Effect of Fenugreek Seed Extract on Some Haematological and Biochemical Parameters in Atrazine Treated Male Rats

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ARTICLE INFO

A B S T R A C T

Atrazine (ATR) is one of the most commonly used herbicide. The present study designed to investigate the effect of boiled aqueous extract of 2.5% and 5% of fenugreek seeds in the diet with 150 mg ATR/kg body weight (B.W.), on B.W., food intake, some haematological and lipid profile parameters, and histological sections of liver and kidney in male albino rats for 4 weeks. The animals were divided equally and randomly into four groups including ATR, ATR+2.5% fenugreek, ATR+5% fenugreek and control group. The animals B.W. in weeks 2 and 3 was decreased significantly in ATR group when compared to control, while it was decreased non significantly in all weeks of ATR+2.5% fenugreek and significantly in week 2 in ATR+5% fenugreek from ATR group. Food intake in ATR group was decreased in all weeks, while it was changed non significantly in ATR+2.5% and ATR+5% fenugreek from ATR group. Non-significant change occurred in haematological parameters of all experimental groups. Total cholesterol in ATR+2.5% fenugreek and in ATR+5% fenugreek were reduced non significantly from ATR group. Meanwhile, cholesterol was increased non significantly in ATR+2.5% fenugreek and significantly in ATR+5% fenugreek from control. Triglyceride TG and LDL in ATR group were increased non significantly from control. While in ATR+2.5% fenugreek and ATR+5% fenugreek they were reduced non significantly from ATR group. HDL was decreased non significantly from control. Whereas in ATR+2.5% fenugreek was increased non significantly from ATR and control as well. Fenugreek decreased degeneration in liver and kidney sections, however the nuclei of some cells in ATR+5% fenugreek were recognized with dark color. In conclusion the present study suggested that fenugreek reduce ATR toxicity, and the aqueous extract of 2.5% of fenugreek is preferred on 5% against ATR.

