

Toxicity Study of Korean Ginseng Herbal medicine

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Abstract: The toxicity studies of Korean ginseng capsules were evaluated in mice through examination of possible biochemical, hematological and histopathological changes. Acute, sub-acute and chronic toxicity studies were undertaken by treating mice with a single dose of 300 mg kg⁻¹ body weight for acute, 100 mg kg⁻¹ body weight administered orally each other day for 7 days for sub-acute and an oral dose of 20 mg kg⁻¹ body weight of the drug daily for 90 days for chronic toxicity studies. In acute, sub-acute and chronic treated mice, biochemical studies revealed a significant decrease in the blood glucose level in all treated animals. Hematological studies revealed an increase in RBC and hemoglobin contents in the treated male group as compared to the control. Absent of signs of visceral toxicity and histopathology result confirmed that all the studied organs were normal compared to the control.

Key words: Korean ginseng, acute, sub-acute, chronic toxicity, RBC, hemoglobin

INTRODUCTION

Ginseng is a well-known medicinal herb in traditional Asian medicine and is considered an adaptogen. Panax ginseng C.A. Meyer (Araliaceae), grows in China and Korea and >30 ginseng saponins (ginsenosides) have been isolated as the main biologically active constituents that include anti-carcinogenic, anti-diabetic and anti-inflammatory effects as well as cardiovascular protection and neuroprotection (Zhang *et al.*, 1996; Yun *et al.*, 2001; Joo *et al.*, 2005; Jung *et al.*, 2005).

Panax ginseng appears to inhibit some characteristics associated with cancer in animal models and human studies (Shin *et al.*, 2000). As cancer is quickly becoming the leading cause of death in the world many reports have focused on chemoprevention trials with Panax ginseng. Most of the pharmacological actions of ginseng are attributed to a variety of ginsenosides which are triterpenoid saponins (Attele *et al.*, 1999; Huang *et al.*, 2005). The physiological and medicinal effects of the various ginsenosides differ and can even be oppositional (Sengupta *et al.*, 2004; Joo *et al.*, 2005).

Since, ginsenosides produce effects that differ from one another and a single ginsenoside initiates multiple actions, the overall pharmacology of ginseng is complex. Thus, ginseng extracts have been studied to examine the final activity of a wide range of biological actions (Tsang *et al.*, 1985; Nishino *et al.*, 2001; Mcelhaney *et al.*, 2004). Korean ginseng contains adaptogens that have been shown to return the body's system levels back to normal by equalizing body's system levels and has been

used for the following: to lower cholesterol increase metabolism, increase energy, stimulate the immune system, alleviate fatigue, reduce nervousness and reduce stress on the body (Bucci, 2000).

Korean ginseng is officially registered in the Ministry of Health (Saudi Arabia) for its anti-diabetic and anti-inflammatory effects as well as cardiovascular protection with a recommended dose of 700 mg twice daily. No toxicity data were available in official files. Therefore, the present study was designed to investigate acute, subacute and chronic toxicity effects of Korean ginseng capsules.

Experimental 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⁻¹ light). The animals were fed with Purina chow diet with free access to water.

Acute toxicity evaluation: The dose selected for acute toxicity was (300 mg kg⁻¹) which is 15 times the therapeutic dose. The drug in each case was suspended separately and homogenously in 1% Carboxymethyl Cellulose solution (CMC) and administered orally (0.5 mL per mouse) 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 100 mg kg⁻¹ 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 ninety days (WHO scientific group) is 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 dose of 20 mg kg⁻¹ 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 substantiated by histopathological studies.

MATERIALS AND METHODS

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

Instuments: Coulter counter Spectrophotometer, Introspect 2 (LKB). American optical Rotary Microtome. Optical microscope centrifuge.

Metabolic measurements: The animals were anesthetized 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 aminotransferase (ALT/GPT), aspartate aminotransferase

(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 normochromatic erythrocytes (PCE/NCE ratio) (Al-Harbi *et al.*, 1994)

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

RESULTS AND DISCUSSION

Acute toxicity: Among the vital organs there was statistically no significant difference in treated male, female and control mice. There was increase in RBC and hemoglobin contents in the treated male group as compared to the control (Table 1 and 2). It was found that Korean ginseng treatment significantly lowered

Table 1: Hematological studies on male mice after acute treatment with K-Gin

Hematological indices (Mean±SE)						
Groups	WBC×10 ⁹ L ⁻¹	RBC×10 ¹² L ⁻¹	Hemoglobin (g dL ⁻¹)	Platelets10 ⁹ L ⁻¹	MCV (fL)	HCT (ratio %)
Control	5.71±0.50	6.1±0.60	12.9±0.35	484±28.0	51.5±0.9	38.70±0.8
K-Gin	5.43±0.30	7.7±0.41*	13.7±0.30*	470±35.0	51.8±0.7	38.34±0.4

