

## ORIGINAL ARTICLE

**Antiandrogenic activities of *Glycyrrhiza glabra* in male rats**

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Abnormal levels of androgens cause many diseases like benign prostatic hyperplasia and hormone dependent cancers. Although the reduction in serum testosterone (T) by *Glycyrrhiza glabra* has been reported, its effects on seminal vesicle (SV) and prostate tissues have never been reported. This study was carried out to investigate different aspects of antiandrogenic properties of this plant. Immature male rats were divided into five groups ( $n = 7$ ): castrated rats without any treatment received only vehicle; castrated rats plus T replacement; three castrated groups with T replacement plus various doses of *G. glabra* extract (75, 150 and 300 mg/kg). All of the injections were carried out once daily in subcutaneous manner for 7 days. On the eighth day, blood samples were collected for total T measurement. Ventral prostate (VP), SV and *levator ani* muscle were dissected and weighed. Slides prepared from prostate were assessed histologically. The variation in the relative and absolute volume of the prostate tissue compartments was determined. Those receiving the doses of 150 and 300 mg/kg showed a significant reduction ( $p < 0.05$ ) in prostate weight, total T and VP epithelium/stroma ratio (V/V). These results in SV and *levator ani* were shown in response to 300 mg/kg of extract. Increasing in T metabolism, down-regulation of androgen receptors or activation of oestrogen receptors could be involved mechanisms. This study showed that alcoholic extract of *G. glabra* has antiandrogenic properties.

**Introduction**

Androgen-mediated diseases such as prostate cancer (PCa), hirsutism, acne, androgenic alopecia and benign prostatic hyperplasia have become serious problems (Bartsch *et al.*, 2002).

*Glycyrrhiza glabra* or liquorice is composed of 25% amid, 10% D-glucose or saccharose and 7% the active principle, glycyrrhizic acid, the hydrolysis of glycyrrhizic acid produces two molecules of D-glucuronic acid and the aglycone 18  $\beta$ -glycyrrhetic acid, which is responsible for most of the metabolic effects of liquorice (Armanini *et al.*, 2002).

*Glycyrrhiza glabra* is rich in flavonoids and isoflavans, which exhibit oestrogen-like activity (Armanini *et al.*, 2002). The configuration of these components could enable them to bind to and activate the oestrogen receptor (ER) (Spignoli, 2000; Armanini *et al.*, 2002). Liquorice

is also able to affect androgen metabolism. Various studies have demonstrated that liquorice blocks the activity of 3- $\beta$ -hydroxysteroid dehydrogenase (3HSD), 17-hydroxysteroid dehydrogenase (17HSD) and 17-20 lyase and stimulates the activity of aromatase (Kroes *et al.*, 1997). Isoflavonoid derivatives present in liquorice include glabridin, galbrene, glabrone, shiapterocarpin, licoisoflavones A and B, formononetin, glyzarin, kumatakenin (Williamson, 2003; Asl & Hosseinzadeh, 2008).

Dibenzoylmethane (DBM), a minor  $\beta$ -diketon constituent of liquorice has shown a potential role in the prevention and treatment of human PCa (Jackson *et al.*, 2002, 2007).

In the traditional system of medicine, the roots and rhizomes of *G. glabra* (family: *Leguminosae*) have been employed clinically for centuries for their anti-inflammatory, antiulcer, expectorant, antimicrobial and anxiolytic activities (Wang & Han, 1993; Asl & Hosseinzadeh,

2008). Liquorice has been shown to have great antioxidant, free radical scavenging (Haraguchi *et al.*, 1998; Di Mambro & Fonseca, 2005) and anticonvulsant activities (Nassiri-Asl *et al.*, 2007).

The prostate and seminal vesicle (SV) are androgen-dependent secretory glands of the male genital tract (Justin *et al.*, 2006). Testosterone, acting via its more potent natural metabolite, dihydrotestosterone (DHT), stimulates maturation of the prostate during sexual development (Allan *et al.*, 2007). The reduction in prostate size that occurs after castration is primarily due to epithelial cells, which undergo apoptosis. As the stroma of prostate does not require androgens for survival, this results in a substantial change in the ratio of epithelial and stromal cells (Morrissey *et al.*, 2002).

