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Effect of *Rhizopus Stolonifer* Metabolic Products on Serum Vitamin C , some elements and catalase in albino male rats

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Abstract

The present study was designed to investigate the effect of *R. stolonifer* metabolic products on some antioxidant defenses and some elements (Cu, Fe, Ca) . The experiment was performed using 25 mature male rats, their age average was about (3-3.5) months and their weight average was about (200-225) gm. The animals were randomly divided into 5 equal groups (five animals for each): control (treated orally with normal saline) and four treatment groups were drenched orally with four concentrations of *R. stolonifer* metabolic products (15, 30, 60,120) µl/kg body weight. The animals were treated with one single dose of the previously described concentrations then left for 15 days. Animals of different groups were sacrificed under light ether anesthesia one day after the end of treatments and blood samples were taken to determine some physiological, biochemical parameters.

The results revealed that the treated groups showed significant decreases ($P< 0.05$) in serum vitamin C and Fe, Cu concentrations. The decrease was proportional with the increase the concentrations of the metabolic products of *R. stolonifer*. On the contrary Catalase activity increased significantly ($P< 0.05$), while Ca concentration didn't differ significantly ($P< 0.05$)

The findings of this study may encourage other researcher for further study especially that we find in our local market that there are many rotted vegetable and fruits which may cause serious problems if consumed for certain period.

Key word: Fungi , Rhizopns stolonifer, antioxidant VitaminC, Serum copper, Serum lron, Serum calcium

Introduction

Fungi, being ubiquitous in distribution, are highly successful in survival because of their great plasticity and physiological versatility. Over the course of history fungi, have been a continuous source of great benefit and risk for human and animals life and significant source of experimental knowledge, thus the importance of kingdom of fungi relates to its interactions on an equal basis with members of plants, animals and prokaryotic microorganism[1]. In terms of abundance of species of fungi with approximately 1.5 million species represent the next major player in the evolutionary drama of the planet [2]. *Rhizopus* belongs to most prominent orders of the Zygomycetes , Mucorales. The Mucorales are mostly saprotrophic and are abundant in soil, on dung and on other organic matter in contact with the soil. They may play an important role in the early colonization of substrata. Sometimes, however, they can behave

as weak pathogens of soft plant tissues [3] . Several species of *Rhizopus* are plant and human pathogens associated [4]. Species of *Rhizopus* are reported from human lesions, and these genera together with species of *Absidia* may also infect domestic animals. *Rhizopus stolonifer* is necrotrophic pathogen cause rots of fruit [3] can cause a rot of sweet potatoes or fruits such as apples, tomatoes and strawberries, such infections may cause spoilage of food [5]. There are about 10 species which grow in soil [6] and on fruits, other foods and all kinds of decaying materials. *Rhizopus stolonifer* (syn. *R. nigricans*) grows rapidly and it is often found on ripe fruits, especially if these are incubated in a moist. Mould infection and mycotoxin contamination of cereal grains can occur in the field during growing, at harvest and during storage. As the grains progress through harvesting and storage, feed manufacture and delivery to farms, therefore public concern on health matters related to food has increased [7,8] and to our knowledge few studies have been carried out to identify the direct relation between these fungi and animal health. Therefore the present study was conducted to investigate the effect of *Rhizopus stolonifer* metabolic products on serum vitamin C, some elements and Catalase in albino male rats.

Methods and Materials

Rhizopus stolonifer

Isolates of *R.stolonifer* cultured in nutrient broth media (from B.D.H) for 21 days. After this period the media was filtered by filter paper (what man no.1) [9] and maintained in clean sterile tubes until used in the experiment.

Animals and Experimental Design

The experiment was performed using 25 mature male rats, their age average was about (3-3.5) months and their weight average was about (200-225) gm. The animals were randomly divided into 5 equal groups (five animals for each): control (treated orally with normal saline) and four treatment groups were drenched orally with four concentrations of *R. stolonifer* metabolic products (15, 30, 60,120) µl/kg body weight. The animals were treated with one single dose of the previously described concentrations then left for 15 days.

Blood Collection

The animals were sacrificed under light ether anesthesia one day after the end of treatment. Blood samples were collected into clean dry centrifuge tubes and left to stand for 30 minutes at room temperature, then centrifuged at 3000rpm for 10 minutes and serum samples were stored in clean tubes at –20C till used for assays.

Vitamin C concentration in serum

Serum vitamin C concentration was analyzed by the method of McCormic (1986) [10]

Total Catalase Activity

The total catalase enzymatic activity was determined manually according to the method described by Aebi (1987) [11]

Determination of elements

Serum iron (Fe), copper (Cu), and calcium (Ca) levels were determined using flame atomic absorption spectrophotometer (GBC 933 plus) and fivefold dilution with de-ionized water. Iron was determined at a wavelength of 284.3 nm, copper at (324.7) nm, and calcium at (319.9) nm. The concentrations of serum Fe, Cu, and Ca were calculated from the standard curves of these elements [12]

Statistical Analysis

The data were analyzed using F test taking $P \leq 0.05$ as the lowest limit of significant difference and Duncan's Multiple Range Test was used to identify group responsible for statistical difference through comparison [13].

Results

Table (1) summarized the results in normal and treated rats in. The treated groups show significant decreases ($P < 0.05$) in serum vitamin C Fe and Cu concentrations. The decreasing was proportional with the increasing of metabolic products concentrations of *R. stolonifer*. On the contrary Catalase activity increased significantly ($P < 0.05$), while Ca concentrations showed insignificant differcef significantly ($P < 0.05$).

