Tr. J. of Biology
23 (1999) 187-197


# Effects of *Fagonia cretica* L. Constituents on Various Endocrinological Parameters in Rabbits

M. Asif SAEED*
Department of Pharmacy, University of the Punjab (Allama Iqbal Campus), Lahore 54000 PAKISTAN
Zaheer-ul-Din KHAN
Department of Botany, Government College Lahore 54000 PAKISTAN
A.W. SABIR
PCSIR Laboratories Complex, Ferozepur Road, Lahore 54000 PAKISTAN

Received: 01.08.1996

**Abstract:** The effects of powdered *Fagonia cretica* plant and its two major triterpenoid saponins (saponin-I and saponin-II) on various blood endocrinological parameters. Prolactin namely, serum prolactin, serum thyrotropin, serum thyroxine and serum cortisol of normal male rabbits were investigated. Two major triterpenoid compounds, saponin-I and saponin-II, were isolated from its ethanolic extract by repeated chromatography on silica gel, sephadex LH-20 and on biogel P-2. These compounds were identified after comparing their values of $^{1}$H NMR and $^{13}$C NMR chemical shifts with previously reported values of similar compounds. Radio-immunological assay was used for the estimation of blood hormones of crude drug and saponin-treated animals using radioactive $I^{125}$. The radioactivity of the standard and the unknown specimen in each case was then measured on NE-1612 gamma scintillation counter for 90 seconds. Both the saponins in 30 mg doses had significant decrease in prolactin and in the serum TSH levels as compared with crude drug treatment and control groups. The thyroxine level was also significantly reduced by saponin-II in a 30 mg dose while the crude drug and saponin-I had non-significant effects on thyroxine after 16 days. A significant increase in serum cortisol occurred with the crude drug in a 1g dose and with both saponins in 30 mg roses. Maximum increase in the serum cortisol occurred with saponin-II after 16 days.

**Key Words:** Triterpenoid saponin glycosides, endocrinological parameters, serum prolactin, serum thyrotropin, serum thyroxine, serum cortisol and *Fagonia cretica* L.

## Fagonia Cretica L. Bileşiklerinin Tavşanların Çeşitli Endokrinolojik Parametreleri Üzerine Etkileri

**Özet:** Toz haline getirilmiş Fagonia cretica bitkisi ve bunun iki esas triterpenoidi (Saponin-I ve saponin-II) nin normal erkek tavşanlarda serum prolaktin, serum tirotropin serum tirokzin ve serum kortizol gibi bazı kan endokrinolojik parametreleri üzerine olan etkileri incelendi. Saponin I ve II bitkinin etanol ekstresinde silika jel, sefadaks LH-20 ve Biojel P-2 üzerinde tekrarlanan kromatografi ile izole edildi. Bu bileşimler H NMR ile ve 13 C NMR ile ilgili kimyasal değişmelerinin değerleri, benzer bileşiklerin

* - To whom all the correspondence should be done.

daha önce kaydedilen değerleriyle karşılaştırıldıktan sonra teşhis edildiler. Ham ilaç verilen ve saponin verilen tavşanların kan hormonlarının tespiti için radyoaktif $I^{125}$ ile radyo-immünolojik tayini yapıldı. Her iki durumdaki standart ve bilinmeyen örneklerin radyoaktivitesi, NE-1612 gamma parıltısı sayacıyla 90 saniye süreli olarak ölçüldü. Kontrol ve ham ilaç gruplarına göre her iki saponinin 30 mg dozunda serum prolaktin ve serum TSH seviyelerini önemli derecede düşürdüğü gözlendi. Ayrıca 16. Günde tiroksin seviyesinin ham ilaç ile saponin-I'ın önemli derecede etkilemediği, saponin-II'nin ise 30 mg dozunda önemli derecede düşürdüğü saptandı. Ham ilacın 1 g dozunda, her iki saponinin ise serum kortizolünü arttırdığı gözlendi. Serum kortizolündeki maksimum artış saponin II ile 16. günde meydana geldi.

**Anahtar Sözcükler**: Triterpenoit saponin glikosit, endokrinolojik parametre, serum prolaktin, serum tyrotropin, serum tiroksin, serum kortizol, Fagonia cretica L.