Although the reduction in serum T by *G. glabra* has been reported (Armanini *et al.*, 1999, 2003, 2004), its effects on SV and prostate tissues have never been reported. In this study, we have demonstrated the anti-androgenic activities of *G. glabra* by using immature rat Hershberger assay.

## Materials and methods

### Animals

Immature male Wistar rats, 3–4 weeks of age (50–70 g), were purchased from the animal house of Razi Institute (Karaj, Iran). They were kept in a controlled environment under a photoperiod of 12 h light: 12 h dark, temperature of  $21 \pm 2$  °C. Food and water were available ad libitum. All animal experiments were carried out according to the Guide for Care and Use of Laboratory Animals, approved by Ethics Committee of the Qazvin University of Medical Sciences.

### Ethanol extract of *Glycyrrhiza glabra*

*Glycyrrhiza glabra* was collected from Vikin (a village in Qazvin provinces, Iran) in 2006 and authenticated by Qazvin Agriculture and National Resources Research Center, Iran (Voucher No. 532).

Dried roots were ground and the 100 g of the powder was extracted with 100 mL ethanol (70%, v/v) at room temperature for 3 days using a blender. The extract was filtered through a filter paper and then concentrated under reduced pressure to the desired volume. The yield of the extract was 10% (w/w).

### Hershberger assay and drug administration

Hershberger assay was performed according to the current draft guidelines for the rodent Hershberger assay (OECD, 2002). Castration was performed via the scrotal route under anaesthesia (ketamine; 60 mg/kg and xylazine; 6 mg/kg intraperitoneally).

Testosterone enanthate was purchased from the Aboureyhan Co. (Tehran, Iran). All treatments were started 1 day after surgery. Animals were divided into five groups ( $n = 7$ ).

Group C – Castrated animals in this group were given only vehicle (olive oil) for 7 days.

Group CT – Castrated animals in this group were treated with 0.15 mg testosterone enanthate in 0.1 mL olive oil, once daily for 7 days to serve as the controls.

Group CTE75 – Castrated animals in this group were treated with 0.15 mg testosterone enanthate in 0.1 mL olive oil plus 75 mg/kg *G. glabra* extract.

Group CTE150 – Castrated rats in this group were treated with 0.15 mg testosterone enanthate in 0.1 mL olive oil plus 150 mg/kg *G. glabra* extract.

Group CTE300 – Castrated rats in this group were treated with 0.15 mg testosterone enanthate in 0.1 mL olive oil plus 300 mg/kg *G. glabra* extract. The injections of the testosterone and *G. glabra* were given subcutaneously at the separate sites.

The doses of testosterone (Vogel, 2002) and *G. glabra* (Dhingra *et al.*, 2004; Parle *et al.*, 2004) were chosen based on the previous studies.

### Total plasma testosterone levels

On the eighth day, the animals were killed by overdosing with ketamin/xylazine. Blood samples were obtained by cardiac puncture. Plasma T levels were estimated by radioimmunoassay (RIA) and were assayed in duplicate. The assay was performed using a commercial kit (Orion Diagnostica, Spectria, Finland). All samples were run in the same assay period.

### Organ weight studies

Ventral prostate (VP) and SV were removed and their wet weights were determined. Seminal vesicles were removed bilaterally. Dissection of the *levator ani* muscle was performed after removal of the skin in the scrotal area between the base of the penis and the anus. The posterior aspect of the perineal body was cleared of connective tissue. The constriction at either end of the levator ani where it joins the *bulbocavernosus* muscle was exposed carefully. *Levator ani* was freed of the rectum and was removed. *Levator ani* muscle weight as well as body weight was recorded. Tissue weights were normalized to whole body weight.

### Stereological analysis

Ventral Prostate sections taken from the prostate, fixed in 10% formalin, dehydrated in gradual ethanol

(50–100%), cleared in xylene and embedded in paraffin. Sections (2–3  $\mu\text{m}$ ) were prepared. Sections were stained with haematoxylin and eosin. Every fifth section spanning the entire VP was used and stereology was performed using Weibel’s system. The volume densities of the epithelium and stroma were determined. An epithelium volume to stroma volume ratio was calculated. According to Deklerk and Coffey, 1 mg fresh prostate tissue has a volume of approximately 1  $\text{mm}^3$ . Consequently, the weight of the VP (mg) may be considered as equivalent to volume ( $\text{mm}^3$ ) (Justulin *et al.*, 2006). Thus, for calculation of the absolute volume of each compartment, the relative volume of the epithelium and stroma were multiplied by the mean VP wet weight from the respective group.