1. INTRODUCTION

Atrazine is a chlorinated s-triazine group of herbicide due to its extensive use, long half-life and various toxic properties, it has very high environmental significance (Ghosh and Philip, 2006). In an attempt Simic et al. (1994) demonstrated that male rats were exposed to 120 mg ATR/kg B.W. for seven days caused significant decrease in B.W. during the period
of treatment. In another study, Fischer rats were treated via intraperitoneal with 60 and 120 mg ATR /kg B.W. twice a week over 60 days showed decreasing in the B.W. (Kniewald et al., 2000). Moreover, investigators exposed rats to 200 mg ATR /kg B.W. orally for 7 and 16 days had their B.W. and feed intake, significantly reduced (Abarikwu et al., 2010). In an investigation, 120 mg/kg B.W. of ATR induced oxidative stress in liver and kidney of mice, decreased activities of various antioxidant enzymes, and increased Lipid peroxidation (LPO) (EL-Shenawy et al., 2011). Other study suggested that exposure to ATR 50 mg/kg B.W. caused a significant elevation in serum total cholesterol, triglyceride (TG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) in addition to significantly decrease HDL in male rats (AL-Attabi and AL-Diwan, 2012). Besides that, ATR decreased red blood cells (RBCs), haemoglobin (Hb), haematocrit (HCT) and increased white blood cells (WBCs) in fish cyprinus carpio (Linn) (Venkatesan et al., 2012). In an experiment, ATR administration affected adversely on the endocrine system and reproductive tissue development in rats (Cooper et al., 2007). Also, 200 mg/kg B.W. of ATR, caused a delay in pubertal progression and reproductive tract development, and altering the secretion of steroids in male rat when administrated during the juvenile and peripubertal period (Stoker et al., 2000). It has been reported that fenugreek as a medicinal plant is well known as antidiabetic plant, has hypoglycemic effect in human being (Sharma, 1986), and in diabetic animals (Khosla et al., 1995; Grover et al., 2002; Vats et al., 2002). It decreases total cholesterol, VLDL and LDL in rats (Petit et al., 1995). Rao et al. (1996) showed that haematological parameters like Hb, PCV and total as well as differential WBC counts were not affected by animal diet supplemented with fenugreek seeds at 5, 10 and 20 gm% level in male and female rats. Another study showed that rats were treated with fenugreek caused non-significant changes in RBC, WBC and Hb (Khalil, 2004). The effect of oral administration of an aqueous extract of fenugreek seeds in various doses 300-900 mg/kg B.W. for 14 days on haematological parameters (Hb, RBC, PCV and WBC) in albino rats, showed a significant increase in the levels of Hb, WBC and PCV after seven days of extract treatment but the levels decreased when treatment continued to the 14th day. While it elevated the WBC counts after 7 and 14 days treatment (Effraim et al., 1999). In addition, fenugreek oil and fenugreek seeds powder showed a significant increase in blood Hb and PCV in normal male albino rats (Helmy, 2011). The study of Stark and madar (1993), demonstrated that purification of the crude extract by dialysis produced an isolated component with haemolytic properties. The dialysate was also found to contain saponins demonstrated by thin-layer chromatography. Also investigations suggested that fenugreek seed extracts could act as potent source of antioxidants (Bukhari et al., 2008). Sur et al. (2001) demonstrated anti-inflammatory and antineoplastic effects of fenugreek seeds extraction in mice. It was reported that, fenugreek high phenolic contents may provide a source of dietary antioxidant (Kaur and Kapoor, 2002). Devasena and Menon (2002) showed that fenugreek exerts its chemo preventive effect by decreasing circulatory LPO and enhancing antioxidant level in male rats during 1, 2-dimethylhydratine-induced colon carcinogenesis. Moreover, fenugreek showed a reversal of the disturbed antioxidant levels and peroxidative damage (Genet et al., 2002). Furthermore, chronic oral ingestion of the aluminum salts induced nephrotoxicity, as well as caused toxicity in the brain and bone. However, the maintenance of a diet supplemented with fenugreek seeds could offer protection for the kidney, bone and brain, at the same time (Belaid-Nouira et al., 2013). Since there is no data deals with the effect of fenugreek against ATR, therefore the research plan was established to investigate the aqueous extract of fenugreek seed against ATR on B.W., some haematological and serum lipid profile parameters, histological sections in liver and kidney in male rats.
2. MATERIALS AND METHODS

The rats (Rattus norvegicus) were inbred in the animal house of Biology department of Education College, Salahaddin University-Erbil, Iraq. The animals acclimatized in an environmentally controlled room at constant temperature 22 ± 2°C, on a lighting schedule 12 h light and 12 h darkness. They were given control diet (Pico Lab. Rodent diet 20) and tap water ad libitum.

According to Pandit et al. (1979) with some alterations the boiled aqueous extract of fenugreek (Trigonella foenum-graecum) seed powder prepared by boiling 25 gm from powder in 250 ml of water for 30 min. then the mixture was filtered through 8 layers of gauze and directly mixed with diet for obtaining the diet containing aqueous extract of 2.5%, and 5% fenugreek seed. (At the same time samples of filtrate from boiled aqueous extract were dried in oven 60°C from each 25 gm fenugreek seed 243 mg net powder was obtained. So the diet with 2.5% fenugreek seed contains 24.3 gm net powder and the diet with 5% fenugreek seed contains 48.6 gm net powder).

The commercial formulation of Atrazine-50%WP each Kg contains ATR 50%W/W active ingredients (Vapco, Jordan, www.vapco.net). It is widely used as selective herbicide in Iraq-Kurdistan region. In the present study the final dose of ATR 150mg/kg B.W. was prepared from ATR 50%. The herbicide was suspended in corn oil (Afia oil company, Saudi Arabia).

2.1. Design of the experiment

Twenty eight adult male rats weighing (253-339) gm were randomly and equally divided into four groups, each group containing 7 males, and each male was individually caged. Group I (control): Rats were received control diet and corn oil 0.3 ml/rat as vehicle orally by needle gavage and tap water ad libitum.

