

## **EFFECT OF GINSENG EXTRACT ON SOME HORMONAL AND IMMUNOLOGICAL PARAMETERS IN MALE NEW ZEALAND WHITE RABBITS**

**KAMEL, A.A. and HODA, M.L. ABD-ALLA**

Animal Health Research Institute - Zagazig

**Received:** 23. 10. 2005

**Accepted:** 31. 10. 2005

### **SUMMARY**

The present study was carried out to investigate the effect of Ginseng extract on some hormones, influencing immune responsiveness as Triiodothyronine (T<sub>3</sub>), Thyroxine (T<sub>4</sub>), Cortisol and Growth Hormone (GH) and some immunological parameters, including total protein, albumin, globulin ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) and immunoglobulins (IgM, IgG) in male white New Zealand rabbits. Fourteen healthy rabbits were assigned to two groups; the control groups, received physiological saline while the other one administrated 50 mg/kg b.w. of Ginseng extract (that dissolved in saline solution) daily for three months. Results showed that Ginseng consumption induced increment in both levels of plasma thyroid hormones (T<sub>3</sub>, T<sub>4</sub>) as well as total serum proteins, globulin,  $\gamma$  globulin and IgG. Though it lowered levels in plasma Cortisol and IgM and did not alter GH level. These findings indicated that the beneficial effect of using Ginseng as immunostimulant, however, through its direct effect on immune parameters or

indirect effect on hormones that potentiate immune response.

### **INTRODUCTION**

Continued use of subtherapeutic dose of antibiotics in animal feeds may result in presence of antibiotic residues in animal products and development of drug-resistant microorganisms in humans. Some of medicinal herbs can maintain health and enhance animal body resistance to many diseases, so it can be used as a substitute for antibiotics. Ginseng (*Panax Ginseng*) is one of the most valued medicinal herb belonging to family Araliaceae. It contains many valuable ingredients such as saponins (known as panaxosides or ginsenosides), antioxidants, peptides, polysaccharides, fatty acids, alcohols, cholesteryl ester-transfer protein inhibitors and vitamins (Huang, 1999). The same author mentioned that Ginseng can enhance potency, relieve stress, improve stamina, regulate blood pressure, lower cholesterol, reduce

the risk of heart attack, control diabetes, protect liver, block inflammation and stimulate healing. Moreover, Jung et al. (2005) reported that the effectiveness of wild Panax Ginseng leaf extract in suppressing lipid peroxidation and detoxifying free radicals in diabetic rats. It has been established that Ginseng treatment reduces bacterial load and lung pathology in both normal and chronically rats infected with mucoid *Pseudomonas aeruginosa* (See et al., 1997 and Song et al., 1997) and contributes to the cows recovery from mastitis (Hu et al., 2001). Furthermore, Nakaya et al. (2004) found that Ginseng radix extract induced production of tumor or necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interferon- $\gamma$  (IFN- $\gamma$ ) in the presence of polymyxin B (an lipopolysaccharide inhibitor). Also Yan et al. (2005) recorded that the polysaccharide ginsan (ethanol-insoluble fraction of Ginseng) constitutes a new noble agent for the improvement of gamma radiation-induced immunosuppression.

Some investigations induced the effectiveness of Ginseng as immunostimulant in rats with infection but it did not affect the rats without infection (Song et al., 1998).

Therefore, the present study was aimed to evaluate the effect of orally administration of Ginseng extract on the immunity of male New Zealand white rabbits by measuring some hormones, that affect immune responsiveness as Cortisol, Triiodothyronine ( $T_3$ ), Thyroxine ( $T_4$ ) and Growth Hormone (GH). In addition total protein, albumin, total alpha, beta, gamma globulins and immunoglobulins (IgM, IgG), in order to get benefit of its useful effect in maintaining health and

resisting diseases to recommend its use in the valuable animals especially racing horses.

## MATERIAL AND METHODS

### Animals:

Fourteen healthy male New Zealand white rabbits about 5 month of age with an average body weight of 2.5 kg were kept in galvanized wire batteries and maintained under identical hygienic condition with temperature  $22\pm 3.5^\circ\text{C}$ . The animals were divided into two groups (seven each), one used as control, that given physiological saline only while the other was given 50 mg/kg body weight of Ginseng extract, that dissolved in saline solution by using mouth tube daily for 3 months (Choi et al., 1999). Rabbits were supplied with a commercial balanced pelleted ration and a fresh clean water adlibitum.