## Introduction

*Fagonia cretica* L. (Family Zygophyllaceae) is a small spiny undershrub, mostly found in dry calcareous rocks throughout Pakistan (1-3). It is reputed to be a medicinal plant in scientific and folkloric literature and its medicinal values are well documented (1-4). An aqueous decoction of the plant is a popular remedy for cancer in the indigenous system of medicine (4). The medicinal properties of the plant are attributed to its variety of active phytochemical constituents. In the last fifteen years, this plant and related species have been investigated mainly for the presence of flavonol and terpenoid glycosides. Most of the flavonol glycosides have been isolated from various Egyptian *Fagonia* species and their phylogenetic affinities have also been investigated (5-11). Several saponin glycosides have been separated and characterized (12, 13). Other constitiunets, such as docosyl docosanoate from hexane extract (14) and water soluble proteins from aqueous extract of air-dried *F. cretica* plants, have been isolated (15); furthermore, nahagenin (16), hederagnin, ursolic acid and pinitol from other *Fagonia* species have also been separated and characterized (17). The antimicrobial activity of its flavonoid compounds has been explored previously (18), while the nutritive values of it and of other species growing wild in the Rajasthan region of India, have also been evaluated (19).

Although the folkloric medicinal literature claims that *F. cretica* is an anticarcinogen, no scientific attempt has yet been made to evaluate the effects of its isolated compounds individualy on different parameters of living organisms. In the first instance, we isolated two mojor triterpenoid saponin glycosides from the aerial parts and investigated their effects on various blood endocrinological parameters, such as serum prolactin, serum thyrotropin (TSH), serum thyroxine ($T_4$) and serum cortisol, in normal male rabbits under normal laboratory conditions.

## Material and Methods

### General

Unless otherwise stated, all the chemicals used were of analytical grade. Concentrations were performed under reduced pressure at bath temperatures not exceeding 55°C. Separations were performed on SE-54 fused-silica capillary columns using authentic hexitol hexa-acetate as a ref-

erence. The TLC spots were visualized under UV lamp (TL 900 Camag Ltd.) The IR spectra of the compounds were determined on Pye-Unicam SP-8-400 and UV spectra were measured on Hitachi-270-30 spectrophotometer. $^{1}$HNMR spectra were obtained at 400 MHz and $^{13}$C NMR spectra at 25 MHz on JEOL GX-400 and JEOL FX-100 spectrometers using tetramethylsilane as an internal reference.

## Plant Materials

### *Collection, Authentication and Pulverization*

The *Fagonia cretica* plants were collected from uncultivated and waste areas of Lahore (i.e., from the central Punjab plain areas of Pakistan), in January/February 1995. These were authenticated by the Herbarium staff, Department of Botany, Unversity of the Punjab, Lahore. The voucher specimen was deposited in the Herbarium of Pharmacognosy Section, Department of Pharmacy, University of the Punjab Lahore for further reference.

### *Extraction*

The aerial parts of the fresh plants (15 kg) were finely chopped and soaked in EtOH (3x151) for 25 days. Extracts were concentrated in a cyclone evaprator with the temperature maintained between 50 and 55°C. A dark green semi-solid residue (96 g) was obtained, which was dissolved in MeOH (4x500 ml). $Me_2CO$ (3x500 ml) was then added, resulting in precipitation. The precipitates were filtered, and washed first with petroleum ether (40-60, 900 ml), then with $CHCl_3$ (800 ml) and EtOAc (500 ml). Finally on drying, it yielded an amorphous material (46.47 g).

### *Isolation of Saponin-I*

Crude amorphuos powder (27 g) was fractionated by flash chromatography on silica gel (450 g) and eluted with $CHCl_3$/MeOH/EtOAc (5:4:8) and $CHCl_3$/MeOH/EtAc/$H_2O$ (5:6:8:2). The pooled fraction obtained with the first solvent system when evaported, yielded a mixture of two saponins (4.26 g), one of which was present in major quntity. The other pooled fraction, obtained with the second solvent system, afforded a mixture of compounds (8.63 g). Four grams of the dried product from the first fraction was re-chromatographed on the second silica gel column (130 g) and eluted with $CHCl_3$/MeOH/EtOAc (5:5:7), yielding almost pure saponin-I (97 mg). This compound exhibited $[\alpha]\ D^{24} = +1$ (*c* 0.4; MeOH) and IR absorption maxima at 2940 (broad and strong), 1700 (strong), 1460, 1380, 1280 (strong), 910 and 810 (medium) $cm^{-1}$. The important $^{1}$H NMR and $^{13}$C NMR signals of this compound have been outlined in Table 1 (Figure 1).

