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Effects of Acetaminophen on the Reproductive Parameters and the Ameliorative Effects of Rutin and Mesenchymal Stem Cells in Male Rats

Rabihah E. A. Elduob [1], Eda M. A. Alshailabi [1*], Samia M. Efkeren [1]

1 Zoology Department- Science Faculty- Omar Al-Mukhtar University.

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الملخص

صممت الدراسة الحالية لتقييم آثار الأسيتامينوفين على العوامل الإنجابية والتأثيرات التحسينية للروتين والخلايا الجذعية الوسيطة في ذكور الجرذان. وقد تم تقسيمهم إلى دراستين، أولاً: تم استخدام عشرين جرذا صغيرا كمصدر للخلايا الجذعية المشتقة من نخاع العظم، ثانياً: تم تقسيم خمسين من ذكور الجرذان البالغة إلى 5 مجاميع: المجموعة الضابطة (G1)، وأعطيت الجرذان الأسيتامينوفين (750 ملجم/كجم من وزن الجسم) كل 72 ساعة لمدة 21 يوماً، ثم تركت لمدة 30 و60 يوماً دون معاملة (G2)، أعطيت الجرذان الأسيتامينوفين لمدة 21 يوماً ثم عولجت بـروتين (25 ملجم/كجم من وزن الجسم) لمدة 30 و60 يومًا (G3)، تم إعطاء الجرذان الأسيتامينوفين لمدة 21 يومًا، وتم حقنها بواسطة خلايا جذعية (1.5× 106خلية في 0.5 من محلول فوسفات البوتاسيوم) في الوريد الذيلي لمدة 30 و60 يومًا (G4)، وتم إعطاء الجرذان الأسيتامينوفين لمدة 21 يومًا، و حقنها بواسطة الخلايا الجذعية في الوريد الذيلي ثم عولجت بالروتين لمدة 30 و60 يومًا (G5). أنتج إعطاء الأسيتامينوفين انخفاضًا ملحوظًا في القيمة المتوسطة لمستويات هرمونات التستوستيرون، والملوتن، والهرمون المنبه للجريب بعد 30 و60 يومًا مقارنةً بالمجموعة الضابطة. حيث أن الجرذان المعالجة بالروتين والخلايا الجذعية معا أظهرت زيادة كبيرة في متوسط قيم مستوى هذه الهرمونات بعد 30 و60 يومًا بالمقارنة مع مجموعة الأسيتامينوفين. وفي الختام، أظهرت هذه الدراسة أن الروتين والخلايا الجذعية عند اعطائهما معًا يحميان من الانخفاض الناجم عن الأسيتامينوفين في مستوى هرمونات الخصية.

الكلمات المفتاحية: وظائف الخصية، روتين، خلايا اللحمة المتوسطة، أسِيتامينُوفين، الجرذان.

Abstract

The present study has been designed to evaluate the effects of acetaminophen on the reproductive parameters and the ameliorative effects of rutin and Mesenchymal stem cells in male rats. The rats were divided into two studies: firstly, twenty young male rats were used as a source of bone marrow-derived MSCs. Secondly, fifty adult male rats were divided into 5 groups: Control group (G1); rats were administrated of AAP (750 mg/kg b.w.) every 72h for 21 days, then left for 30 and 60 days without treatment (G2), rats were administrated of AAP for 21 days then treated with RT (25mg/kg b.w/d) for 30 and 60 days (G3), rats were administrated of AAP for 21 days then the rats were injected by BM-MSCs (1.5x 106 cells in 0.5 PBS) in the tail vein for 30 and 60 days (G4), and rats were administrated of AAP for 21 days then the rats were injected by BM-MSCs in the tail vein, then treated with RT for 30 and 60 days (G5). Administration of AAP produced a significant decrease in the mean value of the Te, LH, and FSH levels after 30 and 60 days as compared to the C groups. Whereas rats treated with RT and MSCs showed a significantly high increase in the mean values of these hormone levels after 30 and 60 days compared with the AAP group. In conclusion, this study demonstrated that RT and MSCs when treated in combination, are protected against the AAP-induced decrease in the hormone level of testicular.