Ginseng Extract (Korean red Ginseng extract) was obtained from Pharco Pharmaceuticals, Alexandria, Egypt, in capsules and each capsule contains 100 mg of Ginseng Extract.

### Samples:

Five blood samples were collected from ear veins into clean tubes with and without anticoagulant after 1st, 2nd and 3rd month of drug administration from each group. Plasma was obtained by centrifuging the blood in tubes with anticoagulant for hormonal assay while other tubes were allowed to clot at room temperature and centrifugated to obtain serum for others biochemical analysis.

**Biochemical analysis:**

Determination of plasma Triiodothyronine (T<sub>3</sub>) and Thyroxine (T<sub>4</sub>) after Abraham (1981), Cortisol according to Kley and Krushemper (1975) and Growth Hormone (GH) after Schalch and Parker (1964) using radioimmunoassay kits from Diagnostic Scientific Lab., U.S.A. using Elecsys 2010, Roch, Germany.

Electrophoresis for serum protein fractionation (albumin, α, β, γ globulins) according to Laemmli (1970).

Immunoglobulins (IgM, IgG) were estimated in serum using special plates (immunology plates)

using radial diffusion methods after Mancini et al. (1965).

The data were statistically analyzed using student "t" test according to Snedecor and Cochran (1969).

**RESULTS**

The data at table (1) showed changes in plasma hormones along different periods of orally Ginseng administration. It has been found that both plasma T<sub>3</sub> and T<sub>4</sub> were significantly increased after 30, 60 and 90 days of Ginseng consumption in compared with the control one.

**Table (1): Plasma levels of Triiodothyronien (T<sub>3</sub>), Thyroxine (T<sub>4</sub>), Cortisol and Growth Hormone (GH) of male rabbits after orally Ginseng administration (50 ml/kg b.w.) daily for 3 successive months.**

Parameters \ Periods of treatment	First month		Second month		Third month	
	Control	Ginseng administration	Control	Ginseng administration	Control	Ginseng administration
T <sub>3</sub> (ng/ml)	1.51± 0.007	1.63± 0.015**	1.50± 0.024	1.69± 0.023**	1.52± 0.022	1.65± 0.017**
T <sub>4</sub> (µg/dl)	3.07± 0.014	3.18± 0.013*	3.06± 0.016	3.22± 0.015**	3.08± 0.018	3.27± 0.011**
Cortisol (µg/dl)	0.682± 0.021	0.712± 0.013	0.681± 0.015	0.596± 0.017	0.680± 0.027	0.590± 0.012*
GH (ng/ml)	0.010± 0.004	0.011± 0.001	0.012± 0.001	0.014± 0.001	0.010± 0.002	0.011± 0.001

\* Significant at P<0.05

\*\* Significant at P<0.01

Regarding, the plasma levels of Cortisol revealed a significant decrease after third month of Ginseng administration but there was no significant changes in their levels after first and second month of administration. Also there was no significant alteration in the plasma levels of GH along periods of Ginseng administration.

Table (2) exhibited a highly significant increase

in the levels of total protein and globulin specially  $\gamma$ -globulin after 30, 60 and 90 days of Ginseng administration as compared with the control group. In the respect of serum levels of IgM and IgG (Table 3), the result revealed a significant decrease in the levels of IgM only at second and third month of Ginseng treated group while IgG showed a highly significant increase in its level, throughout all periods of Ginseng administration as compared to the control.

**Table (2): Total Protein, Albumin, total Globulins and its fractionations of male rabbits after orally Ginseng administration (50 ml/kg b.w.) daily for 3 successive months.**

Parameters \ Periods of treatment	First month		Second month		Third month	
	Control	Ginseng administration	Control	Ginseng administration	Control	Ginseng administration
Total protein (g/dl)	6.3± 0.13	7.8± 0.10**	6.4± 0.09	7.9± 0.03**	6.4± 0.11	8.1± 0.06**
Albumin (g/dl)	4.49± 0.21	4.63± 0.21	4.48± 0.20	4.65± 0.20	4.50± 0.22	4.53± 0.11
Globulin (g/dl)	1.81± 0.009	3.17± 0.016**	1.92± 0.010	3.25± 0.011**	1.90± 0.026	3.53± 0.007**
$\alpha$ -globulin	0.52± 0.013	0.55± 0.014	0.54± 0.016	0.54± 0.012	0.56± 0.020	0.60± 0.020
$\beta$ -globulin	0.80± 0.012	0.84± 0.012	0.83± 0.014	0.84± 0.006	0.82± 0.014	0.85± 0.012
$\gamma$ -globulin	0.48± 0.100	1.75± 0.006**	0.50± 0.022	1.83± 0.013**	0.49± 0.027	1.97± 0.003**