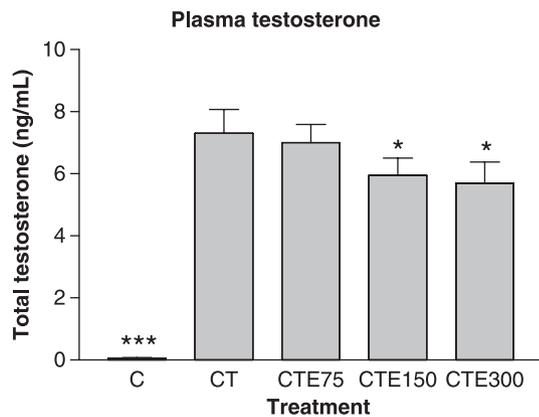
**Statistical analysis**

All data are reported as mean  $\pm$  SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the multiple comparison test of Tukey–Kramer were used for statistical evaluation. A *p* value of  $<0.05$  was considered significant. The statistical tests were performed using the software program SPSS (version 13; SPSS Inc., Chicago, IL, USA).

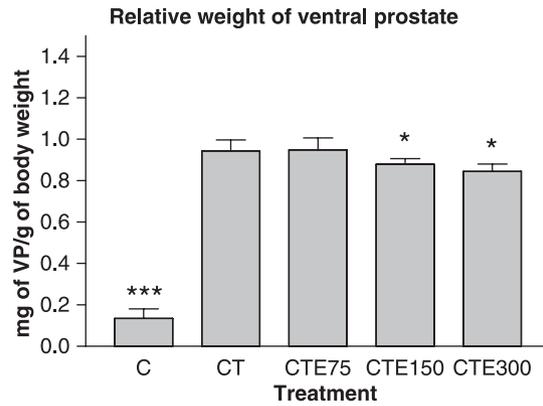
**Results**

**Total plasma testosterone levels**

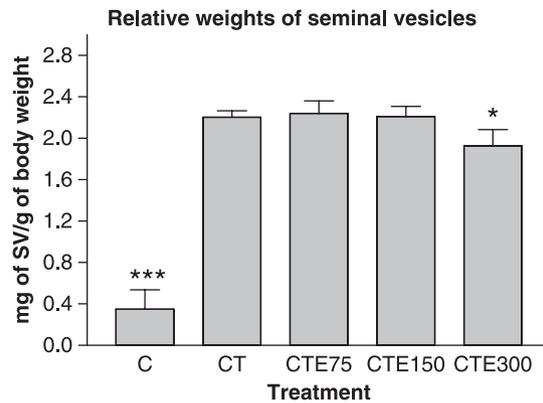
Figure 1 shows the data for total serum T concentration in different groups. Serum levels of T were significantly ( $p < 0.05$ ) decreased at 150 and 300 mg/kg dose levels of *G. glabra* extract compared with the controls (CT).



**Figure 1** Changes in the plasma testosterone level in rats ( $n = 7$  in each experimental group) following *Glycyrrhiza glabra* treatment. The values represent mean  $\pm$  SD. Means statistically significant different from control (CT) group \* ( $p < 0.05$ ) and \*\*\* ( $p < 0.0001$ ).



**Figure 2** Changes in the ventral prostate (mg) weight to body weight (g) ratio in rats ( $n = 7$  in each experimental group) following *Glycyrrhiza glabra* treatment. The values represent mean  $\pm$  SD. Means statistically significant different from control (CT) group \* ( $p < 0.05$ ) and \*\*\* ( $p < 0.0001$ ).



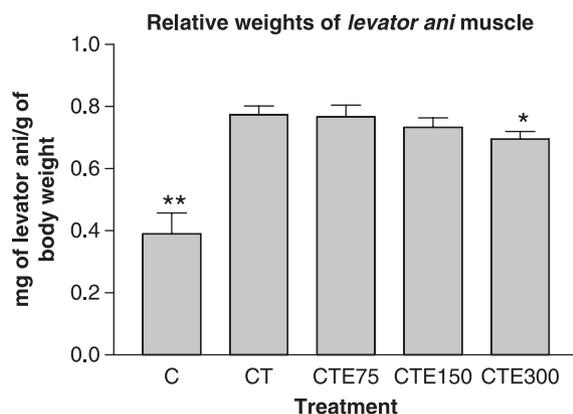
**Figure 3** Changes in the seminal vesicles (mg) weight to body weight (g) ratio in rats ( $n = 7$  in each experimental group) following *Glycyrrhiza glabra* treatment. The values represent mean  $\pm$  SD. Means statistically significant different from control (CT) group \* ( $p < 0.05$ ) and \*\*\* ( $p < 0.0001$ ).

