Effect of fenugreek (**Trigonella foenum-graecum**) seed aqueous extract on testes tissue of anabolic steroid treated adult mice

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The effect of fenugreek seeds aqueous extract on testes tissue of androgens high level mice induced by andriol testocap was investigated. In this study 20 adult white mice were used and divided into 4 groups, the first group treated with normal saline (Control group), the second group treated with (20g/Kg Body Weight) of fenugreek seed aqueous extract, the third group treated with andriol (40 mg/Kg Body Weight) and the forth group treated by a combination of fenugreek with andriol. The treatments made by oral gastric intubation for 21 days, the results showed a significant increase (p≤0.01) in body weight and non-significant increase in testicular tissue weight of andriol treated group in comparison with control group and other groups, a significant differences (p≤0.01) in testes weights among andriol, fenugreek and andriol with fenugreek combination groups. The histological results of testes of andriol group showed a cytoplasmic vaculation in the spermatogenic cells and inhibition of the spermatogenesis. The testicular tissue of fenugreek group showed a normal seminiferous tubules structure and normal spermatogenesis. While the sections of fenugreek and andriol group showed a diverse effect comparing with andriol group changes including stimulation of spermatogenesis and semen production. Our findings suggest a protective effect of fenugreek against androgens and it has a strong effect in the increment of spermatogenesis. It can be used in the treatment of elevated androgen level.

**Key words:** *Trigonella foenum-graecum*, andriol testocap, mice testes, histological study.

**INTRODUCTION**

Androgens excess is the most common endocrine disorder in human at reproductive age, they are produced from adrenal gland, ovaries and testes. The excess amount of androgens can affect different tissues with different clinical feature, such as acne, hirsutism and reproductive dysfunction (Wild et al., 2010).

Administration of androgens for short or long term may elevate blood pressure and high density lipoprotein HDL (Hartgens and Kuipers, 2004). Furthermore it may be the cause of atherogenic disorders in athletes (Friedl, 2000).

Androgens (male sex hormones) are a class of hormones that control the development and maintenance of male characteristics. Testosterone and dihydrotestosterone (DHT) are the most abundant androgens in men. Almost all testosterone is produced in the testicles; a small amount is produced by the adrenal glands (Massie et al., 2011).

Use of plants and their extracts for medicinal purposes has been rapidly increased worldwide (Kaviarasan et al., 2007). (**Trigonella foenum-graecum**) commonly known as fenugreek is one of the oldest medicinal plants originating in India and northern Africa, it has a long history of medical uses in the medicine, and has been used for numerous induction, aiding digestion and a general tonic to improve metabolism and health and also have a hypoglycemic and anti-hyperlipidemic properties (Geetha et al., 2011). Fenugreek has antioxidant and antibacterial effect and also has a high fiber content, alkaid, flavonoids, saponin and volatile content (Meghwai and Goswami, 2012). a lot of studies suggest a protective antioxidant effect of fenugreek extract on different tissues (Lamfon, 2012).
Baquer et al. (2011) referred that fenugreek seed extract reduce serum triglycerides, total cholesterol and low density lipoprotein cholesterol. Some studies were provided evidence for the potential estrogenic activities of chloroform extract of fenugreek seeds, and it could be one of the important dietary ingredients in the treatment of post menopausal health in women (Sreeja et al., 2010). Another study reported that fenugreek seeds prevent Adriamycin-induced cytogenic and testicular damage in albino rats (Sakr et al., 2012). The aim of this study was to investigate the effect of fenugreek extract on testis tissue of animals that suffering from high androgens level induced by andriol testocap.

MATERIAL AND METHODS

Preparation of fenugreek aqueous extract and andriol testocaps

Fenugreek seeds were purchased from the local market, seeds were grind then 20 g of fenugreek powder was melted in 100 ml of distilled water (D.W) for 1 hour, and then the extract was left over night, then filtered and completed to 100ml. The solution kept in the refrigerator in 4°C (Ehteram, 2008). 1ml of the solution used for the treatments of animals. Andriol testocap (40mg) capsules contain (testosterone undecanoate).

Experimental animals

20 adult male albino mice were used in the present study, their average weights were (33.56 ± 1.035) g, and these animals were kept under suitable environmental conditions of 20 - 25 °C in an air conditioned room and photoperiod of 12 hours daily, and has been divided into 4 groups, 5 animals for each.

The first group represented the control group, treated with normal saline. The second group treated with 1ml of fenugreek aqueous extract (20g/Kg body weight). The third group treated with 1ml/ Kg body weight of andriol testocap (40mg) by oral gastric intubation. The fourth group treated with a combination of 0.5 ml/Kg (body weight) fenugreek and 0.5 ml/Kg (body weight) andriol by oral gastric intubation.

Histological study

The animals were dissected and testes were removed and kept in 10% of formaline solution, then specimens were dehydrated in an ascending series of alcohol, cleared twice in xylene and embedded in paraffin. Sections of 5 microns thickness were cut using rotary microtome and mounted on clean slides. For histological and counterstained with eosin (Lefebvre, 2001).

Statistical analysis

Statistical analysis of all values were presented as means ± standard error of the means (SEM) differences between groups means were calculated by a one-way analysis of variance (ANOVA), and Duncan’s multiple range. Results were considered statistically significant under level p ≤ 0.01.

RESULTS

Body weight and testes weight

The results showed that there was a significant increase in body weight and testes weight (P≤ 0.01) in andriol treated mice in comparison with control group, and a significant decrease (P ≤ 0.01) in body weight and testes weight in andriol and fenugreek combination in comparison with andriol group alone (Table 1) (Figure 1) (Figure 2).