Group II: Rats were received control diet, 150 mg/kg B.W. ATR in 0.3 ml/rat corn oil orally by needle gavage, and tap water ad libitum.

Group III: Rats were received diet containing aqueous extract of 2.5% fenugreek, 150 mg/kg B.W. ATR in 0.3 ml/rat corn oil orally by needle gavage, and tap water ad libitum.

Group IV: Rats were received diet containing aqueous extract of 5% fenugreek seeds, 150 mg/kg B.W. ATR in 0.3 ml/rat corn oil orally by needle gavage, and tap water ad libitum.

2.2. Body weight and food intake

At the beginning of the experiment and the end of each week, the weight of animals were recorded in gm, and at the end of the first three weeks the weights of diet also were recorded in gm/rat.

2.3. Collection of blood samples

After 24 hours fasting at the end of treatment period, the male rats of the experiment were anesthetized by a combination of ketamine (90 mg/kg B.W.) and xylazine (10 mg/kg B.W.) intraperitoneally (Keane et al., 1999). Blood samples were collected through cardiac puncture, some of which centrifuged at 3000 rpm (revolutions per min) for 20 min at 4°C (Sanyo Ultra-Low Temperature, Japan) until they assayed (Archer and Jeffcott, 1977). And the remained blood used for haematological parameters.

2.4. Dissection and removal of the organs

After withdrawal of blood samples, animals were dissected. The liver and kidney were removed then fixed in 10% formaline.

2.5. Histological sectioning

Preserved tissue samples from fixative solution exposed to serial processes began with dehydration, clearing and impregnation using a series of graded ethanol in ascending concentrations then immersed in xylene. Finally embedded in paraffin wax and cooled. Paraffin sections were cut by rotary microtome (QPJ-1.) (Drury and Wallington, 1980). Then they were stained with haematoxylin and eosin (Bancroft and Gamble, 2008). Finally photos were taken by novel digital microscope (XZS-N107T, China).

2.6. Measurement of haematological parameters

Haematological parameters including Haemoglobin (Hb), Packed cell volume (PCV), Red blood cells (RBCs), White blood cells (WBCs), platelets (PLT), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular
haemoglobin concentration (MCHC), Lymphocytes (LY), Monocytes (MO), and Granulocytes (GR) for each group were measured by coulter counter (Nihon Kohden, MEK-6410K, Japan) (El Hendy et al., 2001).

2.7. Measurement of serum lipid profile

2.7.1. Total cholesterol

The cholesterol is determined after enzymatic hydrolysis and oxidation. Three tubes prepared for sample, standard and blank, 1ml of working reagent added to three tubes, then 10μL of serum added to sample tube, 10μL of standard added to standard tube, and 10μL of demineralized water added to blank tube, after that contents of tubes mixed and incubated (Memmert, Germany) for 5 min at 37°C. Absorbance of sample and standard were measured at wavelength 546 nm against reagent blank. Serum total cholesterol was calculated by the following equation:

\[ \text{Total chol. conc. (mg/dl)} = \frac{\Delta A_{(s)}}{\Delta A_{(std)}} \times \text{conc.}_{(std)} \]

\( \Delta A_{(s)} \): Absorbency of serum (sample)
\( \Delta A_{(std)} \): Absorbency of standard
\( \text{conc.}_{(std)} \): Concentration of standard = 200

2.7.2. Triglyceride

Serum TG was determined by the enzymatic colorimetric test – CHOD-PAP Method according to the laboratory kit obtained from (Centronic GmbH, Germany). Serum TG was calculated by the following equation:

\[ \text{TG. conc. (mg/dl)} = \frac{\Delta A_{(s)}}{\Delta A_{(std)}} \times \text{conc.}_{(std)} \]

\( \Delta A_{(s)} \): Absorbency of serum (sample)
\( \Delta A_{(std)} \): Absorbency of standard
\( \text{conc.}_{(std)} \): Concentration of standard = 200