Table 2: Hematological studies on female mice after acute treatment with K-Gin

Hematological indices (Mean±SE)						
Groups	WBC×10 ⁹ L ⁻¹	RBC×10 ¹² L ⁻¹	Hemoglobin (g dL ⁻¹)	Platelets10 ⁹ L ⁻¹	MCV (fL)	HCT (ratio %)
Control	5.14±0.40	6.21±0.63	12.5±0.32	464±20.0	51.06±0.45	38.4±0.63
K-Gin	4.50±0.50	7.50±1.24	13.0±0.70	478±33.0	51.96±0.81	38.1±0.55

Table 3: Biochemical studies on male mice after acute treatment with K-Gin

Biochemical indices (Mean±SE)						
Groups	AST (u L ⁻¹)	ALT (u L ⁻¹)	CK-MB (u L ⁻¹)	Creat. (µmol L ⁻¹)	Urea (mmol L ⁻¹)	Glucose (mmol L ⁻¹)
Control	16.0±0.58	11.15±1.50	145.8±15.61	132.86±2.21	5.84±0.35	5.42±0.09
K-Gin	18.6±2.16*	15.96±1.79*	141.3±4.190	125.70±3.55	5.80±0.41	3.71±0.32*

Table 4: Biochemical studies on female mice after acute treatment with K-Gin

Biochemical indices (Mean±SE)						
Groups	AST (u L ⁻¹)	ALT (u L ⁻¹)	CK-MB (u L ⁻¹)	Creat. (µmol L ⁻¹)	Urea (mmol L ⁻¹)	Glucose (mmol L ⁻¹)
Control	15.30±0.52	11.12±0.70	140.5±14.60	99.57±3.61	5.53±0.29	5.45±1.02
K-Gin	16.50±0.95	16.30±1.52*	141.3±15.35	97.30±4.91	5.93±1.04	3.20±0.08*

Table 5: Hematological studies on male mice after chronic treatment with K-Gin

Hematological indices (Mean±SE)						
Groups	WBC×10 ⁹ L ⁻¹	RBC×10 ¹² L ⁻¹	Hemoglobin (g dL ⁻¹)	Platelets10 ⁹ L ⁻¹	MCV (fL)	HCT (ratio %)
Control	5.70±0.45	6.30±0.60	13.00±0.58	517±25.0	52.2±1.80	38.40±0.83
K-Gin	5.60±0.60	7.81±0.55*	14.10±0.45*	524±15.0	52.8±0.53	37.80±0.60

Table 6: Hematological studies on female mice after chronic treatment with K-Gin

Hematological indices (Mean±SE)						
Groups	WBC×10 ⁹ L ⁻¹	RBC×10 ¹² L ⁻¹	Hemoglobin (g dL ⁻¹)	Platelets 10 ⁹ L ⁻¹	MCV (fL)	HCT (ratio %)
Control	5.41±0.40	6.9±0.4	13.0±0.2	535±50.0	51.6±0.7	38.8±2.1
K-Gin	4.60±0.40	7.5±0.9	14.4±0.2*	548±38.0	52.3±0.3	38.9±0.5

*p<0.05 (Student's t-test); Total 5 male mice were used in each group

the blood glucose level in all treated animals (Table 3 and 4). There are no obvious signs of any visceral toxicity and histopathology results confirmed that all the studied organs were normal compared to the control.

Sub-acute toxicity: There were no toxicity signs and symptoms in any treated group. At the end of the treatment, the visceral condition of all the animals in the treated groups (male and female mice groups) was found normal compared to the control groups.

Histopathology studies on animals in different groups confirmed all vital organs in the treated groups were normal and compared to the control. Biochemical studies on the Korean ginseng treated animals, however showed a significant lowering of the blood glucose levels in all treated groups.

Hematological studies after sub-acute treatment, revealed a significant rise in RBC and hemoglobin levels of male animals in the Korean ginseng treated groups compared to the controls. All other studied parameters

remained within the normal range and compared to the control. Korean ginseng was found to be devoid of any clastogenic or cytotoxic effect during the current study.

Chronic toxicity: A subsequent increase in the water intake in all the treated and control groups was observed. The gross and histopathological studies further confirmed all the vital organs of the Korean ginseng treated animals were normal and compared to the control. Hematological studies revealed a significant rise in the RBC contents and hemoglobin levels of the male treated group animals as compared to the control (Table 5 and 6).