### *Isolation of Saponin-II*

The crude amorphous product (18 g) from the solvent extraction was chromatographed on a Sephadex LH-20(5.6x85 cm.) column. Elution was performed with EtOH/$H_2O$ (1:1). The first fraction contained a mixture of saponins. This mixture was passed through a silica gel column (170 g) and eluted with $CHCl_3$/MeOH/EtOAc (5:6:7) to remove any yellow colouring matter. After evaporating the solvent, a white saponin mixture (7.2 g) was obtained. It was re-chromatographed on Biogel P-2 and eluted with $H_2O$. Pure saponin-II (92 mg) was obtained from a

polled farction after evaporating the solvent. This compound exhibited [$\alpha$] $D^{24}$ = +1 (*c* 0.3; MeOH); UV $\lambda_{max}$ = 230 nm and IR absorption maxima at 3480, 2940 (strong), 1700 (strong), 1650, 1460, 1380, 1260 (strong), 1050, 820 and 730 (medium) $cm^{-1}$. The important $^{1}H$ NMR and $^{13}C$ NMR signals of this compound have been outlined in Table 1 (Figure 1).

## Biological Assay

### *Procurement and Maintenance of Animals*

Forty-two healthy adult male rabbits of albino strain (species *Oryctolagus cuniculus* and subspecies *Caprolagus hispidus*) weighing 1.0-1.5 kg were purchased from an animal supplier and acclimatized in the Department of Pharmacy, University of the Punjab, Lahore, for one week. The animals were provided with fresh green fodder (clover) and tap water *ad libitum.* They were put into seven groups of six animals each by randoms selection.

The arrangement of animals in these groups is as follows:

(1)-- labeled as C (For untreated control group);

(2 and 3)-- labeled as $CT_1$ and $CT_2$ (For 1st and 2nd crude drug treated groups);

(4 and 5)-- labeled as $S_1T_1$ and $S_1T_2$ (For 1st and 2nd dose of saponin-I treated groups);

(6 and 7)-- labeled as $S_2T_1$ and $S_2T_2$ (For 1st and 2nd dose of saponin-II treated groups).

### *Dosage Form*

Each rabbit in group C was orally administered 20 ml of distilled water through Ryle's tube. The doses of crude *F. cretica* powder for $CT_1$ and $CT_2$ were calculated on the basis of 1.0 g/kg and 2 g/kg body weight, respectively. For both saponin-I and saponin-II treated groups i.e., for $S_1T_1$, $S_1T_2$ and for $S_2T_1$, $S_2T_2$, the doses were calculated on the basis of 20 mg/kg and 30 mg/kg body weight, respectively. Each sample of the powdered crude drug dose and its isolated compound's dose was suspended in 20 ml of distilled water and administered to the animals through Ryle's tube.

### *Blood Sampling*

Blood sampling was done from the ear veins of the rabbits at zero hours (before treatment), and subsequently at the end of the Ist, 2nd, 4th, 8th and 16th day of the treatment. At each sampling, 4 ml of blood was collected in a centrifuge tube and centrifuged at 3000 rmp for 15 minutes. Serum was separated and stored at-20°C.

### *Radio-Immuno-Assay for Hormones*

For radio-immunological assay of hormones, radioactive $I^{125}$ was used for the estimation of serum prolaction, serum thyrotropin, serum thyroxine and serum cortisol in the standard and in various samples of blood serum of the drug-treated animals. The radioactivity of the standard and the unknown specimen in each case was measured on an NE-1612 gamma scintillation counter for 90 seconds. The detailed method used for the estimation of each hormones is as follows:

*Estimation of Prolactin*

50 µl prolactin standard or the sample serum was pipetted into a incubation tube and 100 µl prolaction antiserum was added and mixed well with the help of a vortex mixer. The tubes were incubated for 30 minutes at 37°C in a water bath. 100µl $I^{125}$-prolaction solution was then added and, after mixing , these were again incubated for two hours at 30°C . 500µl goat-anti-rabbit-gamma-globulin/PEG solution was then mixed and again incubated for 30 minutes at room temperature. After centrifugation for 30 minutes at 3000 rpm, supernatant was removed carefully by a pasture pipette connected to a water jet pump, The activity in the precipitate of all the incubation tubes was measured.

*Estimation of Thyrotropin (TSH)*

Thyrotropin in the standart and unknown serum samples were determined in duplicate. The first sixteen tubes were assigned as follows: 1-2 for NSB (nonspecific binding), 3-4 for zero standard, 5-6 for 0.8 uu/ml standard, 7-8 for 2.5 uu/ml standard, 9-10 for uu/ml standard, 11-12 for 10 uu/ml, 13-14 for 25 uu/ml standard and 15-16 for 50 uu/ml standard. The remaining tubes were assigned for unknown serum samples such that successive pairs of tubes corrosponded to a successive number of samples.

