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Toxicity Studies of Vicotoria Capsules Herbal Medicine

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Abstract: The acute, sub-acute and chronic toxicity were conducted on Victoria 1500 and 2000 capsules which contains royal jelly, panax ginseng and yohimbe park, all individually have not shown any toxicity on long term administration. Study was planned to assess the safety of the combination by doing toxicity studies. Toxicity studies were done in two animal models by using mice and rats. Acute, sub-acute and chronic toxicity studies were undertaken by treating nimals with a single dose of 15 times the recommended dose for acute, 5 times recommended dose administered orally each other day for 7 days for sub-acute and a daily recommended dose for 6 months for chronic toxicity studies. In acute and sub-acute toxicity studies histopathological results confirmed all vital organs to be normal. The results of the biochemical and hematological studies revealed some changes in some parameters of the treated animals. In sub-acute an increase in RBC and hemoglobin levels was shown. Treatment showed no spermatotoxic activity. During chronic toxicity study, the visceral condition of the animals and histopathological investigations were found to be normal. There were no drastic changes in the biochemical and hematological parameters and the results of the sperm count, sperm motility and sperm viability, respectively were found to be: $\geq 14\times10^{6}$ mL^{-1}, ≥ 50 and $\geq 50\%$.

Key words: Panax ginseng, royal jelly, yohimbine, acute, sub-acute, chronic toxicity, Sauid Arabia

INTRODUCTION

Victoria capsules contains Royal jelly, *Panax ginseng*, Bee pollen and Yohimbe bark powder as major ingredients. Royal jelly is a milky white gelatinous material secreted in the salivary glands of worker bees (*Apis mellifera*) for the sole apparent purpose of stimulating the growth and development of queen bees. Royal jelly is considered to extend life span and in general, reinvigorate the body. It is said to increase appetite and general vigor; retard aging; boost longevity; accelerate healing and strengthen the immune system. It exhibits antibiotic and antiviral properties. Royalisin, a protein isolated from royal jelly was found to possess a strong antibacterial activity against gram-positive bacteria (Supabphol, 1995; Fujiwara *et al*., 1990). Royal jelly showed antioxidative action.

Ginseng is a well-known medicinal herb in traditional Asian medicine. Ginsenosides are active compounds of ginseng and they are usually used as marker compounds for the quality control work on ginseng products. *P. ginseng* contains adaptogens; it is believed to be an anti-aging herb and is a favorite today because of its ability to be used for long-term without much toxic effects on the body. Ginseng possesses anti-cancer properties (Helms, 2004; Shin *et al*., 2000). *P. ginseng* has been used for the following: improve psychologic function, to lower cholesterol, increase metabolism, increase energy, stimulate the immune system, alleviate fatigue, control seizers, reduce nervousness and improve conditions associated with diabetes (Reay *et al*., 2006; Kiefer and Pantuso, 2003). The recommended dosage for extracts containing 4-8% of ginsenosides is 100 mg once or twice daily (Lian *et al*., 2005; Gale Group, 2001; Hobbs, 1996). Yohimbine is one of the active alkaloids found in Yohimbe bark (*Pausinystalia yohimbe* Pierre) previously known as *Corynanthe yohimbe*; family Rubiaceae. Yohimbine is a selective competitive α2-adrenergic receptor antagonist and is used in the treatment of male erectile dysfunction (Anonymous, 1998; Cremona, 1998; Nessel, 1994). In higher doses, yohimbine may lead to rapid heart rate, high blood pressure and anxiety.

Experinental animals: Toxicity studies were conducted using male and female mice adopting acute, sub-acute and chronic modes. Swiss albino mice (SWR) aged 6-7 weeks and weighing 20-25 g (home breed) were used. The animals were maintained under standard conditions of humidity, temperature and light (12 h dark/12 h light). The animals were fed with Purina chow diet with free access to water.

Acute toxicity evaluation: The dose selected for acute toxicity was 15 times the therapeutic dose. The drug in each case was suspended separately and homogenously in 1% Carboxymethyl Cellulose solution (CMC) and

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administered orally (0.5 mL mouse^{-1}) in a single acute dose. The control group received equal amount of vehicle. The animals were observed for 7 days after treatment. Each of the control and treated groups contained randomly allotted 10 male and 10 female mice kept separately. The animals were observed for any sign of toxicity.