Keywords: testes functions; rutin; mesenchymal cells; acetaminophen; rats.

1. INTRODUCTION

The properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites [1]. Rutin (RT) has several pharmacological properties, including antioxidant, anticarcinogenic, cytoprotective, vasoprotective, cardioprotective and neuroprotective activities [2, 3]. Moreover, RT is a flavonoid of the flavonol type that is found in many typical plants, such as buckwheat, passion flower, apple and tea. It is also an important dietary constituent of foods and plant-based beverages [4, 5].

***Correspondence**: Eda M. A. Alshailabi

eda.muftah@omu.edu.ly

On the other hand, RT is known as Vitamin P, and it has been extensively studied and known to exhibit multiple pharmacological activities including antibacterial and antiviral, antiprotozoal, antitumor, antiallergic, anti-inflammatory, and antiplatelets [6, 7].

Acetaminophen (AAP) is the drug of choice for patients who cannot use nonsteroidal anti-inflammatory drugs (NSAIDs) because of hypersensitivity to aspirin, gastric ulcers, impaired blood coagulation, pregnancy, breastfeeding, or fever accompanying disease in children [8]. AAP, also known as (paracetamol or N-acetyl-p-amino-phenol) is the most commonly used antipyretic and pain reliever, and since 1955 it has been a widely analgesic medication in many countries [9,10]. Moreover, AAP is metabolized in the liver by cytochrome P450 (CYP450) enzymes, to N-acetyl-p-benzoquinone imine

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(NAPQI). NAPQI reacts with glutathione (GSH), therefore, overdoses of paracetamol may result in a depletion of hepatocellular GSH [11, 12]. Acute overdose of AAP might cause toxicity of testis in humans and experimental animals [13]. Furthermore, the long-term use of AAP causes toxicity effects in the organs, including hepatotoxicity, renal toxicity, and testicular toxicity. In addition, it affects the blood chemistry and reproductive parameters, [14, 15] such as semen quality, particularly sperm morphology and its fertilizing ability [16]. Also, [14] it was found that the long-term AAP caused an increased risk of oxidative stress, testicular tubules, blood cells, and causing hypertension and heart infarction. However, the administration of AAP is one of the most common causes of toxicity worldwide [17]. Moreover, the AAP-toxicity is the production of the reactive intermediate NAPQI by CYP450 which is removed by conjugation to glutathione in the therapeutic doses, so the higher doses of AAP lead to the drop of cellular content GSH which allows NAPQI to combine with the cellular proteins and induction of lipid peroxidation, leading to toxicity [18, 13, 19].

Bone marrow mesenchymal stem cells (MSCs) are a type of mesoderm-derived stem cells that can differentiate into a variety of cell types, including osteoblasts, chondrocytes, adipocytes, and muscle cells [20], where MSCs have the ability to differentiate into germ cells [21]. Moreover, MSC-secreted factors may protect the spermatogenic dysfunction after busulfan treatment by reducing the apoptosis of spermatogenic cells and enhancing the expression of intercellular adhesion molecules [22]. MSCs are used in the repair and reconstruction of tissues and organs, treatment of degenerative diseases, and anti-aging treatment due to their self-renewal and multidirectional differentiation potential [23, 24].

So, this study aimed to evaluate the effects of acetaminophen on the reproductive parameters and the ameliorative effects of rutin and Mesenchymal stem cells in male rats.

2. MATERIALS AND METHODS:

Chemicals:

- Acetaminophen ($C_8H_9NO_2$) was used. It was purchased from Sigma Chemical Company (USA) [25].
- Rutin ($C_{27}H_{30}O_{16}$) the natural antioxidant was purchased from Sigma Chemical Company (USA) [26].
- Bone marrow-derived stem cells, one important source of mesenchymal stem cells (MSCs), have been isolated and cultured at the Medical Research Center, Aleibbasiuh, Ain Shams University.