**Organ weight studies**

Figures 2–4 show the organ weight/body weight ratio. This study demonstrated that administration of *G. glabra* at 150 and 300 mg/kg dose levels reduced prostate weight significantly ( $p < 0.05$ ) (Fig. 2). In contrast, seminal vesicles (Fig. 3) and *levator ani* (Fig. 4) weight were decreased significantly ( $p < 0.05$ ) only in the 300 mg/kg treatment group.

**Stereological analysis of the VP**

Treatment with *G. glabra* resulted in a significant reduction ( $p < 0.05$ ) in the volume density of the epithelium and an



**Figure 4** Changes in the *levator ani* muscle (mg) weight to body weight (g) ratio in rats ( $n = 7$  in each experimental group) following *Glycyrrhiza glabra* treatment. The values represent mean  $\pm$  SD. Means statistically significant different from control (CT) group \* ( $p < 0.05$ ) and \*\* ( $p < 0.001$ ).

**Table 1** Changes in the volume density of the ventral prostate epithelium, stromal compartment volume and the epithelium to stroma ratio in rats ( $n = 7$  in each experimental group) following *Glycyrrhiza glabra* treatment

| Treatment | Stroma volume (mm <sup>3</sup> ) | Epithelium volume (mm <sup>3</sup> ) | Epithelium/stroma ratio |
|-----------|----------------------------------|--------------------------------------|-------------------------|
| C         | 3.68 $\pm$ 0.74***               | 1.35 $\pm$ 0.29***                   | 0.38 $\pm$ 0.11***      |
| CT        | 23.51 $\pm$ 1.48                 | 46.14 $\pm$ 1.65                     | 1.96 $\pm$ 0.13         |
| CTE75     | 23.37 $\pm$ 1.22                 | 47.07 $\pm$ 1.50                     | 2.01 $\pm$ 0.11         |
| CTE150    | 25.98 $\pm$ 1.76*                | 42.28 $\pm$ 2.3*                     | 1.62 $\pm$ 0.09*        |
| CTE300    | 26.1 $\pm$ 1.89*                 | 39.58 $\pm$ 3.063**                  | 1.52 $\pm$ 0.22*        |

Values are mean  $\pm$  SD.

\* $p < 0.05$  vs. control (CT).

\*\* $p < 0.001$  vs. control (CT).

\*\*\* $p < 0.0001$  vs. control (CT).

increase in the stromal compartment (Table 1) at the doses of 150 and 300 mg/kg. The ratio of the prostate epithelium volume to prostate stroma volume was reduced significantly at the doses of 150 and 300 mg/kg (Table 1).

Castrated rats treated with T (CT group) revealed a simple, cylindrical epithelium with basal nuclei. The stroma was scarce. But, treatment with high doses of *G. glabra* resulted in shorter epithelial cells and the stroma was prominent (Fig. 5).

## Discussion

The data presented in this study demonstrate a reduction in serum T level, as a result of liquorice alcoholic extract administration. Various studies have demonstrated that liquorice block the activity of 3  $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ HSD), 17  $\beta$ -hydroxysteroid dehydrogenase

(17 $\beta$ HSD) and 17-20 lyase (Latif *et al.*, 1990; Armanini *et al.*, 1999, 2003, 2004). All these enzymes are involved in the synthesis and/or metabolism of androgens and oestrogens (Armanini *et al.*, 2004).

As, in our study, the main source of endogenous serum T (i.e. testis) was removed surgically, the reduction in serum T level could not be because of reduction in T synthesis. Therefore, T reduction could be resulting from increase in T metabolism by *G. glabra*.