200 µl aliquots of the standrads and unknown serum samples were pipetted into the appropriate tubes according to the protocol, and serum diluent was added to tubes 1 and 2. 100 µl anti-TSH serum solution was added to all tubes except 1 and 2, to which 100 µl NSB reagent was added. The contents of all the tubes were thoroughly mixed by means of a vortex mixer, covered with plastic film and incubated at 20°C for 16 hours. 100 µl $I^{125}$-labeled TSH solution was then added to each of the tubes and after mixing, these were again incubated at 37°C for 2 hours. 1.0 ml of Amerlex-M second antibody reagent was dispensed into each of the tubes and, after thorough mixing, again incubated for 10 minutes at room temperature (25±3.7°C). All the tubes were centrifuged for 10 minutes at 1500 rpm. Following centrifugation, tubes were inverted in decantation racks, supernant liquid was poured off and radioactivity was measured.

*Estimation of Thyroxine ($T_4$)*

50 µl aliquots of the standard and unknown specimen were pipetted into the appropiate tubes. Zero standard was used for NSB reagent tubes. 500 µl $I^{125}$-thyroxine solution was added to each of the tubes, followed by the addition of 500 µl Amerlex-M NSB reagent. After thorough mixing, all of the tubes were incubated at room temperature (25±3.5°C) for 45 minutes and centrifuged at 1500 rpm for 15 minutes. After centrifugation, the tubes were placed in decantation racks and supernatant liquid was discarded. The radioactivity in each tube was then measured.

*Estimation of Cortisol*

For the estimation of cortisol, first lyophilized $I^{125}$-cortisol was reconstituted by adding 1.0 ml of distilled water and citrate buffer solution and mixed thoroughly. 10 µl cortisol standard or samples were pipetted out into the SPAC tubes and 1000 µl of reconstituted lyophilized $I^{125}$-cortisol was added and mixed well with a vortex mixer. All the test tubes were incubated at room

temperature (23±2.5°C) for 6 hours. The solution from each tube was removed by decantation and washed with 1.5 ml saline solution. The radioactivity in each tube was then measured.

### Statistical Analysis

In all the experiments, data was expressed as the Mean ± SEM (Standard error of the mean) (Tables 2 to 5). Significant differences between the control and treatment groups were calculated by Student's t test (20, 21).

## Results and Discussion

The ethanolic extract of the aerial parts of *F. cretica* afforded a fraction containing triterpenoid saponins upon precipitation with acetone. One of the triterpenoid saponins was isolated and purified by repeated chromatography on silica gel. This saponin was designated saponin-I. Another triterpenoid saponin was isolated and purified after repeated chromatgraphy on Sephadex LH-20 and Biogel P-2. This saponin was designated saponin-II. Both saponins were analyzed by IR, UV, $^{1}$H NMR and $^{13}$C NMR spectroscopy. These compounds were identified after

Table 1. Some Important $^{1}$H NMR and $^{13}$C NMR* signals of saponin-I and saponin-II from *Fagonia cretica* (Coupling constants ($J_{H,H}$) are given in parentheses)

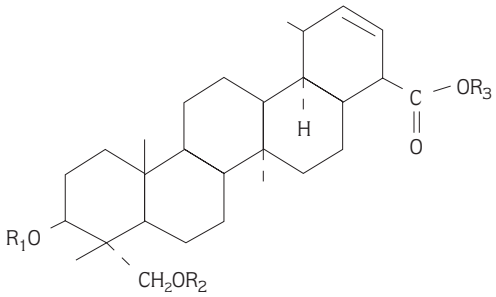
| | 1HNMR (σ and *J*) | | $^{13}$CNMR chemical shifts (σ) | | |
|---|---|---|---|---|---|
| | Saponin-I | Saponin-II | | Saponin-I | Saponin-II |
| H-1'± | 6.17 (7.9) | 6.16 (8.3) | C-1'± | 95.2 | 94.9 |
| H-1"± | 4.80 (7.4) | 5.14 (7.6) | C-1"± | 104.9 | 104.8 |
| H-21 | 5.34 (6.3) | 5.37 (6.7) | C-3 | 71.8 | 82.1 |
| | | | C-23 | 74.0 | 68.9 |
| | | | C-20 | 142.8 | 142.7 |
| | | | C-21 | 117.4 | 117.3 |
| | | | C-18 | 173.9 | 173.8 |

* -- Spectra were obtained in pyridine-d5 at 90 ($^{1}$H) and 60 ($^{13}$C).
± -- H-1' = H-1 from the 28-O-β-D-Glep group; H-1" = H-1 from the 3- or 23-O-β-D-Glep group.