2.7.3. High density lipoprotein

Serum HDL-Cholesterol was determined using the precipitation with phototuncsic acid Method according to the laboratory kit obtained from (Centronic GmbH, Germany). Calculation:

\[ \text{HDL – Chol. conc. (mg/dl)} = 325.1 \times A_{(s)} \]

\( A_{(s)} \): Absorbency of serum (sample)

2.7.4. Low density lipoprotein

Calculation of low-density lipoprotein

\[ \text{LDL} \left( \frac{\text{mg/dl}}{\text{dL}} \right) = \text{Chol} - \left( \text{HDL} + \frac{\text{TG}}{5} \right) \] (Friedwald et al., 1972)

\( \text{LDL} \): Concentration of serum low-density lipoprotein
\( \text{HDL} \): Concentration of serum HDL
\( \text{TG} \): Concentration of serum triglyceride

2.8. Statistical analysis of results

Data were analyzed statistically by one-way Analysis of Variance (ANOVA) and Duncan test by using Statistical Package for the Social Sciences (SPSS) version 16.0 with significant level fixed at \( p < 0.01 \) and \( p < 0.05 \). To present the result in tables and figures. Data are expressed as mean ± standard error (mean ± S.E.).

3. RESULTS AND DISCUSSION

3.1. Effect of fenugreek on body weight and food intake in male rats treated with atrazine

The effect of ATR, ATR+2.5% fenugreek, ATR+5% fenugreek on B.W. and food intake are shown in table 1 and 2 respectively. Treatment with ATR decreased the B.W. significantly in week 2 \( p < 0.01 \), and in week 3 \( p < 0.05 \). Also food intake in ATR group was decreased significantly \( p < 0.05 \) in all weeks when compared to control. This declination is supported by the findings of (Simic et al., 1994) who showed that exposure of rats to 120 mg ATR/kg B.W. for 7 days caused significant loss of B.W. during the period of treatment. Also the reduction in B.W. of ATR treated rats is confirmed by (Trentacoste et al., 2001; Victor-Costa et al., 2010). Furthermore the alteration in B.W. is supported by reduction in food intake in all three weeks when compared to control (Table 2). However, Farombi et al. (2013) reported that ATR had non-significant effects on feed intake and B.W. in male rats. This controversy may be either return to dose difference between the present and previous studies or to inert compounds of ATR 50%. At the same time our explanation is supported by other investigators who reported that the B.W. loss by ATR due to the reduction in food consumption (Stoker et al., 2000; Akuna et al., 2011). Besides that, the role of ATR in
pituitary weight loss by degradation leads to reduction in growth hormone and hence B.W. loss (Stoker et al., 2000; Fan et al., 2007). Also may affect orexin A and ghrelin levels. Therefore more studies are needed to confirm and interpretation the results.

In contrast, the B.W. in ATR+2.5% fenugreek and ATR+5% fenugreek were decreased from control in all weeks of treatment. Treatment with ATR+2.5% fenugreek non significantly changed the B.W. in all weeks when compared to ATR treated rats. Whereas ATR+5% fenugreek decreased B.W. in week 2 significantly \( p < 0.01 \) as compared to ATR treated rats. In great extent, alterations in B.W. is supported by significant decrease in food intake of ATR, ATR+2.5% fenugreek, ATR+5%fenugreek in all 3 weeks from control, except ATR+2.5% fenugreek in which was decreased non significantly in week 3 (Table 2).