\* Significant at  $P < 0.05$

\*\* Significant at  $P < 0.01$

**Table (3): Immunoglobulins IgM and IgG of male rabbits after orally Ginseng administration (50 ml/kg b.w.) daily for 3 successive months.**

Parameters \ Periods of treatment	First month		Second month		Third month	
	Control	Ginseng administration	Control	Ginseng administration	Control	Ginseng administration
IgM (mg%)	11.49±0.29	11.22±0.28	11.45±0.30	9.27±0.39*	11.46±0.28	9.40±0.27**
IgG (mg%)	122±0.59	130±0.63**	123±0.91	175±0.77**	125±1.17	139±1.02**

Significant at P<0.05      \*\* Significant at P<0.01

## DISCUSSION

The thyroid hormones are capable of influencing immune responsiveness independent of their interaction with GH (March, 1994). The present study showed elevation in the levels of both T<sub>3</sub> and T<sub>4</sub> after first, second and third orally Ginseng administration. In accordance with this result Dai et al. (1999) found that the increased T<sub>3</sub> and T<sub>4</sub> levels of patients suffering from congestive heart failure following Ginseng injection and Due et al. (1991) suggested that ginsenosides have a stimulating effect on thyroid gland via the stimulation of the anterior pituitary. It has been shown that thyroid hormones directly stimulate the thymic epithelium (Villa-Verde et al., 1992) and have an intriguing effect on interleukin-2 (Chandratilleke and Marsh, 1993). Hence Ginseng extract may be potentiated the cell-mediated immunity through-out their effects on thyroid hormones.

Concerning, plasma Cortisol levels, the obtained data recorded a significant decrease in its levels at

third month of Ginseng treated group. This result agree with the previous result of Gaffney et al. (2001a,b) who suggested that Panax Ginseng inhibits 11-beta hydroxysteroid dehydrogenase one enzyme and catalyse the degradation of stress hormones into inactive compounds. Contrary to the present result, Luo et al. (1993) recorded that an increase in plasma concentration of ACTH and corticosterone in rats receiving ginsenosides. The biphasic effect of Ginseng of sometimes increasing and sometimes decreasing the stress response from increased occupancy of positive and negative feedback stress hormone receptors by its natural ligand due to inhibition of specific enzyme which function to limit receptor occupancy (Gaffney et al., 2001b).

The data present here did not showed any changes in the plasma levels of GH between the control and Ginseng treated group. Similarly Youl Kang et al. (2002) recorded that GH, testosterone, Cortisol and insulin-like growth factor were not significantly changed following Ginseng consump-

tion and do not support the use of Ginseng to promote an anabolic hormonal status following resistance exercise.

The present finding indicated that total protein, globulin specially  $\gamma$ -globulin increased significantly in serum of rabbits after Ginseng administration. These findings are similar to that obtained by Kim et al. (1993) who reported that Ginseng extracts acts directly on body cells promoting DNA and protein synthesis and potentiate the cell-mediated immunity and enhance the natural body resistance. Also Li et al. (1980) added that Ginseng can stimulate the production of specific antibodies in animals, resulting in an increase in immune function.

It is obvious from Table (3) that IgM decreased while IgG increased significantly following Ginseng administration. The result is concomitant with the result of Song et al. (2002) who recorded that Ginseng treatment resulted in lowering serum IgM, upregulated serum IgG2a and higher lung IgA. Whereas Liou et al. (2004) showed increased both IgM and IgG levels at the highest dose of panax Ginseng administration in mice (4 g/kg b.w.), indicating that the herbal drug Ginseng was able to regulate antibody production by augmenting Th1 (IL-2, IFN- $\gamma$ ), and Th2-type (IL-4, IL-10) cytokine production.

In conclusion, our observation in this study demonstrated that Ginseng extract possesses beneficial effects as immunostimulant, however, through its direct effect on immune parameters or indirect effect on hormones that potentiate im-

mune response. So, the obtained data encourages the used of Ginseng as natural medicine may have the potential to be a promising alternative supplement for the treatment or in conjunction with other forms of treatment to enhance body resistance of animals.