During biochemical studies a significant decrease in the blood glucose levels of the treated groups as compared to the control was observed (Table 7 and 8). At the end of the treatment, the visceral condition of the Korean ginseng treated group animals was found normal compared to the control. Korean ginseng was found to show no clastogenic or cytotoxic effect during the current study (Table 9). In the current study, K-Gin capsule

Table 7: Biochemical studies on male mice after chronic treatment with K-Gin

Biochemical indices (Mean±SE)						
Groups	AST (u L ⁻¹)	ALT (u L ⁻¹)	CK-MB (u L ⁻¹)	Creat. (µmol L ⁻¹)	Urea (mmol L ⁻¹)	Glucose (mmol L ⁻¹)
Control	16.40±2.40	12.80±1.90	96.00±22.00	83.00±13.05	4.55±0.50	5.11±0.30
K-Gin	18.65±3.91	12.90±3.70	83.90±14.50	79.50±10.10	4.46±0.43	4.00±0.41*

Table 8: Biochemical studies on female mice after chronic treatment with K-Gin

Biochemical indices (Mean±SE)						
Groups	AST (u L ⁻¹)	ALT (u L ⁻¹)	CK-MB (u L ⁻¹)	Creat. (µmol L ⁻¹)	Urea (mmol L ⁻¹)	Glucose (mmol L ⁻¹)
Control	15.50±0.66	12.30±1.63	98.50±9.610	135.61±2.25	4.26±0.35	5.40±0.14
K-Gin	16.05±0.75	13.00±1.50	105.00±16.13	138.00±4.24	4.38±0.31	3.45±0.51*

Table 9: Effects of chronic treatment with K-Gin on the femoral cells in mice

Groups	Polychromatic Erythrocytes (PCE) screened	Micronucleated Polychromatic Erythrocytes (MPE) (%)	Normochromatic Erythrocytes (NCE) screened ⁻¹	PCE/NCE ratio
Control	5200	0.23±0.03	5060	1.04±0.07
Adriamycin	5000	5.32±0.33**	7596	0.67±0.04*
K-Gin	5645	0.28±0.03	7050	0.81±0.06

*p<0.05 (Student's t-test); Total 5 male mice were used in each group

contents (Panax ginseng) were subjected to acute, sub-acute and chronic toxicity studies in mice. During acute toxicity evaluation, ginseng treatment caused reduction in blood glucose levels in both male and female treated groups compared to mice in the control groups which may be attributed to panaxans, ginsenosides and some other chemical constituents of ginseng (Sotaniemi *et al.*, 1995). These findings substantiated the earlier reports about the antidiabetic potential of ginseng (Kiefer and Pantuso, 2004; Sotaniemi *et al.*, 1995). Ginsenosides are the main active components of P. ginseng which possess a variety of beneficial effects including anti-inflammatory, antioxidant and anticancer effects (Shen *et al.*, 2007; Chang *et al.*, 2003). Some clinical studies conducted earlier showed that P. ginseng may improve psychologic function, immune function and conditions associated with diabetes (Kiefer and Pantuso, 2004).

During current study, a significant increase in RBC and hemoglobin levels of the treated male animals compared to the control was observed. At the end of the treatment, there were no signs of visceral toxicity and all vital organs were found normal. Histopathological studies proved all vital organs were normal compared to the control. At the end of sub-acute treatment, visceral condition and vital organs of all mice in the treated groups were normal compared to the control. Biochemical studies revealed a significant reduction in blood glucose levels of the treated animals as compared to the control. Hematological studies showed rise in RBC and hemoglobin levels only in the male treated group as compared to the control. These hematological changes in treated male mice may be attributed to the elevation of androgen levels in male mice that is capable of stimulating erythropoiesis (Qureshi *et al.*, 1992). K-Gin treatment was

found to be devoid of any clastogenic or cytotoxic effect during current study and support the earlier reports (Wang *et al.*, 2006). The results of the present toxicity studies showed reduction in blood glucose levels of mice in the treated groups and support the earlier claims about the antidiabetic potential of this plant (Sotaniemi *et al.*, 1995). At the end of chronic treatment, the visceral condition of mice in the treated groups was normal compared to the control. Hematological studies revealed increase in the RBC and hemoglobin levels in the male treated group as compared to the control.

Biochemical studies revealed a significant decrease in the blood glucose levels of the animals in the treated groups as compared to the control. This reduction in blood glucose levels in the treated groups may be attributed to panaxans, ginsenosides and some other chemical constituents of ginseng which are known to reduce blood sugar levels (Sotaniemi *et al.*, 1995). Ginseng chronic treatment showed no clastogenic or cytotoxic effect during current study. The increase in RBC and hemoglobin levels in male mice observed during current acute, sub-acute and chronic toxicity studies may be due to the known properties of ginseng such as it increases body metabolism and energy and supports the immune function. However, the altered red cell production may be attributed to the constituents of ginseng that may influence the androgen levels in the body. Testosterone and related androgenic derivatives are known to be potent hormones capable of stimulating erythropoiesis (Adamson, 1980; Qureshi *et al.*, 1992).

CONCLUSION

The toxicity data obtained during current study demonstrated ginseng treatment to possess low toxicity.

Furthermore, it is interesting to observe that the data obtained during current acute, sub-acute and chronic toxicity studies support the folklore claims about ginseng such as antidiabetic, antioxidant and its possible use in male fertility problems. However, further detailed investigations are needed to confirm these claims.

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