Table (1): Effect of ATR, ATR+2.5% fenugreek, ATR+5% fenugreek on body weight in male rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>ATR</th>
<th>ATR + 2.5% fenugreek</th>
<th>ATR + 5% fenugreek</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment (gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>319.71429±</td>
<td>316.500±</td>
<td>314.28571±</td>
<td>300.85714±</td>
<td></td>
</tr>
<tr>
<td>10.756425 (^a)</td>
<td>15.117398 (^a)</td>
<td>11.279383 (^a)</td>
<td>12.892280 (^a)</td>
<td></td>
</tr>
<tr>
<td>Week 1* (gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>323.28571±</td>
<td>285.37500±</td>
<td>279.42857±</td>
<td>248.14286±</td>
<td></td>
</tr>
<tr>
<td>13.001308 (^a)</td>
<td>14.0444 (^a)</td>
<td>15.844268 (^b)</td>
<td>9.585278 (^b)</td>
<td></td>
</tr>
<tr>
<td>Week 2** (gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>342.28571±</td>
<td>295.50000±</td>
<td>290.71429±</td>
<td>251.42857±</td>
<td></td>
</tr>
<tr>
<td>11.736622 (^c)</td>
<td>13.961682 (^b)</td>
<td>13.714286 (^b)</td>
<td>11.975883 (^a)</td>
<td></td>
</tr>
<tr>
<td>Week 3* (gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>349.85714±</td>
<td>305.75000±</td>
<td>293.42857±</td>
<td>269.85714±</td>
<td></td>
</tr>
<tr>
<td>10.777906 (^c)</td>
<td>12.720000 (^b)</td>
<td>14.235000 (^b)</td>
<td>10.944511 (^a)</td>
<td></td>
</tr>
<tr>
<td>Week 4* (gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>360.00000±</td>
<td>310.00000±</td>
<td>301.28571±</td>
<td>286.00000±</td>
<td></td>
</tr>
<tr>
<td>13.520707 (^c)</td>
<td>13.88858 (^a)</td>
<td>13.985902 (^a)</td>
<td>14.200939 (^a)</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as mean ± S.E. the same letters mean non-significant differences while the different letters mean significant differences *\( p<0.05 \) **\( p<0.01 \)

Table (2): Effect of ATR, ATR+2.5% fenugreek, ATR+5% fenugreek on food intake in male rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>ATR</th>
<th>ATR + 2.5% fenugreek</th>
<th>ATR + 5% fenugreek</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1* (gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90.500000±</td>
<td>78.60000±</td>
<td>80.80000±</td>
<td>82.60000±</td>
<td></td>
</tr>
<tr>
<td>2.997221 (^c)</td>
<td>2.976575 (^ab)</td>
<td>3.929377 (^a)</td>
<td>1.630951 (^a)</td>
<td></td>
</tr>
<tr>
<td>Week 2* (gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>118.00000±</td>
<td>86.00000±</td>
<td>88.00000±</td>
<td>89.80000±</td>
<td></td>
</tr>
<tr>
<td>11.509416 (^b)</td>
<td>3.492850 (^a)</td>
<td>12.177849 (^a)</td>
<td>8.187796 (^a)</td>
<td></td>
</tr>
<tr>
<td>Week 3* (gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>114.00000±</td>
<td>84.60000±</td>
<td>110.20000±</td>
<td>101.80000±</td>
<td></td>
</tr>
<tr>
<td>4.250490 (^c)</td>
<td>7.082372 (^a)</td>
<td>9.409570 (^ab)</td>
<td>6.374951 (^ab)</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as mean ± S.E. The same letters mean non-significant differences \( * p<0.05 \) **\( p<0.01 \)
3.2. Effect of fenugreek on haematological parameters in male rats treated with atrazine