## REFERENCES

- Abraham, G.E. (1981): Radioassay system in clinical endocrinology: Marcel Dekker, Inc. New York.
- Chandratilleke, D. and Marsh, J.A. (1993): Effects of dietary Triiodothyronine and in vitro chicken Growth Hormone on avian interleukin-2 production. Poultry Sci. 72 (Suppl. 1) 100 (Abstr.).
- Choi, Y.D.; Rha, K.H. and Cho, H. K. (1999): Effect of Korean red Ginseng on the rabbit corpus cavernosal smooth muscle. Int. J. Impot. Res., 10 (1): 37-43.
- Dai, X.; Zhou, Y. and Yu, X. (1999): Effect of Ginseng injection in treating congestive heart failure and its influence on thyroid hormones. Zhongguo Zhong Xi Yi Jie He Zhi 19 (4): 209-211.
- Due, W. et al. (1991): Chin J. Appl. Physiol. 7: 125-128.
- Gaffney, B.T.; Hugel, H.M. and Rich, P.A. (2001a): The effects of *Eleutherococcus senticosus* and *Panax Ginseng* on steroidal hormone indices of stress and lymphocyte subset numbers in endurance athletes. Life Sci. 70 (4): 431-442.
- Gaffney, B.T.; Hugel, H.M. and Rich, P.A. (2001b): *Panax Ginseng* and *Eleutherococcus senticosus* may exaggerate an already existing biphasic response to stress via inhibition of enzymes which limit the binding of stress hormones to their receptors. Med. Hypotheses 56 (5): 567-572.
- Han, S.K.; Song, J.Y.; Yun, Y.S. and Yi, S.Y. (2005): Ginseng improved Th1 immune response inhibited by gamma radiation. Arch. Pharm. Res. 28 (3): 343-350.

- Hu, S.; Concha, C.; Johannisson, A.; Meglia, G. and Waller, K.P. (2001): Effect of subcutaneous injection of Ginseng on cows with subclinical *Staphylococcus aureus* mastitis. *J. Vet. Med. B. Infect. Dis. Vet. Public Health* 48 (7): 519-528.
- Huang K.C. (1999): The pharmacology of Chinese Herbs with multiple actions. CRC Press, Boca Raton, London, New York, Washington, pp. 17-51.
- Jung, C.H.; Seog, H.M.; Choi, I.W.; Choi, H.D. and Cho, H.Y. (2005): Effects of wild Ginseng (*Panax Ginseng* C.A. Meyer) leaves on lipid peroxidation levels and antioxidant enzyme activities in streptozotocin diabetic rats. *J. Ethnopharmacol.* 98 (3): 245-250.
- Kim, S.H.; Chack, V. and Kim, T.H. (1993): In vivo radioprotective activity of *Panax Ginseng* and diethyl dilhyl dithiocarbonate. *In vivo*, 7 (5): 467-470.
- Kley, H.K. and Krushemper, H.L. (1975): Georg Thieme verlay, Stuttgart pp. 20.
- Laemmli, V.K. (1970): Structural protein during the assembly of the head of bacteriophage T4. *Nature*, 227 (15): 680.
- Li, S. et al. (1980): *Jilin Chinese Med. Mat. Med.* 1: 67-71.
- Liou, C.J.; Li, M.L. and Tseng, J. (2004): Intraperitoneal injection of Ginseng extract enhances both immunoglobulin and cytokine production in mice. *Am. J. Chin. Med.* 32 (1): 75-88.
- Luo, Y.M. et al. (1993): *Chin J. Pharm. Toxicol.*, 14: 401.
- Mancini, G.; Carbonara, A.O. and Hermons, S. (1965): Immunochemical quantitation of antigen by single radial immune diffusion. *Immunochem.* 2: 235-241.
- March, J.A. (1994): Neuroendocrine-immune interactions. *Poultry Science* 73: 1049-1061.
- Nakaya, T.A.; Kita, M.; Kuriyama, H.; Iwakura, Y. and Imanishi, J. (2004): *Panax Ginseng* induces production of proinflammatory cytokines via toll-like receptor-24 (2): 93-100.
- Sendef, D. and Parker, M. (1964): A sensitive double antibody immunoassay for Growth Hormone in plasma. *Nature*, 203: 1141.
- Seo, D.J.; Broumand, B.; Sahl, L. and Tilles, J.G. (1997): In vivo effects of echinacea and Ginseng on natural killer and antibody-dependent cell cytotoxicity in healthy subjects and chronic fatigue syndrome or acquired immunodeficiency syndrome patients. *Immunopharmacology* 35: 229-235.
- Sendecor, G.W. and Cochran, W.G. (1969): *Statistical Methods*. 6th Ed. Iowa State University Press. Ames Iowa, U.S.A.
- Song, Z.; Kharazmi, A.; Wu, H.; Faber, V.; Moser, C.; Johnsen, H.K.; Rygaard, J. and Hoiby, N. (1998): Effects of Ginseng treatment on neutrophil chemiluminescence and immunoglobulin G subclasses in a rat model of chronic *Pseudomonas aeruginosa* pneumonia.
- Song, Z.; Wu, H.; Mathee, K.; Hoiby, N. and Kharazmi, A. (2002): Gerimax Ginseng regulates both humoral and cellular immunity during chronic *Pseudomonas aeruginosa* lung infection. *J Altern. Complement Med.* 8 (4): 459-466.
- Song, Z.J.; Johansen, H.K.; Faber, V.; Moser, C.; Kharazmi, A.; Rygaard, J. and Hoiby, N. (1997): Ginseng treatment reduces bacterial load and lung pathology in chronic *Pseudomonas aeruginosa* pneumonia in rats. *Antimicrob. Agents Chemother.* 41: 961-964.
- Villa-Verde, D.M.S.; Dfresne, M.P.; Vannierdos Santos, M.A.; Dassault, J.H.; Boniver, J. and Savino, W. (1992): Identification of nuclear Triiodothyronine receptors in the thymic epithelium. *Endocrinology* 131: 1313-1320.
- Youl Kang, H.; Hwan Kim, S.; Jun Lee, W. and Byrne, H. K. (2002): Effects of Ginseng injection on Growth Hormone testosterone, Cortisol and insulin-like growth factor I responses to acute resistance exercise. *J. Strength Cond. Res.* 16 (2): 179-183.

