial cells, also hyperpigmentation of connective tissue between the semniferous tubules (Figure 4B).

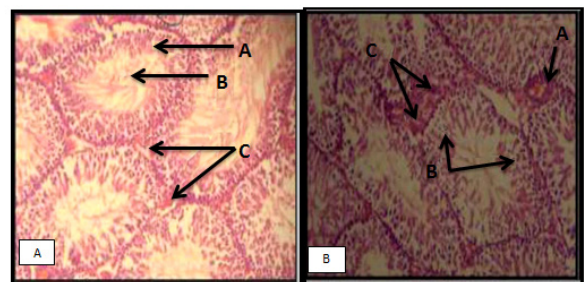


Figure 4. A-The semniferous tubules in testes (A), the sperms (B), and spate them the connective tissue (C) (H&E400X). B-The bloody congestion (A), necrosis of epithelial cell (B), Hyperpigmentation of connective tissues between tubules (C) (H&E400X).

Discussion

Verily giving hydrogen peroxide to animals leads to oxidative stress resulting in the generation of free radicals including the active oxygen classes [18,19]. The weakening of the antioxidant defense systems is a great indication that the cells in a state of oxidative stress [20]. Free radicals affect body tissues and cells and cause many diseases including testicle cells [3,21], increasing free radical production lead to cells need from both enzymatic and non-enzymatic antioxidants, the reason can be attributed to the production of active oxygen classes which lead to a defect in the DNA of energy houses (Mitochondria) which results in genetic mutations that lead to a defect in building proteins. Thus, it leads to an imbalance in the electron transport chain, thereby causing a decrease in energy production (ATP) which affects the functions of cells [22].

The improvement in the shape, size and nature of cells in the testicle due to the use of *Cucurbitaeae* plant extract due to the extract containing vitamin E and this type of vitamin has the ability to capture free radicals such as single oxygen consequently, it leads to inhibiting the activity of free radicals at the level of membranes in their primary stages consequently, it leads to inhibiting the level of membranes in their primary stages consequently, it leads to inhibiting the activity of free radicals at the level of membranes in their primary stages [23,24], also *Cucurbitaeae* extract contain many antioxidant materials and other active ingredients [5]. And that it contains vitamin C gives it an effective role as an antioxidant and it contains from Zinc [9]. Element give it important role on reducing the harmful effects of free radicals, also Zinc improve from sperm motility and increase in number

due to the normal state of testicle cells due to the use of pumpkin extract [25-27].

Conclusion

Food of all kinds is a primary source for body building, and also important energy source to do vital functions that includes reproduction and growth. Therefore eating a variety of foods with suitable concentrations has a major role in maintaining these jobs perfectly. The improvement in the shape, size and nature of cells in the testicle due to the use of *Cucurbitaceae* plant extract due to the extract containing vitamin E and this type of vitamin has the ability to capture free radicals such as single oxygen consequently, it leads to inhibiting the activity of free radicals at the level of membranes in their primary stages consequently.

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