The Effect of ATR, ATR+2.5% fenugreek and ATR+5% fenugreek on haematological parameters (WBC, RBC, Hb, PCV, MCV, MCH, MCHC, PLT, LY, MO and GR) are shown in table 3. An administration of ATR caused non-significant change in all haematological parameters when compared to control. Indeed, there is little information concerning with the effect of ATR on haematological parameters in laboratory animals. ATR against some blood parameters was investigated with fish common carp *Cyprinus carpio*. The results showed a declination in RBC and Hb in contrast the WBC was enhanced (Ramesh *et al.*, 2009). Also in an attempt, the toxicity effect of ATR was observed in changing the blood indices in other type of fish, *Tilapia mossambica* (Prasad *et al.*, 1991). On the other hand, the adverse effects of ATR from avian species performed in male Japanese quail Coturnix japonica induced significant changes in nucleus of erythrocytes (Hussain *et al.*, 2012). The controversy between the above mentioned information and the present study most probably return to dose exposure period and species differences. Also non-significant changes occurred in all haematological parameters in ATR+2.5% fenugreek, and ATR+5% fenugreek groups when compared to ATR treated rats and control. The present study is supported by the findings of Rao *et al.*, (1996) who demonstrated that fenugreek seeds have no effect on Hb, PCV as well as differential WBCs counts in male and female rats. Also Khalil (2004) confirmed no effect of fenugreek seeds on RBC, WBC and Hb in normal male rats. Whereas, Sharaf (1997) showed significant increase in Hb concentration in male rats treated with fenugreek. Another study demonstrated that there was significant increase in the level of Hb, WBC, and PCV after 7 days of extract treatment but the levels decreased when treatment continued to the 14th day, WBC count were significantly higher after 7 days of extract treatment as compared to control and 14 days treatment (Effraim *et al.*, 1999). According to the observations above, the alteration in haematological parameters by fenugreek may depend on methods of processing and experimental period.
Table 3: Effect of ATR, ATR+2.5% fenugreek, ATR+5% fenugreek on haematological parameters in male rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Control</th>
<th>ATR</th>
<th>ATR + 2.5% fenugreek</th>
<th>ATR + 5% fenugreek</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WBC (10^3/µL)</td>
<td>9.63000 ± 0.441529&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.87143 ± 1.299948&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.15714 ± 1.276315&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.94286 ± 1.575536&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>RBC (10^6/µL)</td>
<td>7.65714 ± 0.144250&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.87143 ± 1.299948&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.15714 ± 1.276315&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.94286 ± 1.575536&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Hb (g/dL)</td>
<td>14.65714 ± 0.249626&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.67143 ± 0.390186&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.70000 ± 0.367747&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>13.64286 ± 1.246492&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PCV (%)</td>
<td>42.45714 ± 0.887709&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.08571 ± 0.162164&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.62857 ± 1.060933&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>39.70000 ± 3.343081&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>MCV (fL)</td>
<td>55.44286 ± 0.39133&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>56.27143 ± 0.579291&lt;sup&gt;c&lt;/sup&gt;</td>
<td>54.55714 ± 0.938554&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>56.88571 ± 0.710274&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>MCH (pg)</td>
<td>19.14286 ± 0.177089&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.61429 ± 0.285714&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.88571 ± 0.431734&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.40000 ± 0.252605&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>MCHC (g/dL)</td>
<td>35.4286 ± 0.319119&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.84286 ± 0.287840&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.58571 ± 0.431734&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.15714 ± 0.252605&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PLT (10^3/µL)</td>
<td>525.1428 ± 88.446225&lt;sup&gt;a&lt;/sup&gt;</td>
<td>543.5714 ± 83.43423&lt;sup&gt;a&lt;/sup&gt;</td>
<td>608.000 ± 81.026157&lt;sup&gt;a&lt;/sup&gt;</td>
<td>706.2857 ± 38.39253&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>LY (%)</td>
<td>67.62000 ± 4.428702&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.05000 ± 7.717351&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.85000 ± 10.645382&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.60000 ± 6.040364&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>MO (%)</td>
<td>15.34000 ± 2.181880&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.12500 ± 2.745737&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.45000 ± 2.65870&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.66000 ± 1.317042&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>GR (%)</td>
<td>17.04000 ± 6.166247&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.82500 ± 9.711366&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.75000 ± 12.4633&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.74000 ± 6.307583&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data presented as mean ± S.E. The same letters mean non-significant differences while the different letters mean significant differences * = p<0.05  ** = p<0.01