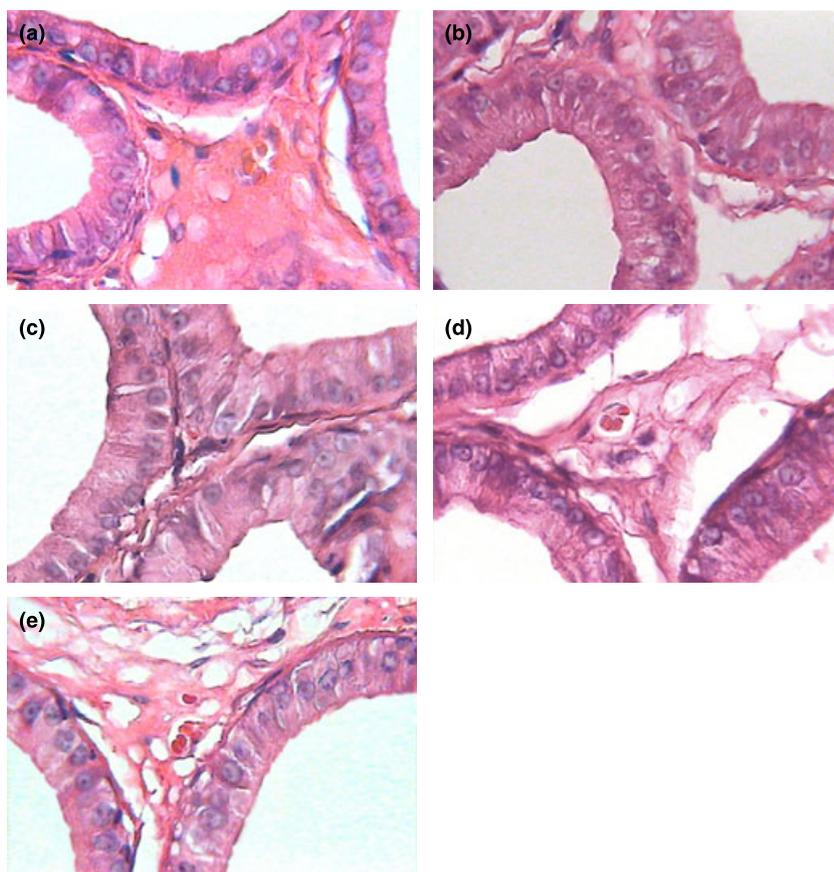
The data demonstrate significant decrease in VP and SV weight. But, there was a difference between two glands. Data showed that reduction in prostate weight was significant in 150 and 300 mg/kg doses. But SV weight was reduced only in rats treated with 300 mg/kg of liquorice.

The prostate and SV are androgen-dependent, therefore serum T reduction could result in prostate and SV weight reduction.

Down-regulation expression of the androgen receptor (AR) could be another possible mechanism for VP weight reduction. Glycyrrhetic and glycyrrhizic acid do not bind to AR (Armanini *et al.*, 2002), but a constituent of liquorice, DBM, inhibits the growth of androgen-responsive human PCa by down-regulation expression of the AR (Jackson *et al.*, 2007). DBM also has a potential role in the prevention and treatment of PCa by deregulation of the cell cycle, which correlated with cytostatic effects of DBM in prostate carcinoma cells (Jackson *et al.*, 2002). Down-regulation of AR by phytoestrogens like flavonoids and isoflavans has been suggested in previous studies (Lund *et al.*, 2004). Down-regulation of AR by liquorice phytoestrogens could be another factor involved in antiandrogenic properties of this plant.

Liquorice is rich in flavonoids and isoflavans, which exhibit oestrogen-like activity, because of their structural similarity to endogenous oestrogens and the configuration could enable them to bind to and to activate the E receptor (Armanini *et al.*, 2002). Two types of the ER are expressed in the prostate. ER $\alpha$  localized in prostatic stromal cells and ER $\beta$  primarily expressed in prostate epithelium. It is believed that stromal proliferation may be mediated through stromal ER $\alpha$ , while ER $\beta$  has an anti-proliferative role in the prostate epithelium (Prins & Korach, 2008). The weight reduction of VP could be because of more epithelial cell atrophy or cell loss in contrast to a mild stromal proliferation.

The data demonstrate a significant decrease in *levator ani* muscle weight. *Levator ani* muscle in rats has been found to be highly responsive to androgen. Studies in rats also indicate that androgen acts directly on this muscle to regulate its size via AR (Johansen *et al.*, 2007). Serum T reduction could be the main cause of the *levator ani* weight decreasing.