Table 2. Effect of Crude Powdered *Fagonia cretica*, Saponin-I and Saponin-II on Serum Prolactin Level (miu/L) In rabbits±

| | TREATMENTS | | | | | | |
|---|---|---|---|---|---|---|---|
| No of Days | Controlled | Crude Drug | | Saponin-I | | Saponin-II | |
| | | 1g | 2g | 20 mg | 30mg | 20 mg | 30 mg |
| 0 | 1312.0±6.7 | 1251.1±8.8** | 1290±7.6 | 1310.2±2.3 | 1316.5±1.2 | 1306.5±2.5 | 1305.6±6.3 |
| 1 | 1412.7±6.5 | 1280.7±5.0** | 1214.6±6.0* | 1206.6±3.6 | 1200.6±3.2 | 1203.9±3.7 | 1205.3±6.2 |
| 2 | 1215.5±5.2 | 1162.8±8.0* | 1192.2±7.1* | 1206.8±5.2* | 1180.6±5.2** | 1100.5±6.5 | 1130.5±5.2 |
| 4 | 1340.6±6.1 | 1132.4±4.5** | 1251.7±4** | 1104.6±3.1 | 1038.8±3.1*** | 1072.8±5.2** | 1100.7±6.5** |
| 8 | 1213.4±4.3 | 1208.8±4.0 | 1167±6.1*** | 1101.6±6.1 | 1032.5±2.1 | 1061.5±2.5 | 1080.8±5.3** |
| 12 | 1250.2±7.0 | 1240.2±6.7 | 1109.8±5*** | 1100.5±3.1 | 1031.0±4.1 | 1041.9±6.2** | 1040.9±3.2*** |
| 16 | 1233.3±6.0 | 1165.7±9*** | 1160.4±7*** | 1060.8±4.2** | 1030.5±2.3** | 1040.2±2.1 | 1015.7±4.2*** |

* - p<0.05 ** - p<0.01 *** - p<0.001




| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| Saponin-I | H | β-D-Glep. | β-D-Glep. |
| Saponin-II | β-D-Glep. | H | β-D-Glep. |

Figure 1. Structures of the Compounds isolated from *Fagonia cretica*

comparing their values of $^{1}H$ NMR and $^{13}C$ NMR chemical shifts (values of $\sigma$) and their coupling constant (values of *J*) with the previously reported chemical shifts and *J* values of similar compounds (12-17) (Table-1; Figure 1).

### Effect on Prolactin

It is known that in the radio-immunological determination of prolactin when RIA-rat prolactin was used, $I^{125}$-prolactin and serum prolactin compete for the binding sites of a specific antibody (22, 23). The higher the prolactin concentration, the less $I^{125}$-prolactin will be bound to the antibody (22). With the aid of a second antibody, in combination with PEG, the prolactin bound by the first antibody is precipitated. The unbound prolactin remaining in the supernatant is aspirated and the remaining activity bound to the second antibody is measured. The prolactin concentration of the sample serum can be calculated with the help of comparison with the standards.

The results indicated that the crude drug and both of its saponins exhibited more marked effects on the serum prolactin than the controlled group (Table 2). Both saponins further showed significant decrase in prolactin levels after 16 days, as compared to the crude drug treatment group and the control group (Table 2). The results also demonstrated that the effects of different doses (i.e. 20 mg and 30 mg) in both of the saponins is not a significant factor. Both doses behaved similarly in reducing the amounts of serum prolactin (Table 2).

### Effects on Thyrotropin (TSH)

The radio-immunoassay method also depends on the concentration for a limited number of binding sites on a TSH-specific antibody, between TSH in the serum and $I^{125}$-labeled TSH. The proportion of $I^{125}$-labeled TSH bound to the antibody is inversely related to the concentraiton of TSH present in serum (22, 23). The antibody-bound TSH is then reacted with Amerlex-M reagent, which contains a second antibody that is bound to magnetizable polymer particles. Separation of the antibody-bound fraction is effected by either magnetic separation or centrifugation of suspension and decantation of the supernatant. By measuring the proportion of $I^{125}$-labeled TSH bound in the presence of reference standard solutions containing known amounts of TSH, the concentration of TSH present in the known samples can be interpolated. The anti-TSH serum and the TSH in the samples or standards are allowed to react together before the $I^{125}$-labeled TSH is added. This enhances the sensitivity of the assay, allowing low concentrations of TSH in serum to be measured with better precision.