Histological results

The histological results of Andriol treated group showed a damage effects on the testicular tissue, there was a cytoplasmic vaculation, degeneration and inhibition of the germinal cells of the seminiferous tubule, and inhibition of spermatogenesis (Figure 4), in comparison with normal seminiferous tubules shape and spermatogenesis of control group (Figure 3).

While the histological results of fenugreek treated group showed a normal seminiferous tubules shape with normal spermatogenesis (Figure 6) in comparison with control group (Figure 3). Whereas, in andriol and fenugreek combination group, the results showed an improvement effect of fenugreek on seminiferous tubules and the spermatogenesis increased (Figure 5).

DISCUSSION

In the present study, fenugreek showed a weight lowering effect in treated groups (Table 1), that’s related to the fact that fenugreek contain diosgenin, which is a lipid lowering potential structure, diosgenin decreased the elevated cholesterol in serum, LDL and HDL cholesterol in fed rats, by the inhibition of cholesterol absorption and suppress it’s accumulation in liver. A lot of evidence proved the ability of fenugreek to control the metabolic diseases like diabetes and obesity (Ulbricht et al., 2007) by lowering adipocytes size and reduce the accumulation of lipids (Uemura et al., 2010; Sauviar et al., 2000). Whereas the histological results showed a normal seminiferous tubules, this attributed to the fact that fenugreek seeds are reported to contain lysine, phytic
Table 1. Effect of different treatments on body weight and testes weights of mice.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Animal number</th>
<th>Body weight (g)</th>
<th>Testes weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>5</td>
<td>33.56±1.035</td>
<td>0.53±0.070</td>
</tr>
<tr>
<td>Fenugreek</td>
<td>5</td>
<td>31.10±0.190</td>
<td>0.42±0.042</td>
</tr>
<tr>
<td>Andriol</td>
<td>5</td>
<td>39.09±0.464*</td>
<td>0.75±0.667*</td>
</tr>
<tr>
<td>Andriol &amp; Fenugreek</td>
<td>5</td>
<td>35.25±0.982</td>
<td>0.38±0.031*</td>
</tr>
</tbody>
</table>

* Significantly different from control (P ≤ 0.01).

Figure 1. The effect of different treatments on body weight.

Figure 2. The effect of different treatments on testes weight.

Figure 3. Testis section of control group, showing normal seminiferous tubule shows normal spermatogenesis (H&E) 400X.

Figure 4. Testis section of andriol testocap (anabolic steroid) treated mice shows cytoplasmic vaculation, degeneration, and inhibition of spermatogenesis (H&E) 400X.

Acid, minor steroidal saponins and galactomannans, these galactomannans have a unique structure that responsible for some of the therapeutic properties attributed to fenugreek (Billaud, 2001; Puthenpura et al., 2006).

Andriol leads to significant increase in body weight and testes weight, that’s due to the fact that one of the anabolic actions of anabolic steroids is the effect on
protein metabolism by stimulation of protein synthesis and inhibition of protein breakdown (Hartgens and Kuipers, 2004). Also androgens increase gene transcription in adipose tissue through the activation of the androgens receptors (Mammi et al., 2012). The histological results showed a cytoplasmic vaculation, degeneration of germinai layer cells and inhibition of spermatogenesis (Figure 4) in comparison with control group (Figure 3) these results can be explained by the fact that androgen receptors signaling mediates many physiological and pathophysiological processes in various tissues/organs, people with hyper-androgenemia also have higher risk of developing visceral obesity, metabolic syndrome, type 2 diabetes and cardiovascular diseases, in addition to hirsutism and infertility (Chang et al., 2013).

Histological results showed that fenugreek ameliorated the changes of andriol in testes tissues of treated groups, there was an improvement in seminiferous tubules shape, stimulation of spermatogenesis (Fig 5), that’s may be related to the fact that fenugreek seed extracts exhibited antioxidant property that could ameliorate the alterations induced in mice treated with andriol testocaps, the seminiferous tubules look normal with normal spermatogenesis.

These results agreed with a previous studies which refered to using of fenugreek in combination with different materials that known to cause damage to testes tissue. Lamfom (2012) found that the usage of fenugreek with carbendazim pesticides showed marked improvement in the mean diameter and in germ cell height in comparison with carbendazim treated animals, also after aspirin usage (Chaloob et al., 2010) and cadmium – induced testicular damage (Arafa et al., 2014). Fenugreek may increase testosterone level, by increasing the secretion of FSH and LH hormones from the pituitary gland to increase semen and ova production, and the usage of fenugreek extract may be a useful treatment for increasing sexual arousal and desire in women by increasing testosterone level (Rao et al., 2015).

Fenugreek has antiandrogen activities, due to beta-sitosterol, palmitic-acid and stearic-acid, and also has the ability to lower total cholesterol, LDL, VLDL cholesterol and triglycerides significantly according to several reports. *Trigonella* seed extract contains saprogenic and diosgenin, which are precursor of progesterone and has anti-gonadotropine and anti-androgenic character, fenugreek extract exerts both antifertility and antiandrogenic activities (Al-janabi, 2011).

Our findings disagree with another study which stated that the usage of fenugreek extract lead to a significant reduction in number of graafian follicle in female mice in dose dependent manner (Modaresi et al., 2012).

**Conclusion**

In conclusion, fenugreek seed extract could ameliorate the histological changes induced by excessive androgens. It’s recommended to use of fenugreek extract during androgens intake in order to reduce the side effects of androgens on body tissues. Depending on human and animal studies, fenugreek had been considered by many researchers as a potential medicinal herb. However, many of its effects are subjected to lack of clinical trials confirmation. Therefore, detailed clinical studies are needed to strengthen these findings.

**REFERENCES**