**Figure 5** Hematoxylin and eosin stained sections of the ventral prostate. Castration resulted in a short epithelium and a prominent stroma (a). Administration of T in castrated rats resulted in a cylindrical epithelial cells (b). The same results are seen in the T treated plus 75 mg/kg of *Glycyrrhiza glabra* (c). Reduction in epithelium height and increase in stroma are seen at the doses of 150 and 300 mg/kg of *G. glabra* (d and e). Magnification  $\times 640$ .

Down-regulation expression of the AR by DBM, flavonoids and isoflavans could be other possible mechanisms for *Levator ani* weight reduction.

Histological analysis of the rat VP showed that castrated rats treated with T (CT group) revealed a simple, cylindrical epithelium with basal nuclei. The stroma was scarce. But, treatment with *G. glabra* resulted in shorter epithelial cells and the stroma was prominent. The relative volume of epithelium to stromal compartment was reduced significantly. The prostate epithelial cells are androgen-dependent and as stroma dose not require androgen for survival (Morrissey *et al.*, 2002), these result in a substantial change in the ratio of epithelium to stroma volume. The more prominent stroma in the treated groups (high doses) could be because of ER $\alpha$  activation by liquorice phytoestrogens. On the other hand, epithelium volume reduction, as our results have shown, could be because of anti-proliferative properties of ER $\beta$ , activated by these ingredients.

The importance of androgen and AR in the development and progression of PCa has been discussed in the previous studies. The role of androgen in PCa is supported by the observations that PCa rarely occurs in eunuchs or in men with a deficiency in 5 $\alpha$ -reductases, the

enzymes that convert testosterone to its active metabolite DHT. Androgen-ablation therapies are the standard treatments for invasive metastatic PCa because a large percentage of the cancer cells are still responsive to androgen (Lu *et al.*, 2007). As previously mentioned, inhibition of the growth of PCa by down-regulation expression of the AR by DBM has been shown in previous studies (Jackson *et al.*, 2007). Based on the aforementioned matters liquorice could be considered as a chemotherapeutic agent for PCa in future studies.

In conclusion, the results presented in this study suggest an anti-androgenic activity of *G. glabra* in male rats.

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### References

- Allan, G., Lai, M. T., Sbriscia, T., Linton, O., Haynes-Johnson, D., Bhattacharjee, S. *et al.* (2007) A selective androgen receptor modulator that reduces prostate tumor size and