The results presented in Table 3 show that both saponins in 30 mg doses were associated with much greater decreases in the serum TSH level than occurred in the control and crude drug treatment groups. Furthermore, as far as the serum TSH is concerned, there seemed to be a pronounced effect of the dose of both saponins. 30 mg dose of both the saponins was more effective than 20 mg dose. Over all, the saponin-II was more effectual than saponin-I in this regard, during the whole of the study period (Table 3).

### Effects on Thyroxine ($T_4$)

The radioimmunoassay method depends on the competition between thyroxine in the surem and $I^{125}$-thyroxine for a limited number of binding sites on a $T_4$-specific antibody (22, 23). The proportion of $I^{125}$-thyroxine bound to the antibody is inversely related to the concentration of the thyroxine present in the serum. The antibody suspension contains antibodies bound to magetisable polymer particles. Separation of the antibody fraction is effected by centrifugation of magnetic separation, followed by decantataion of the supernant. By measuring the proportion of $I^{125}$-thyroxine bound in the presence of reference standard sera containing various

Table 3. Effect of Crude Powdered *Fagonia cretica*, Saponin-I and Saponin-II on SerumThyrotropin (TSH) Level (miu/L) In Rabbits±

| | | TREATMENTS | | | | | |
|---|---|---|---|---|---|---|---|
| No of | Controlled | Crude Drug | | Saponin-I | | Saponin-II | |
| Days | | 1g | 2g | 20 mg | 30mg | 20 mg | 30 mg |
| 0 | 35.2±1.2 | 27.6±1.5* | 28.2±2.1 | 27.6±6.3 | 21.6±1.4 | 23.6±6.5 | 22.2±3.5 |
| 1 | 33.7±0.6 | 23.5±2.3* | 25.5±3.4 | 25.6±2.4 | 20.6±3.0 | 22.0±2.4 | 20.8±2.3 |
| 2 | 31.9±0.8 | 19.8±3.5** | 20.6±2.5* | 25.5±4.2 | 14.8±2.5*** | 15.5±3.1** | 17.6±2.5*** |
| 4 | 32.8±0.7 | 20.2±1.2 | 22.2±5.2 | 25.4±3.2 | 12.3±2.1 | 14.6±4.2** | 14.6±5.6*** |
| 8 | 30.7±0.6 | 16.9±2.3*** | 18.4±1.4** | 23.6±2.1 | 11.5±2.0* | 13.8±1.1 | 12.8±3.6 |
| 12 | 32.5±0.9 | 21.2±3.4* | 20.2±2.7* | 21.5±3.1 | 11.4±3.6 | 13.3±2.1** | 10.5±3.5** |
| 16 | 33.2±0.7 | 22.3±3.2* | 21.5±2.2* | 20.7±3.4 | 10.5±2.0*** | 12.5±3.5* | 9.6±2.5*** |

* - $p<0.05$ ** - $p<0.01$ *** - $p<0.001$

known amounts of $T_4$, the concentration of $T_4$ present in unknown samples can be interpolated.

The results of thyroxine estimation (Table 4) demonstrated that both the crude drug (in 1g and 2g doses) and saponin-I (in both 20 and 30 mg doses) have equal effects and there is no significant difference between the two materials as regards the activity of the serum thyroxine level in the blood is concerned, when compared with the controlled group of animals. On the other hand, saponin-II in a 30mg dose has a signficant effect in reducing the quantity of thyroxine in the blood serum (Table 4).

Table 4. Effect of Crude Powdered *Fagonia cretica,* Saponin-I and Saponin-II on Serum Thyroxine ($T_4$) Level (nanomole/L) In Rabbits±

| | TREATMENTS | | | | | | |
|---|---|---|---|---|---|---|---|
| No of Days | Controlled | Crude Drug | | Saponin-I | | Saponin-II | |
| | | 1g | 2g | 20 mg | 30mg | 20 mg | 30 mg |
| 0 | 37.7±2.5 | 50.3±1.8** | 49.8±2.1** | 49.7±2.1** | 51.6±2.6** | 48.9±2.5** | 37.7±1.9 |
| 1 | 23.5±3.6 | 33.5±2.5* | 40.2±3.6* | 35.2±3.2* | 48.7±1.4** | 47.5±3.6 | 35.4±2.5 |
| 2 | 38.6±6.2 | 24.6±3.6 | 32.5±1.0 | 33.9±4.2 | 40.2±3.2 | 48.7±2.4 | 31.9±5.2 |
| 4 | 37.2±1.2 | 31.5±3.4 | 23.4±2.4 | 35.7±2.0 | 38.5±2.4 | 43.5±1.6 | 20.6±1.4** |
| 8 | 24.6±2.3 | 32.5±1.2 | 27.4±3.6 | 34.5±1.0 | 41.5±3.5 | 33.5±3.4 | 19.6±2.3 |
| 12 | 25.9±1.9 | 41.8±2.0 | 21.5±2.0 | 33.1±2.1 | 34.8±2.4 | 31.5±2.4 | 18.7±1.2* |
| 16 | 24.6±2.1 | 45.7±1.4 | 20.1±2.5 | 20.8±3.6*** | 20.5±1.6 | 24.2±1.3*** | 17.5±3.5*** |