Experimental Animals:

A total number of 70 male albino rats (*Rattus norvegicus*) were used. They were divided into two main studies: (S1) consisted of twenty young male albino rats of average weight 100 g that were used as a source of bone marrow-derived MSCs, and (S2) consisted of fifty adult male albino rats that were divided into 5 groups with an average weight of (150-160g) for the experimental study. The rats were obtained from Animal House of El-Salam Farm, Giza-Cairo, Egypt, and were acclimatized to the laboratory conditions for two weeks prior to the start of the experiment. They were housed in metabolic cages at a temperature of 24-27 °C, 12 hours dark/ light cycle, and received standard food and water ad-libitum with fresh daily supplies. The experimental procedures complied with the guidelines of the Committee on Care and Use of Experimental Animal Resources, Ain Shams University, Cairo, Egypt.

Experimental Design:

The first study (S1): Preparation of Bone Marrow-derived Mesenchymal Stem Cells (MSCs):

Twenty young male albino rats of average weight 100 g (6 weeks old) were used as a source of bone marrow-derived MSCs [27]. The rats were injected with BM-MSCs (1.5x 106 cells in 0.5 PBS) [28] in the tail vein [29]. The cultured BM-MSCs were characterized by using NAVIOS flow cytometer by BECKMAN COULTER in the Medical Research Center of Ain Shams University [30].

The second study (S2):

Fifty adult male rats were randomized into five groups 10 rats in each:

Group (1): In the control group (C), the rats were left as the normal control rats with no treatment.

Group (2): Acetaminophen treated group (AAP); the rats were orally administrated with a dose of AAP (750 mg/kg b.w./ every 72h) for 21 days. Then, they were left for 30 and 60 days without any treatment.

Group (3): Acetaminophen with rutin group (AAP+RT), the rats received oral doses of AAP (750 mg/kg b.w./ every 72h) for 21 days. Then, they were treated orally with RT at a dose of (25mg/kg b.w./d) for 30 and 60 days.

Group (4): Acetaminophen with stem cells group (AAP+MSCs), the rats received oral doses of AAP (750 mg/kg b.w./ every 72h) for 21 days. Then, the rats were injected with BM-MSCs (1.5x106 cells in 0.5 PBS) in the tail vein for 30 and 60 days.

Group (5): Acetaminophen with stem cells and rutin group (AAP+MSCs+RT), the rats received oral doses of AAP (750 mg/kg b.w./ every 72h) for 21 days. Then, the rats were injected with BM-MSCs (1.5x106 cells in 0.5 PBS) in the tail vein with treated orally by rutin at a dose of (25mg/kg b.w./d) for 30 and 60 days.

At the end of the experimental period, the rats were overnight fasted after the last dose and blood samples were collected.

Determination of Serum Hormone Levels:

Blood samples were taken into clean and dry screw-capped centrifuge tubes and then centrifuged at 3000rpm for 15 minutes in order to separate clear serum samples. They were then stored at -20ºC until used for the determination of different biochemical parameters. Sera were used for the determination of male sex hormones analysis such as the testosterone hormone (Te), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), were evaluated to determine the sex hormones activities of the control group, and the experimental groups were estimated according to the method of [31] for Te, and [32] for FSH [33] for LH.

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Statistical Analysis:

Results were expressed as mean standard error (SE). The parameters were analyzed using significance by one-way ANOVA. Means were detached using Tukey's test at $P < 0.05$. Also, using the T-test to compare between two means. All statistical procedures were performed with (Minitab version 17).