- prevents orchidectomy-induced bone loss in rats. *Journal of Steroid Biochemistry and Molecular Biology* 103, 76–83.
- Armanini, D., Bonanni, G. & Palermo, M. (1999) Reduction of serum testosterone in men by licorice. *New England Journal of Medicine* 341, 1158.
- Armanini, D., Fiore, C., Mattarello, M. J., Bielenberg, J. & Palermo, M. (2002) History of the endocrine effects of licorice. *Experimental and Clinical Endocrinology & Diabetes* 110, 257–261.
- Armanini, D., Bonanni, G., Mattarello, M. J., Fiore, C., Sartorato, P. & Palermo, M. (2003) Licorice consumption and serum testosterone in healthy man. *Experimental and Clinical Endocrinology & Diabetes* 111, 341–343.
- Armanini, D., Mattarello, M. J., Fiore, C., Bonanni, G., Scaroni, C., Sartorato, P. & Palermo, M. (2004) Licorice reduces serum testosterone in healthy women. *Steroids* 69, 763–766.
- Asl, M. N. & Hosseinzadeh, H. (2008) Review of pharmacological effects of *Glycyrrhiza* sp. and its bioactive compounds. *Phytotherapy Research* 22, 709–724.
- Bartsch, G., Rittmaster, R. S. & Klocker, H. (2002) Dihydrotestosterone and the concept of 5 $\alpha$ -reductase inhibition in human benign prostatic hyperplasia. *World Journal of Urology* 19, 413–425.
- Dhingra, D., Parle, M. & Kulkarni, S. K. (2004) Memory enhancing activity of *Glycyrrhiza glabra* in mice. *Journal of Ethnopharmacology* 91, 361–365.
- Di Mambro, V. M. & Fonseca, M. J. (2005) Assays of physical stability and antioxidant activity of a topical formulation added with different plant extract. *Journal of Pharmaceutical and Biomedical Analysis* 37, 287–295.
- Haraguchi, H., Ishikawa, H., Mizutani, K., Tamura, Y. & Kinoshita, T. (1998) Antioxidative and superoxide scavenging activities of retrochalcones in *Glycyrrhiza inflata*. *Bioorganic & Medicinal Chemistry* 6, 339–347.
- Jackson, K. M., Deleon, M., Verret, C. R. & Harris, W. B. (2002) Dibenzoylmethane induces cell cycle deregulation in human prostate cancer cells. *Cancer Letters* 178, 161–165.
- Jackson, K. M., Frazier, M. C. & Harris, W. B. (2007) Suppression of androgen receptor expression by dibenzoylmethane as a therapeutic objective in advanced prostate cancer. *Anti-cancer Research* 27, 1483–1488.
- Johansen, J. A., Breedlove, S. M. & Jordan, C. L. (2007) Androgen receptor expression in the levator ani muscle of male mice. *Journal of Neuroendocrinology* 19, 823–826.
- Justulin, L. A. Jr, Ureshino, R. P., Zannoni, M. & Felisbino, S. L. (2006) Differential proliferative response of the ventral prostate and seminal vesicle to testosterone replacement. *Cell Biology International* 30, 354–364.
- Kroes, B. H., Beukelman, C. J., van den Berg, A. J., Wolbink, G. J., van Dijk, H. & Labadie, R. P. (1997) Inhibition of human complement by beta-glycyrrhetic acid. *Immunology* 90, 115–120.
- Latif, S. A., Conca, T. J. & Morris, D. J. (1990) The effects of the licorice derivative, glycyrrhetic acid, on hepatic 3 $\alpha$ - and 3 $\beta$ -hydroxysteroid dehydrogenases and 5 $\alpha$ - and 5 $\beta$ -reductase pathways of metabolism of aldosterone in male rats. *Steroids* 55, 52–58.
- Lu, J., Kim, S. H., Jiang, C., Lee, H. & Guo, J. (2007) Oriental herbs as a source of novel anti-androgen and prostate cancer chemopreventive agents. *Acta Pharmacologica Sinica* 28, 1365–1372.
- Lund, T. D., Munson, D. J., Adlercreutz, H., Handa, R. J. & Lephart, E. D. (2004) Androgen receptor expression in the rat prostate is down-regulated by dietary phytoestrogens. *Reproductive Biology and Endocrinology* 2, 5.
- Morrissey, C., Buser, A., Scolaro, J., O'Sullivan, J., Moquin, A. & Tenniswood, M. (2002) Changes in hormone sensitivity in the ventral prostate of aging Sprague–Dawley rats. *Journal of Andrology* 23, 341–351.
- Nassiri-Asl, M., Saroukhani, S. & Zamansoltani, F. (2007) Anticonvulsant effects of aqueous extract of *Glycyrrhiza glabra* root in PTZ-induced seizure in mice. *International Journal of Pharmacology* 3, 432–434.
- Organization for Economic Cooperation and Development (OECD) (2002) Environment directorate. Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology. OECD Series on Testing and Assessment. OECD, Paris.
- Parle, M., Dhingra, D. & Kulkarni, S. K. (2004) Memory-strengthening activity of *Glycyrrhiza glabra* in exteroceptive and interoceptive behavioral models. *Journal of Medicinal Food* 7, 462–466.
- Prins, G. S. & Korach, K. S. (2008) The role of estrogens and estrogen receptors in normal prostate growth and disease. *Steroids* 73, 233–244.
- Spignoli, G. (2000) Protective effects of dietary flavonoids on cardiovascular system and circulation. *European Bulletin of Drug Research* 8, 1–8.
- Vogel, H. G. (2002) Drug Discovery and Evaluation: Pharmacological Assays, p. 1180. Springer-Verlag, Berlin.
- Wang, G. S. & Han, Z. W. (1993) The protective action of glycyrrhiza flavonoids against carbon tetrachloride hepatotoxicity in mice. *Yao Xue Xue Bao* 28, 572–576.
- Williamson, E. M. (2003) Licorice. In: Potter's Cyclopedic of Herbal Medicine (ed. C. W. Daniels), pp. 269–271. Saffron Walden, London.