3.3. Effect of fenugreek on serum lipid profile in male rats treated with atrazine

The effect of ATR, ATR+2.5% fenugreek and ATR+5% fenugreek on serum lipid profile are shown in table 4 and figures (1 and 2). The rats treated with ATR showed significant p < 0.05 increase of serum total cholesterol when compared to control. The present indirectly is supported by study of EL-Shenawy et al. (2011) who reported that ATR induced LPO in liver and kidney of mice. Total cholesterol in both groups ( ATR+2.5% fenugreek and ATR+5% fenugreek ) were reduced non significantly from ATR group. Meanwhile, cholesterol was increased non significantly in ATR+2.5% fenugreek and significantly p < 0.05 in ATR + 5% fenugreek from control. Triglyceride and LDL were increased non significantly in ATR group when compared to control. While in ATR+2.5% fenugreek and ATR+5% fenugreek they were reduced non significantly when compared to ATR group. HDL was decreased non significantly when compared to control. While in ATR + 2.5% fenugreek...
fenugreek was increased significantly from ATR group and non significantly from control. Whereas in ATR + 5% fenugreek was increased non significantly from ATR group and control as well. These alterations in the above mentioned parameters are supported by the findings of (Sharma et al., 1990) who demonstrated that fenugreek diet significantly reduced serum total cholesterol, LDL, and TG in diabetic patients. However HDL cholesterol fraction remained unchanged.

Table (4): Effect of ATR, ATR+2.5% fenugreek, ATR+5% fenugreek, on serum lipid profile in male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>ATR</th>
<th>ATR + 2.5% fenugreek</th>
<th>ATR + 5% fenugreek</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol* (mg/dL)</td>
<td>32.30769±a</td>
<td>72.43590±b</td>
<td>54.39560±abc</td>
<td>58.07692±bc</td>
</tr>
<tr>
<td>TG* (mg/dL)</td>
<td>3.064880a</td>
<td>8.666894b</td>
<td>6.048117abc</td>
<td>7.868182bc</td>
</tr>
<tr>
<td>HDL* (mg/dL)</td>
<td>36.27907±a</td>
<td>48.50498±b</td>
<td>41.86047±ab</td>
<td>42.17054±abc</td>
</tr>
<tr>
<td>LDL* (mg/dL)</td>
<td>2.225225a</td>
<td>5.060314b</td>
<td>4.902756c</td>
<td>3.410852c</td>
</tr>
<tr>
<td>HDL* (mg/dL)</td>
<td>24.70760±a</td>
<td>22.21517±b</td>
<td>36.64341±ab</td>
<td>34.18194±abc</td>
</tr>
<tr>
<td>LDL* (mg/dL)</td>
<td>1.720268ab</td>
<td>4.726576b</td>
<td>4.014196a</td>
<td>3.806143ab</td>
</tr>
</tbody>
</table>

Data presented as mean ± S.E. The same letters mean non-significant differences while the different letters mean significant differences * =p<0.05 ** =p<0.01

3.4. Effect of fenugreek on histological sections in male rats treated with atrazine

3.4.1. Liver

Histological sections from control (Plates 1 and 2) showed normal histological appearances of the liver, included blood sinusoids spaces, central vein, hepatocytes and kupffer cells.

Histological sections of the liver in the rat treated with ATR revealed some alterations represented mainly by liver nonhomogeneous architecture, dilated sinusoids, necrotic hepatocytes, degeneration of the hepatocytic nuclei. Also vacuolar degeneration, and dilatation of some blood vessels congested with blood cells were revealed (Plates 3 and 4). In
the present study the histological alteration in the liver is supported by Singh et al. (2011) who suggested that ATR increased Superoxide Dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GSTs) in male rats. Rats treated with ATR+2.5% fenugreek retained some histological features including decreased the sinusoidal dilation, decreased degeneration in hepatocytic cells, and increased the number of kupffer cells (Plates 5 and 6). Rats treated with ATR+5% fenugreek showed marked retaining the normal histological characteristics in the sinusoid dilation, homogenous architecture and increased the number of normal hepatocytes, however, 5% fenugreek in some hepatocytes increased nuclear density recognized with dark color (plates 7 and 8). The effects of fenugreek on liver in previous study has been demonstrated that it increased hepatocyte viability and reduced apoptotic nuclei in rats (Kaviarasan and Anuradha, 2007).