Sub-acute toxicity evaluation: The study on sub-acute treatment involved repeated dose exposures for short term and provide information on cumulative effects, latency period for development of toxicity, reversibility of toxicity and dose response relationship. A dose of 5 times the therapeutic dose was given orally each other day for 7 days. The control group received vehicle in the same dose (0.5 mL). The parameters included in this study were based on standard toxicological screening program (Robin *et al*., 1982; Chan *et al*., 1982) and included screening on general toxicity systems, mortality, body and organ weight, hematology, biochemistry, genotoxicity and histopathology.

Chronic toxicity evaluation: For chronic toxicity test the prolonged treatment for a period of 90 days are suggested to be sufficient in short lived rodents in order to predict the hazards of long term low dose exposure of a particular drug (Mossberg and Hayes, 1989). A therapeutic dose was given orally for 90 days. The purpose of this investigation was to evaluate the effect of prolonged treatment on the target organs and the physiological and metabolic tolerance of the drug product at low doses. The parameters included in the study were based on the standard toxicological screening program (Robin *et al*., 1982; Chan *et al*., 1982; Mossberg and Hayes, 1989). The findings were sub-stantiated by histopathological studies.

MATERIALS AND METHODS

Ether (Sigma-aldrich) haematoxylin eosin test combination reagents (Boehringer Mannheim GmbH, Diagnostica-Germany). Formalin, methanol (Sigma-aldrich).

Instruments: Coulter counter spectrophotometer, introspect II (LKB), American optical rotary microtome, optical microscope, centrifuge.

Metabolic measurements: The animals were anesthesized with ether and blood was taken from the heart by direct puncture.

Biochemistry: The blood was collected, serum samples were separated, stored at -20°C and analyzed for Alanine aminotrasnferase (ALT/GPT), Aspartate aminos-trasnferase (AST/GOT), alkaline phosphatase, enzyme MB of Creatine Kinase (CK-MB), glucose, urea and creatinine. The parameters were analyzed by an enzymatic colorimetric method using test combination reagents (Boehringer Mannheim GmbH, Diagnostica, Germany). The measurements were carried out in a spectrophotometer, introspect II (LKB).

Hematology: The blood was analyzed on a Coulter counter for the quantification of different hematological indices such as WBC, RBC, hemoglobin, haematocrit, platelets and MCV.

Histopathological procedures: Tissue samples of liver, heart and kidney were preserved in 10% buffered formalin and processed for routine paraffin block preparation. Using an American optical rotary microtome, sections of thickness of about 5 μm were cut and stained with haematoxylin and eosin. The preparations were analyzed using an optical microscope compared to control animal preparation.

Genotoxic studies: The adhering soft tissue and epiphyses of both the tibiae were removed. The marrow was aspirated from each femur in fetal calf serum and transferred to centrifuge tubes. After centrifugation at 1000 rpm for 5 min. the supernatant was discarded and the residual cells were spread on slides and air dried. The slides were fixed in methanol, stained in May-Gruenwald solution followed by Giemsa stain. The coded slides were screened for the presence of micronuclei in polychromatic erythrocytes which indicated non-disjunction, chromosomal breaks and structural or numerical changes in the chromosomes. The bone marrow depression (mitotic index) was evaluated on the basis of the ratio of polychromatic to normochromati erythrocytes (PCE/NCE ratio) (Al-Harbi *et al*., 1994).

Semen examination: To study the effects of each drug product on male fertility, the chronically treated mice were used. At the end of chronic treatment 10 animals from the control and 10 animals from the treated groups were killed (by cervical dislocation or by ether anesthesia); the lower abdomen was opened, the caudae epididymis and the vas difference from each animal were dissected out and placed in a Petri dish (containing EBSS) to obtain the sperms as described earlier. The sperms were examined for:

- Sperm count using a hemocytometer after dilution 1:20
- Sperm motility (after dilution 1:6)
- Sperm viability using the differential staining of live and dead sperms

- Sperm abnormality by placing one drop of sperm suspension on a microscope slide and looking for amorphous, flatted, rotated head, microcephali and swollen chromosomes of the sperms

The results were evaluated with respect to those obtained from control non-treated male mice.

Statistical analysis: For the evaluation of results obtained during acute, sub-acute and chronic toxicity studies: Student's t-test and Chi-square (χ^2) test were used to assess the significance of the values obtained in the treated groups as compared to controls.