3. RESULTS: -

Determination of the Testosterone Hormone Level (Te): -

Averages of the Te level were given in Table (1), it has shown a significant decrease ($P < 0.05$) in the mean value of the Te level after 30 and 60 days in the AAP groups (0.8200±0.37&1.2000±0.07) as compared to the C group (4.340±0.246 & 4.340±0.25). Whereas there was no significant ($P < 0.05$) between the AAP and AAP+RT groups (1.2000±0.031 & 1.6600±0.051) in the mean value of the Te level after 30 and 60 days. Also, the mean values of the Te level after 30 and 60 days showed a significant increase in the treated group with the AAP+MSCs groups (1.8200±0.058 & 2.600±0.0511) when compared with the AAP groups. Moreover, the treated groups by AAP+MSCs+RT in the mean value of the Te level after 30 and 60 days showed a significantly high increase (2.5000±0.044 & 2.820±0.153) when compared with AAP groups.

Determination of the Luteinizing Hormone (LH) Level: -

Data recorded for the mean values of the LH level of control and experimental rats were obtainable in Table (1), a significant decline ($P < 0.05$) was found in the mean value of LH level after 30 and 60 days in the AAP rats (0.5200±0.037 & 1.2600±0.075) as compared to the C rats (25.20±1.358 & 5.10±0.23). Although, there was a significant increase ($P < 0.05$) in the AAP+RT group (1.3000±0.031) in the mean value of LH level after 30 days when compared to the AAP rats (0.5200±0.037). No significant effects were observed on the AAP+RT rats (2.1400±0.051) in the mean value of LH level after 60 days as compared to the AAP rats (1.2600±0.075). In addition, the mean values of the LH level after 30 and 60 days revealed a significant increase in the AAP+MSCs groups (1.6800±0.037& 2.680±0.455) compared to the AAP rats in the mean value of LH level after 30 and 60 days. Furthermore, the mean value of LH level after 30 and 60 days demonstrated a significantly high increase in the AAP+MSCs+RT rats (2.9200±0.066 & 4.180±0.199) when compared with AAP groups.

Determination of the Follicle-stimulating Hormone (FSH) Level: -

On measuring the FSH level, the data presented in Table (1) showed a significant decrease ($P < 0.05$) in the mean value of the FSH level in the AAP groups after 30 and 60 days (0.6400±0.040 &1.4000±0.070) compared to the C group (4.560±0.213 & 4.60±0.21). Additionally, the mean value of FSH level showed a significant increase in the AAP+RT group (1.5600±0.087) after 30 days when compared with the AAP rats (0.6400±0.040). Moreover, there were no remarkable changes between the mean value of the FSH level after 60 days in the AAP group (1.4000±0.070) and the AAP+RT group (3.2200±0.20). While the AAP+MSCs rats (1.8400±0.024 & 3.200±0.170) presented a significant increase ($P < 0.05$) in the mean value of the FSH level after 30 and 60 days when compared with the AAP groups. However, the mean values of the FSH level after 30 and 60 days revealed a notably high increase in the AAP+MSCs+RT groups (2.680±0.120 & 4.640±0.301) when compared with AAP groups.

Table 1: Average mean values of creatinine, urea and albumin levels in control and experimental groups.

Duration	Parameter	C	AAP	AAP + RT	AAP + MSCs	AAP+ MSCs+ RT
30 days	Te (ng/ml)	4.340± 0.246^{A}	0.8200±0.37^{D}	1.2000± 0.031^{D}	1.8200± 0.058^{B}	2.5000± 0.044^{C}
	LH (mlU/ml)	5.100± 0.219^{A}	0.5200±0.037^{D}	1.3000± 0.031^{C}	1.6800± 0.037^{C}	2.9200± 0.066^{B}
	FSH (mlU/ml)	4.560± 0.213^{A}	0.6400±0.040^{D}	1.5600± 0.087^{C}	1.8400± 0.024^{C}	2.680± 0.120^{B}
60 days	Te (ng/ml)	4.340± 0.25^{A}	1.2000±0.07^{D}	1.6600± 0.051^{CD}	2.600± 0.0511^{C}	2.820± 0.153^{B}
	LH (mlU/ml)	5.10± 0.23^{A}	1.2600±0.075^{C}	2.1400± 0.051^{BC}	2.680± 0.455^{B}	4.180± 0.199^{A}
	FSH (mlU/ml)	4.60± 0.21^{A}	1.4000±0.070^{C}	2.1000± 0.083^{C}	3.200± 0.170^{B}	4.640± 0.301^{A}