Discussion

Fungi were extensively studied, to determine their modifying effects on animal health [5]. Many studies demonstrated different effects and mechanisms of action. Some authors have raised the theory that their action may be a result of disruption of the balance between oxidants and antioxidant. Serum vitamin C was regarded as one of the extracellular antioxidant which neutralizes the free radicals [14].It is a primary antioxidant in plasma and within cells and may contact with plasma membrane [15]. Therefore, the decrease in serum vitamin C concentration may be due to that it acts as a sacrificial antioxidant, since it is able to inhibit the generation of hydroxyl radicals from systems containing copper ions and hydrogen peroxide by a mechanism that involve the immediate attack of vitamin C itself by free radicals, so they become no longer free in solution [16].

The elevation in serum Catalase activity observed in this study can be explained by the fact that this enzyme considered as one of the enzymatic antioxidants inside and outside of the cells [17, 18], thus foreign substances triggers the sequence of biochemical and cellular events associated with inflammation, which include stimulation of activity of the above mentioned enzyme and many other enzymes which have related functions [19].

Trace elements are essential for human and animals. They are constituents of, or interact with, enzymes and hormones that regulate the metabolism of much larger amounts of biochemical substrates [20]. Also they are found at the active sites of many enzymes and have the ability to facilitate the transfer of single electrons to molecular oxygen to produce superoxide anion radical which in turn is converted to hydroxyl radical through Haber-Weiss reaction or Fenton reaction [21]. Decreasing the concentrations of serum Fe and Cu ions may explained on the basis of the roles of these metals, especially their importance in the activity of enzymes [20].

Serum Ca concentration didn't change compared with the other two elements. This may be explained by the fact that most of the calcium in the body is stored in the bones [21], thus any expected alteration in calcium state in the cell, bones may provide an extra amount of needed ions.

The results of the present study indicate that there are alterations in serum concentrations of some elements, vitamin C and catalase , suggesting that they may play a role in the pathophysiology of the fungus.

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Table (1): Effect of *Rhizopus stolonifer* metabolic products on serum vitamin C , some elementsand catalase in albino male rats

treatment / Parameters	C Normal saline	15 µl/kg *R. stolonifer*	30 µl/kg *R. stolonifer*	60 µl/kg *R. stolonifer*	120 µl/kg *R. stolonifer*
Vitamin C (ppm)	9.22 ±0.13 a	9.01±0.18 a	8.79±0.07 a	7.50 ±0.19 b	6.68±0.18 c
Catalase activity (u/l)	143.56±17.72 a	146.7±17.7 a	166.28±4.97 b	183.40 ±15.28 c	185.72±14.2 c
Cu (µg/dl)	145.05±2.03 a	140.00±0.9 a	126±1.00 b	102±3.12 c	85.71±0.77 d
Fe (µg/dl)	140±4.00 a	138.30±1.05 a	131±2.10 a	112±4.65 b	95.00±4.07 c
Ca (mg/dl)	9.95±0.63 e	9.87±0.23 e	9.53±0.71 e	8.86±1.00 e	8.83±1.30 e

*values represented averages ± standard error.

*Different letters mean that there are significant differences (P<0.05).

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تأثير المنتجات الايضية لـ*Rhizopus stolonifer* على تركيز فيتامين C وبعض العناصر وفعالية أنزيم الكاتاليز في مصل دم ذكور الجرذان البيض.

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الخلاصة

صممت الدراسة الحالية من اجل التعرف على تأثير المنتجات الايضية للفطر *Rhizopus stolonifer* في بعض مضادات الأكسدة، والعناصر ، مثل النحاس، والحديد، والكالسيوم. تم تقسيم 25 من ذكور الجرذان، بمعدل عمر تراوح بين(3–3.5) شهراً ومعدل وزن تراوح بين (200–225) غرام، عشوائيا إلى 5 مجاميع متساوية(كل مجموعة ضمت 5 حيوانات): مجموعة السيطرة التي جرعت فمويا بالمحلول الملحي الفسيولوجي واربع مجاميع جرعت فمويا بأربعة تراكيز من المنتجات الايضية للفطر (15، 30، 60، 120) مايكروليتر/ كيلوغرام من وزن الجسم ، وأعطيت هذه التراكيز مرة واحدة فقط ثم تركت مدة 15 يوما. ثم قتلت الحيوانات في المجاميع المختلفة بعد تخديرها بالايثرعند نهاية مدة التجربة بيوم واحد وأخذت نماذج الدم لقياس بعض المعايير.

أظهرت النتائج وجود انخفاض معنوي في تركيز كل من فيتامين C والحديد والنحاس في المصل مع زيادة تركيز المعاملة، بينما شهدت فعالية إنزيم الكاتاليز زيادة معنوية في الحيوانات المعاملة في حين لم يتأثر تركيز الكالسيوم في المصل معنويا.

أن نتائج هذه الدراسة يمكن أن تشجع باحثين آخرين لدراسات أخرى مستفيضة في هذا الموضوع لا سيما أننا نجد في كثير من أسواقنا المحلية العديد من الخضراوات والفواكه المتعفنة أو التالفة التي يمكن أن تسبب الكثير من المشاكل الصحية إذا استهلكت مدة من الزمن.

الكلمات المفتاحية:فطريات ،مضادات الاكسدة ،فيتامين سي،سيروم كالسيوم،سيروم نحاس،سيروم حديد

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