RESULTS AND DISCUSSION

Acute and sub-acute toxicity: During studies all animals, treated with Victoria 1500 and 2000 capsules separately were found to be normal compared to the animals in the respective control groups. At the end of the treatment, visceral condition was normal, histopathological results confirmed all vital organs to be normal compared to the control. The results of the biochemical and hematologica studies revealed some changes in some parameters of the treated animals as compared to the respective control. During sub-acute treatment, the animals treated showed increase in RBC and hemoglobin levels as compared to the control (Table 1 and 2). These findings are in agreement with the claims that bee pollen have some beneficial effects on hemolytic anemia and hemopoietic system (Wong *et al*., 1993). Treatment also showed no spermatotoxic activity.

Chronic toxicity: During study, there was a significant increase ($p<0.01$) in the post-treatment body weight of the animals as compared to the pre-treatment body weights and these changes were comparable to the control. There was also a significant rise ($p<0.01$) in180 water intake of animals which was found to be proportional to the body weight gain after chronic treatment studies (Table 3 and 4). All the animals were found healthy and no animal died in the treated groups. At the end of the chronic treatment, the visceral condition of the animals was found to be normal compared to the control. Histopathological investigations confirmed all vital organs of the male and female mice in the treated groups

Table 1: The effect of different dose treatments with Victoria 2000 capsules on water intake and body weight of tested animals

			Male mice			Female mice			Female rat	
Indices	Treatments	Animals group	Acute	Sub-acute	Chronic	Acute	Sub-acute	Chronic	Acute	Sub-acute
Body weight (g^{-1})	Before	Control	20.4±1.4	20.5±1.0	20.2±0.8	19.8±0.7	20.4±0.7	20.6±0.9	259±6.5	258±4.5
		Treated	20.3±0.8	20.1±0.7	19.4±0.8	18.8±0.6	20.1±0.6	19.1±0.3	255±3.6	255±3.0
	After	Control	22.9±1.2	24.7±0.8	39.0±1.2	21.9±1.0	24.2±0.6 3	36.3±0.9	265±6.5	274±5.0
		Treated	23.9±0.9	25.8±0.8	43.8±0.6	22.2±1.0	25.3±0.4	40.4±0.6	262±3.7	270±3.0
Water intake (mL^{-1})	Before	Control	NR	NR	3.9±0.1	NR	NR	3.8±0.1	NR	NR
		Treated	NR	NR	3.8±0.1	NR	NR	3.7±0.1	NR	NR
	After	Control	NR	NR	5.6±0.1	NR	NR	4.8±0.1	NR	NR
		Treated	NR	NR	5.7±0.2**	NR	NR	5.1±0.2**	NR	NR

Results are expressed as men±SEM; $p>0.05$: non-significant; *$p<0.05$: significant; **$p<0.01$: highly significant; compared with the control groups using paired student's t-test. NR = Not Required

Table 2: Biochemical and hematological indices of tested animals after different dose treatments with Victoria 2000 capsules