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Data are expressed as mean ± SE of rat within each row, means with different superscript (A, B, C & D) were significantly different at $P < 0.05$, were means superscripts with the same letters mean that there is no significant difference ($P < 0.05$).

*C= Control group, AAP= Acetaminophen treated group, AAP+RT= Acetaminophen with rutin group, AAP + MSCs = Acetaminophen with stem cells group, and AAP+MSCs +RT = Acetaminophen with stem cells and rutin group.

4. DISCUSSION:

The results showed that AAP produced a significant decrease ($P < 0.05$) in the mean value of the Te, LH, and FSH levels after 30 and 60 days as compared to the C group. Similar results were obtained by [13, 17] who found that the administration of AAP revealed a significant decrease in reproductive parameters. A significant reduction in the Te, LH, and FSH levels in treated groups by AAP indicates that the AAP may cause testicular toxicity and impaired fertility. Another study investigated the toxic effects of a high dose of AAP on the reproductive system of male rabbits and mice, which induced several changes and harmfully affected the histological structure of the seminiferous tubules [34, 35, 13]. So, the results suggested that AAP can potentially cause reproductive toxicity when high and long-term treatment.

The AAP+MSCs rats obtained a significant increase ($P < 0.05$) in the mean value of the Te, LH, and FSH levels after 30 and 60 days when compared with the AAP rats. These are in agreement with the results of [36, 37]. These effects may be due to MSCs differentiation into germ cells, where the mechanisms involved in the restorative effects of MSCs on testicular functions are due to the ability of transplanted MSCs to stimulate the production of substances capable of inhibiting ROS**,** cell death, inflammation, and mutagenic activities [36]. On the other hand, MSCs play a role in restoring spermatogenesis by differentiating into sperm cells or maintaining spermatogonial stromal cells [38]. Hence, MSCs could be an essential and powerful approach to treating infertility [36].

In the present study, animals treated with both MSCs and RT after being treated by AAP showed a significantly high increase in the mean values of the Te, LH, and FSH levels after 30 and 60 days were markedly improved when compared with the AAP alone. Combined administration of MSCs and RT reversed the levels of these hormones almost back to nearly normal. Many studies recorded that MSCs or RT treatment resulted in a rapid reversal of testicular injury and marked restoration of normal histological structure of testes [38, 39, 36, 40]. RT administration is an important defensive antioxidant enzyme in tissues, and it preserves the morphology of the testis and spermatogenesis. Moreover, RT is a strong antioxidant and has potent pharmacological capabilities including, anti-inflammatory, immunomodulatory antiviral, anti-angiogenic, anti-mutagenic, and antidiarrheal [40]. Also, [41] reported that the RT detoxifies the oxidative stress produced in the body by various drugs and chemicals, and showed RT has inhibitory effects against the generation of ROS and membrane lipid peroxidation. On the other hand, [42] it demonstrated that the specific responses of MSCs to oxidative stress may play a crucial role in the regulation of tissue homeostasis as well as regeneration of organs after oxidative damage. Also, they said, that the MSCs might have a potential role in treating male infertility and testosterone deficiency. Furthermore, Wang et al. [21] found that MSCs can regulate testicular and ovarian function.

5. CONCLUSION:

In conclusion, this study demonstrated that RT and MSCs when administered in combination, protected against the AAP-induced decrease in the hormone level of testicular, suggesting that RT and MSCs exhibited anti-inflammatory potencies against inflammatory toxicities and testicular function changes induced by AAP.

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