Plate (1): Section in liver of control showing sinusoids and central vein. (Stain: H&E.100X).

Plate (2): Section in liver of control showing hepatocytes, sinusoids and kupffer cells. (Stain: H&E.400x).

Plate (3): Section in liver of rat treated with ATR showing dilation of central vein and sinusoids and degeneration of hepatocytes. (Stain: H&E.100X).

Plate (4): Section in liver of rat treated with ATR showing dilated central vein, dilated sinusoids and necrotic hepatocytes. (Stain: H&E.400X).

Plate (5): Section in liver of rat treated with ATR+2.5% fenugreek showing retained of some tissue architecture and decrease of central vein diameter. (Stain: H&E.100X).
3.4.2. Kidneys

The histological examination of the kidney sections from the control (plates 9 and 10) showed normal histological features, glomeruli, bowman’s capsule and kidneys tubules. While rats treated with ATR revealed several histological alterations including degeneration of kidney tubular cells, absence of glomerular tuft, necrosis of kidney tubular cells, increasing bowman’s space (plates 11, 12 and 13). Alteration in the kidney by ATR is supported by studies of AL-Attabi and AL-Diwan, (2012) who reported that ATR impair kidney function in adult male rats. Whereas treatment with ATR+2.5% fenugreek showed normal size of bowman’s capsules, decreased bowman’s space and decreased degeneration in epithelial cells (plates 14 and 15). Treatment with ATR+5% fenugreek retained normal cells, decreased bowman’s space, decreased degeneration in glomeruli, however, 5% fenugreek in some cells increased nuclear density recognized with dark color (plates 16 and 17). In the present study kidney protection by fenugreek against ATR is supported by studies of (Belaïd -Nouira et al., 2013) who demonstrated that fenugreek could offer protection for kidney in the rat.
Plate (10): Section in kidney of control showing Bowman’s capsule, Bowman’s space and kidney tubules. (Stain: H&E.400X).

Plate (11): Section in kidney of rat treated with ATR showing atrophy, absence of glomerulus tuft, increased Bowman’s space and kidney tubular necrotic cells. (Stain: H&E.100X).

Plate (13): Section in kidney of rat treated with ATR showing absence of glomerular tuft. (Stain: H&E.400X).

Plate (12): Section in kidney of rat treated with ATR showing more lobules structure of glomerulus, glomerulus atrophy, kidney tubules, degeneration the kidney tubular cells and increased Bowman’s space. (Stain: H&E.400X).

Plate (14): Section in kidney of rat treated with ATR+%2.5% fenugreek showing proliferation of epithelial cells and decreased glomerular diameter. (Stain: H&E.100X).
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Plate (15): Section in kidney of rat treated with ATR+2.5% fenugreek showing decreased degeneration in glomerulus and kidney tubules. (Stain: H&E.400X).

Plate (16): Section in kidney of rat treated with ATR+5% fenugreek showing decreased degeneration of epithelial cells and increased nuclear density. (Stain: H&E.100X).

Plate (17): Section in kidney of rat treated with ATR+5% fenugreek showing decreased Bowman’s space and proliferated epithelial cells. (Stain: H&E.400X).

4. CONCLUSION
The effects of aqueous extract of 2.5% and 5% fenugreek seeds in the diet against ATR on reduction of serum total cholesterol, TG and LDL, and its role in HDL rise is clearly observed. However aqueous extract of 2.5% fenugreek on total cholesterol and HDL is more effective than that of aqueous extract of 5% fenugreek against ATR. Also both doses of aqueous extract of fenugreek decreased degeneration and tissue damage in liver and kidney against ATR, however some cells were recognized with dark nuclei in ATR+5% fenugreek. Therefore, in the present study the effects of aqueous extract of 2.5% fenugreek is preferred.

REFERENCES


DEVASENA, T. AND MENON, V.P. 2002. Enhancement of circulatory antioxidants by fenugreek during 1,2-dimethylhydrazine-