	Male mice				Female mice				Female rat		
Indices	Control	Acute	Sub-acute	Chronic	Control	Acute	Sub-acute	Chronic	Control	Acute	Sub-acute
Biochemical											
Glucose (mmol L^{-1})	5.4±0.30	5.2±0.40	5.5 ±0.30	5.5±0.30	5.1±0.30	4.8±0.40	5.3±0.40	5.3±0.40	6.3.±0.6	6.4±0.4	6.7±0.4
Urea (mmol L^{-1})	6.1±0.40	6.3± 0.20	6.0 ±0.20	6.2 ±0.40	6.0±0.30	6.1±0.30	5.8±0.30	6.2±0.30	7.5±.30	7.2±0.5	7.7±0.3
Creatinine (µmol L^{-1})	28.7±2.0	29.0 ±0.4	29.0 ±1.2	29.8± 2.0	26.8±1.3	26.8±1.8	28.0±1.2	28.4±1.1	62.0±2.0	65±6.0	58±5.0
Uric acid (µmol L^{-1})	62±6.00	60± 4.00	56 ±5.00	66± 3.00	59±3.00	56±5.00	60±2.00	63±3.00	118±10.0	121±1.0	126±9.0
Calcium (mmol L^{-1})	1.92±0.1	1.92±0.03	1.98±0.06	2.06±0.05	1.86±0.1	1.96±0.06	1.88±0.06	1.92±0.09	2.7±0.10	2.6±0.1	2.8±0.1
AST (U L^{-1})	100±14.0	97 ±10.00	85 ±8.00	96 ±6.00	100±12.0	94±6.00	106±10.0	97±5.00	85±7.00	90±8.0	93±6.0
ALT (U L^{-1})	56±7.00	53 ±6.00	57 ±5.00	56± 5.00	54±7.00	50±8.00	55±5.00	60±4.00	55±5.0 0	55±5.0	57±5.0
ALP (U L^{-1})	93±8.00	95 ±6.00	92± 9.00	89± 8.00	91±12.0	88±11.0	99±9.00	98±4.00	91±8.00	94±9.0	99±6.0
Hematological											
WBC ($\times10^9$ L^{-1})	5.6±0.40	6.2± 0.40	5.8± 0.20	5.7± 0.40	5.2±0.30	5.0±0.20	5.2±0.10	5.5±0.20	10.6±1.1	11.1±0.9	11.5±0.9
RBC ($\times10^{12}$ L^{-1})	6.4±0.50	6.6± 0.30	6.8 ±0.30	7.7 ±0.2*	6.5±0.40	6.6±0.40	6.7±0.10	8.0±0.3*	7.7±0.40	8.0±0.3	8.5±0.2*
Hg (g dL^{-1})	12.9±0.5	13.1± 0.5	13.0± 0.5	14.7±0.3*	12.7±0.5	12.8±0.2	13.0±0.2	14.6±0.3*	13.5±0.6	13.8±0.5	14.2±0.2*
Platelets ($\times10^9$ L^{-1})	503±17.0	483± 23.0	491± 27.0	494± 11.0	485±24.0	487±17.0	479±19.0	490±34.0	630±53.0	655±52.0	633±26.0
MCV fl	51.7±1.1	51.9± 0.7	51.2± 0.8	50.9 ±0.8	51.1±0.7	51.4±0.7	50.7±0.7	51.0±0.5	55.0±1.2	55.2±0.7	55.4±0.9
HCT (%)	38.7±0.7	39.3 ±0.8	38.8± 0.2	38.4± 0.3	38.8±1.0	39.2±0.5	38.6±0.2	40.0±0.3	40.6±1.6	40.6±1.4	40.9±0.9

Results are expressed as mean±SEM; $p>0.05$: non-significant; *$p<0.05$: significant; **$p<0.01$: highly significant; compared with the control groups using Paired student's t-test

Table 3: The effect of different dose treatments with Victoria 1500 capsules on water intake and body weight of tested animals

Indices	Treatments	Animals group	Male mice			Female mice			Female rat	
			Acute	Sub-acute	Chronic	Acute	Sub-acute	Chronic	Acute	Sub-acute
Body weight (g^{-1})	Before	Control	20.4±1.4	20.5±1.0	20.2±0.8	19.8±0.7	20.4±0.7	20.6±0.9	259±6.5	258±4.5
		Treated	20.2±0.9	20.0±1.0	20.4±0.4	18.8±0.6	20.3±1.2	19.5±0.5	255±3.0	255±3.0
	After	Control	22.9±1.2	24.7±0.8	39.0±1.2	21.9±1.0	24.2±0.6 3	36.3±0.9	265±6.5	274±5.0
		Treated	24.2±0.7	25.8±0.8	44.6±0.7	21.9±0.4	25.5±0.7	40.8±0.6	262±3.0	268±5.0
Water intake (mL^{-1})	Before	Control	NR	NR	3.9±0.1	NR	NR	3.8±0.1	NR	NR
		Treated	NR	NR	3.8±0.1	NR	NR	3.8±0.1	NR	NR
	After	Control	NR	NR	5.6±0.1	NR	NR	4.8±0.1	NR	NR
		Treated	NR	NR	5.7±0.1**	NR	NR	5.1±0.2**	NR	NR

Results are expressed as men±SEM; p>0.05: non-significant; *p<0.05: significant; **p<0.01: highly significant; compared with the control groups using Paired student's t-test. NR = Not Required

Table 4: Biochemical and hematological indices of tested animals after different dose treatments with Victoria 1500 capsules