## تأثير خلاصة نبات الجنسنج على بعض الهرمونات والمؤشرات المناعية فى ذكور الأزانب النيوزيلاندى البيضاء

عادل على كامل - هدى محمد لطفى عبد الله

معهد بحوث صحة الحيوان - الزقازيق

تم إجراء هذا البحث لإستبيان مدى تأثير خلاصة نبات الجنسنج على بعض الهرمونات ذات التأثير المناعى مثل هرمون الترائى أيدوثيرونين ، الثيروكسين ، الكورتيزول وهرمون النمو إلى جانب بعض المؤشرات المناعية والتي تشمل البروتين الكلى والجلوبيولين الكلى ومشتقاته وأيضاً الأجسام المناعية من نوع IgG ، IgM فى ذكور الأزانب النيوزيلاندى البيضاء . وقد أستخدم فى هذا البحث ١٤ من ذكور الأزانب الصحيحه جسمانياً حيث تم تقسيمهم إلى مجموعتين متساويتين . الأولى تم جريعتها بمحلول ملح فسيولوجى يومياً وأستخدمت كمجموعة ضابطة بينما المجموعة الثانية تم جريعتها يومياً بجرعة مقدارها ٥٠ ملجرام/كجم من وزن الجسم من نبات الجنسنج والمذاب فى محلول ملح فسيولوجى ولمدة ٣ شهور .

وقد أظهرت النتائج أن المجموعة التى جُرعت بخلاصة نبات الجنسنج قد أظهرت زيادة فى مستوى هرمونات الغدة الدرقية (تراى أيدوثيرونين ، الثيروكسين) بالإضافة إلى مستوى البروتين الكلى والجلوبيولين الجاما جلوبيولين علاوة على الجسم المناعى من نوع IgG بينما إنخفض مستوى كل من هرمون الكورتيزول والجسم المناعى من نوع IgM ولم يحدث تغير فى مستوى هرمون النمو وذلك بالمقارنة بالمجموعة الضابطة .

وقد إتضح من نتائج هذه الدراسة أن نبات الجنسنج له تأثيرات مفيدة كمحفز مناعى سواء بالتأثير على الجهاز المناعى أو غير المباشر على الهرمونات التى لها دور فى الإستجابة المناعية . وهذه النتائج تشجع على إستخدام هذا النبات كدواء طبيعى له من القدرة على أن يكون بديل واعد فى العلاج أو الإقتران بالأشكال الأخرى من العلاج لرفع مقاومة جسم الحيوان.