* - $p<0.05$ ** - $p<0.01$ *** - $p<0.001$

### Effects on Contisol

The amount of cortisol in the blood was also determined by radio immunological assay. This was based on the assumption that $I^{125}$-cortisol and cortisol in the sample compete for the binding sites of a specific antibody (22, 23). The higher the cortisol concentration in the sample or in the standard, the less $I^{125}$-cortisol will be bound to the antibody (22, 23). The free cortisol fraction was then removed and tubes were counted in the gamma counter.

The results showed that a significant increase in the serum cortisol level of the blood occur under the influence of all the three types of materials (Table 5). The significant and maximum effects exhibited by the crude drug were seen with a 1g dose, and that exhibited by saponins were with 30 mg dose. 30 mg doses of both the isolated compounds, sahonin-II was more effective and exhibited a maximum increase in serum cortisol after 16 days of the experimental period (Table 5).

On the basis of results, we concluded that 30 mg doses of triterpenoid saponin-I and saponin-II, isolated from *F. cretica,* had greater decreasing effects on prolactin and serum TSH levels of the blood of rabbits than 1 and 2g doses of crude powdered *F. cretica* and with the same parameters in controlled animal's groups. The serum thyroxine level was also significantly reduced by a 30mg dose of saponin-II, while the crude powdered *F. cretica* drug and saponin-

I had no significant effects on the serum thyroxine level, even at the end of the experimental period of 16 days. On the other hand, serum cortisol was significantly incerased with 1 g of crude powdered *F. cretica* drug and with 30 mg doses of both saponins. Furthermore saponin-II seemed to have a greater influnece on this parameter of serum than the other saponin. A maximum increase in the serum cortisol level of the blood of rabbits was noticed with saponin-II after 16 days. Most probably, the chemical differences in the structures of these isolated compounds were related to such differences in their activities, as far as serum cortisol is concerned. A side chain in the molecule of saponin-II seemed to be involved in this activity, since the basic rings of both the saponins are the same (Figure 1). The mechanism of action of these saponins on various endocrinological parameters of blood, however, needs further elucidation in future studies, particularly in relation to the structure-activity relationship of these compounds.

Table 5. Effect of Crude Powdered *Fagonia cretica*, Saponin-I and Saponin-II on Serum Cortisol Level (nanomole/L) In Rabbits±

| | TREATMENTS | | | | | | |
|---|---|---|---|---|---|---|---|
| No of Days | Controlled | Crude Drug | | Saponin-I | | Saponin-II | |
| | | 1g | 2g | 20 mg | 30mg | 20 mg | 30 mg |
| 0 | 42.5±2.5 | 43.5±4.9 | 41.5±1.2 | 42.5±1.0 | 40.1±3.6 | 42.9±2.4 | 51.2±3.6 |
| 1 | 27.8±3.6 | 55.4±6.0** | 69.8±1.0 | 61.5±2.1 | 42.9±2.4 | 40.2±1.5 | 64.2±2.0 |
| 2 | 33.5±5.3 | 62.7±3.6** | 74.3±3.2 | 66.2±3.2 | 39.5±1.0 | 62.3±2.6* | 78.5±1.2** |
| 4 | 39.8±2.4 | 66.2±1.2** | 79.5±3.7** | 79.5±4.2 | 71.6±3.1* | 85.2±3.1* | 97.6±2.0** |
| 8 | 45.8±1.7 | 98.2±2.1*** | 91.5±2.4** | 81.9±2.0 | 91.2±1.0** | 89.4±2.4* | 104.2±1.4*** |
| 12 | 52.8±3.1 | 107.8±3.2*** | 104.7±1.3*** | 83.2±3.2** | 123.5±1.3*** | 96.2±3.0** | 124.6±2.5*** |
| 16 | 66.8±4.1 | 134.1±1.2*** | 108.3±2.4*** | 102.3±1.2*** | 148.2±2.5*** | 108.5±2.5*** | 153.8±6.3*** |