Indices	Male mice				Female mice				Female rat		
	Control	Acute	Sub-acute	Chronic	Control	Acute	Sub-acute	Chronic	Control	Acute	Sub-acute
Biochemical											
Glucose (mmol L^{-1})	5.4±0.30	5.7±0.20	5.6±0.30	5.5±0.30	5.1±0.30	5.3±0.4	5.1±0.20	4.9±0.3	6.3±0.6	6.4±0.5	6.0±0.40
Urea (mmol L^{-1})	6.1±0.40	5.7±0.10	5.8±0.2 0	6.1±0.20	6.0±0.3 0	6.1±0.4	5.9±0.3 0	5.9±0.3	7.5±.0.3	7.7±0.3	7.4±0.30
Creatinine (µmol L^{-1})	28.7±2.0	29.0±2.3	29.2±1.0	29.0±2.0	26.8±1.3	26.8±0.6	28.2±2.2	28.6±1.3	62.0±2.0	59±4.0	64±4.00
Uric acid (µmol L^{-1})	62±6.00	59±1.00	57±2.00	57±3.00	59±3.0	58±2.00	0 60±3.0	58±4.00	118±10.0	113±7.0	110±13.0
Calcium (mmol L^{-1})	1.92±0.10	2.04±0.05	2.02±0.07	2.02±0.06	1.86±0.1	2.02±0.03	1.90±0.10	1.94±0.05	2.7±0.10	2.6±0.1	2.8±0.10
AST (U L^{-1})	100±14.0	91±8.00	93±6.00	98±10.0	100±12.0	89±4.00	94±9.00	99±13.0	85±7.00	83±9.0	89±9.00
ALT (U L^{-1})	56±7.00	42±6.00	55±5.00	51±6.00	54±7.0	52±8.00	58±3.00	52±7.00	55±5.00	53±4.0	49±6.00
ALP (U L^{-1})	93±8.00	87±5.00	91±5.00	97±7.00	91±12.0	88±7.00	93±8.00	90±7.00	91±8.00	95±5.0	89±4.00
Heamatological											
WBC ($\times 10^9$ L^{-1})	5.6±0.40	5.8±0.20	5.8±0.20	5.9±0.40	5.2±0.3	5.3±0.40	5.0±0.20	5.3±0.20	10.6±1.1	10.6±1.1	11.4±0.6
RBC ($\times 10^{12}$ L^{-1})	6.4±0.50	6.5±0.30	6.9±0.20	7.6±0.2*	6.5±0.4	6.3±0.30	6.6±0.20	8.1±0.2*	7.7±0.40	8.0±0.3	8.4±0.3*
Hg (g dL^{-1})	12.9±0.5	13.1±0.1	13.4±0.4	14.5±0.4*	12.7±0.5	12.7±0.2	13.0±0.3	14.1±0.4*	13.5±0.6	13.5±0.6	14.2±0.3*
Platelets ($\times 10^9$ L^{-1})	503±17.0	506±23.0	504±28.0	483±41.0	485±24.0	491±17.0	485±12.0	493±20.0	630±53.0	651±28.0	649±34.0
MCV fl	51.7±1.1	51.5±1.1	51.1±1.2	51.6±0.9	51.1±0.7	50.8±0.6	50.9±0.3	52.2±1.1	55.0±1.2	55.4±0.9	55.8±1.0
HCT (%)	38.7±0.7	39.0±0.2	38.9±0.6	39.0±0.4	38.8±1.0	28.0±0.7	38.7±0.2	38.6±0.8	40.6±1.6	40.1±0.7	41.1±1.1

Results are expressed as mean±SEM; p>0.05: non-significant; *p<0.05: significant; **p<0.01: highly significant;compared with the control groups using Paired student's t-test

to be normal compared to the control. There were no drastic changes in the biochemical and hematological parameters of the animals in the treated groups. At the end of the chronic treatement, the results of the sperm count, sperm motility and sperm viability, respectively were found to be: $\geq 14\times 10^6$ mL^{-1}, ≥50 and ≥50%, respectively which were comparable to the respective control animals. It is worth mentioning that Royal jelly which is one of the major constituent of Victoria 1500 and 2000 was found to provoke some allergic reaction in susceptible individual suffering from asthma or anaphylaxis (Leung *et al*., 1997; Thien *et al*., 1966). Furthermore, yohimbine (an active alkaloid found in Yohimbe bark) in higher doses was reported to increase the heart rate, blood pressure and anxiety. Therefore, Victoria 1500 and 2000 may be used with care by susceptible persons having such complaints.