* - $p<0.05$ ** - $p<0.01$ *** - $p<0.001$

## References


1. Chopra, R.M., Handa, K, L; Kapur, L, D., and Chopra, I.C., Indigenous Drugs of INdia. 2nd ed. Academic Publisher, New Delhi 1982, pp. 507.
2. Chopra, R.N., Nayar, S.L., and Chopra, I.C., Glossary of Indian Medicinal Plants. CSIR, New Delhi 1956, pp. 116.
3. Hooker, J.D., Flora of British India. Reeva and London 1, 1975, pp. 425.
4. Saeed, M.A; Hamdard Pharmacopoeia of Eastern Medicine, Hamdard Academy, Karachi 1969, pp. 41-43.
5. Al-Wakeel, S.A.M., and Shahnaz, A.M., Significance of flavonoid chemistry in the Egyptian Fagonia glutinosa and F. Isothricha complex. Biochem Syst. Ecol. 20, 259-64 (1992).
6. Al-Wakeel, S.A.M., El-Garf, I.A., and Salen, N.A.M., Distribution of flavonoids in Fagonia thebica complex. Biochem. Syst. Ecol. 16, 57-58 (1988).
7. Al-Wakeel, S.A.M., El-Nagoumy, S.I., El-Hadidi, M.N., and Saleh, N.A.M., flavonoid pattern in Fagonia mollis complex. Biochem. Syst. Ecol. 15, 459-460 (1987).

8. El-Hadidi, M.N., Al-Wakeel, S.A.M., El-Garf, I.A., Systematic significance of the flavonoid constituents in Fagonia indica complex. Biochem. Syst. Ecol. 16, 293-297 (1988).

9. El-Negoumy, S.I., Al-Wakeel, S.A.M., El-Hadidi, M.N., and Saleh, N.A.M., The flavonoids of the Fagonia arabica complex. Phytochem. 25, 2423-4 (1986).

10. Saleh, N.A.M., El-Hadidi, M.N., Ay-Wakeel, S.A.M., andShahnaz, A.M., Phytochemistry and phylogenetic affinities among Egyptian species of Fagonia. Biochem. Syst. Ecol. 18, 49-52 (1990)

11. Saleh, N. A. M., El-Hadidi, M.N., and Al-Wakeel, S.A.M., Phytochemistry and the evolution of Fagonia species. Bull. Liaison-Groupe Polyphenols 14, 46-49, (1988).

12. Ansari, A.A., Kenne, L., Atta-ur-Rehman., and Wehler, T., Isolation and characterization of a saponin from Fagonia indica. Phytochem. 27, 3979-82 (1988).

13. Ansari, A.A., Kenne, L., Atta-ur-Rehman., and Wehler, T., Isolation and charcterization of two saponins from Fagonia indica. Phytochem. 26, 1487-90 (1987).

14. Hamid, A., Majid, A. C. M., and Atta-ur-Rehman., Isolation of docosyl docosanoate from Fagonia cretica Linn. Arab Gulf J. Sci. Res. A.7, 29-34 (1989).

15. Shaukat, G.A., Malik, M.A., and Ahmad, M.S., Water-soluble protein from Fagonia cretica Linn. Pak. J. Bot. 13, 99-101 (1981).

16. Atta-ur-Rahman; Ansari, A.A., Drexlex, S.A., and Clardy, J., The isolation and structure of nahagenin. Heterocycles 19, 217-20 (1982).

17. Atta-ur-Rahman; Ansari, A.A., and Kenne, L., Hederagenin, ursolic acid and pinitol from Fagonia indica. J. Nat. Prod. 47, 186-7 (1984).

18. Hash, M.L., and Nag, T.N., Flavonoids with antimicrobial activities of arid zone plants. Geobios 15, 32-5 (1988).

19. Harash, M.L., Purohit, G.R., Mathur, C.S., and Nag, T.N., Nutritive value of dried terrestrial plants growing in Rajasthan -- Chemical composition. Comp. Physiol. Ecol. 6, 30-2 (1981).

20. Milton, J.S., and Tsokos, J.D., Statistical Methods in Biology and Health Sciences. McGraw-Hill, Kogakusha LTD, Tokyo 1983.

21. Snedecor, G.W., and Cochran, W.G., Statistical Methods. 6th edition, Iowa State University Press, Ames, Iowa U.S.A. 1967.

22. Green, J.H., The Blood. In; An introduction to human physiology. Oxford University Press New York U.S.A. pp. 5-22, 1978.

23. Hunter, D. and Bomford, R.R., Hutchison's Clinical Methods, 15th ed. Bailliere Tindall & Cassel, London pp. 120-124, 1968.