CONCLUSION

Based on the results of the current toxicity studies, it is observed that Victoria 1500 and 2000 possess low toxicity and could be used in the labelled dose without any remarkable side effect.

REFERENCES

Al-Harbi, M.M., S. Qureshi, M.M. Ahmed, S. Rafatullah and A.H. Shah, 1994. Effect of commiphora molmol (aloe-gum-resin) on cytological and chemical changes induced by cyclophophamide in mice. Am. J. Chin. Med., 32: 77-82.

Anonymous, 1998. Yohimbine for male sexual dysfunction. Med. Lett. Drugs Ther., 36: 115-116.

Chan, P.K., G.P. O'Hara and A.W. Hayes, 1982. Principles and Methods of Acute and Sub-Chronic Toxicity. In: Principles and Methods of Toxicology, Hayes, A.W. (Ed.). Raven Press, New York, ISBN: 0890044708, pp: 1-51.

Cremona, T., 1998. Getting beyond Viagra: Natural sex enhancers. Nat. Pharm., 2: 14-15.

Fujiwara, S., J. Imai, M. Fujiwara, T. Yaeshima, T. Kawashima and K. Kobayashi, 1990. A potent antibacterial protein in Royal Jelly. Purification and determination of the primary structure of royalisin. J. Biol. Chem., 265: 11333-11337.

Gale Group, 2001. Gale Encyclopedia of Alternative Medicine. Thomson Gale, USA., ISBN-13: 9780787649999, pp: 1937.

Helms, S., 2004. Cancer prevention and therapeutics: *Panax ginseng*. Alternative Med. Rev., 9: 259-274.

Hobbs, C., 1996. Ginseng: The Energy Herb. Love Land Co., Botanica Press, USA.

Kiefer, D. and T. Pantuso, 2003. Panax ginseng. Am. Fam. Physician, 68: 1539-1542.

Leung, R., A. Ho, J. Chan, D. Choy and C.K. Lai, 1997. Royal jelly consumption and hypersensitivity in the community. Clin. Exp. Allergy, 27: 333-336.

Lian, X.Y., Z.Z. Zhang and J.L. Stringer, 2005. Anticonvulsant activity of ginseng on seizures induced by chemical convulsants. Epilepsia, 46: 15-22.

Mossberg, A.T. and A.W. Hayes, 1989. Sub-chronic Toxicity Testing. In: Principles and Methods of Toxicology, Hayes, A.W. (Ed.). 2nd Edn., Raven Press, New York, pp: 221-236.

Nessel, M.A., 1994. Yohimbine and pentoxifylline in the treatment of erectile dysfunction. Am. J. Psychiatry, 151: 453-453.

Reay, J.L., D.O. Kennedy and A.B. Scholey, 2006. The glycaemic effects of single doses of *Panax ginseng* in young healthy volunteers. Br. J. Nutr., 96: 639-642.

Robin, J.F., J.J. Jomer and R.R. Schueler, 1982. Methods in Testing for Carcinogenicity. In: Principles and Methods of Toxicology, Hayes, A.W. (Ed.). Raven Press, New York, pp: 79-105.

Shin, H.R., J.Y. Kim, T.K. Yun, G. Morgan and H. Vainio, 2000. The cancerpreventive potential of *Panax ginseng*: A review of human and experimental evidence. Cancer Causes Control, 11: 565-576.

Supabphol, R., 1995. Antibacterial activity of Royal Jelly royalisin: Potent antibacterial protein from Royal Jelly. Mahidol Univ. J. Pharm. Sci. Varasarn Paesachasarthara, 22: 33-38.

Thien, F.C., R. Leung, B.A. Baldo, J.A. Weiner, R. Plomley and D. Czarny, 1966. Asthma and anaphylaxis induced by Royal Jelly. Clin. Exp. Allergy, 26: 216-222.

Wong, M.S., H.F. Fan and H.J. Xu, 1993. Effects of bee pollen on blood and hemopoietic system in mice and rats. Chin. Tradit. Herbs Drugs, 24: 588-591.