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Role of Ginseng as Hepatoprotective, Antioxidant and Anti-Inflammatory against Methotrexate Induced Liver Injury in Rats.

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ABSTRACT

Background Ginseng, an ancient and famous medicinal herb in the Orient, has been used as a valuable tonic and for the treatment of various diseases including hepatic disorders. Ginseng extracts and individual ginsenosides have shown a wide array of beneficial role in the regulation of regular liver functions and the treatment of liver disorders.

Objective: This study tries to determine the hepatoprotective , antioxidant and anti-inflammatory effects of ginseng on Methotrexate (MTX)-induced hepatotoxicity

Materials and Methods: forty rats (weigh 150-180 g) were used. The rats were kept in animal house for one week and had access to water and food . Temperature was kept at 37 °C. After one week, the rats were randomly divided into four equal groups: Group (A) (control) received normal saline ; group (B)received Ginseng(1.8 ml/kg/day) orally ; group(C) received MTX (100 μg/kg) intraperitoneally and group (D) received MTX (100 μg/kg) intraperitoneally with ginseng (1.8 ml/kg/day) orally. After six weeks, the rats were decapitated and evaluation of liver function was done.

Results: Ginseng treatment markedly suppressed the serum alanine aminotransferase (ALT) , aspartate aminotransferase (AST) and serum gama glutamil transpeptidase (λGTP) activiteis . Ginseng was attributed to stimulate anti-oxidant protein contents, such as glutathione peroxidase (GPX). The marked increase of proinflammatory cytokines (TNF α) in MTX treated rats group was additionally attenuated by ginseng,

Conclusion : Ginseng effectively prevent liver injury, mainly through down regulation of oxidative stress and inflammatory response.

Key words: Ginseng ,methotrexate, liver, anti-inflammatory activity, antioxidant.

INTRODUCTION

The pharmacological effects of ginseng can be understood in the light of their polyvalent actions as demonstrated by ginseng saponins with their positive anti- mutagenic, anticancer, protective action against mammalian tumors cell lines , anti-diabetes and.[1,2] Ginseng has an immune-stimulatory, anti-inflammatory effects and antihepatoxicity effects.[3] It is well known strong antioxidant effect, for its antioxidant property due to its ability to scavenge free radicals and to neutralize, ions-induced peroxidation.[4]

Methotrexate (MTX) is a potent hepatotoxic agent. The hepatic malfunction is probably due to a direct toxic action of the methotrexate, since most reaction is dose dependent.[5]

Liver diseases represent a major health burden worldwide, with liver cirrhosis being the ninth leading cause of death in Western countries.[6] Therapies developed along the principles of western medicine are often limited in efficacy, carry the risk of adverse effects.[7] Therefore, treating liver diseases with plant-derived products which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive.[8]

The present work was to introduce the multifaceted pharmacological effects and related mechanisms of ginseng on hepatic functions.

MATERIALS AND METHODS

The present study was carried out on forty adult male albino rats, weighing 150-180 gm. They were housed in clean properly ventilated cages under the same environmental condition, with free access to food and water throughout the period of the experiment which was ten days. The animals were divided equally into four groups:

Group I: Control group.(received normal saline)

Group II: Ginseng group. Animals received a daily oral dose of ginseng extracts for 6 weeks, used in a soft gelatin capsules each capsule contain ginseng extracts 100mg provided by Arab Co. for Pharmaceutical & Medicinal plant. The experimental dose daily prepared by 100mg of ginseng extract dissolved in 0.5 ml olive oil , according to Paget and Barnes given each rat about (1.8 ml/kg/day) by oesophygeal tube.[9]

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Group III: methotraxate group. We used 1 mL of MTX (1000 mg/10 mL) produced by Miracalus Pharma PvtLtd and diluted it in 99 mL of normal saline and then 1 mL of product was diluted in 9 mL of normal saline (100 µg/mL MTX). It was injected by insulin syringes.[10]

GroupIV: ginseng methotraxate group. Animals received oral doses of ginseng extracts and intraperitonial methotraxate as in the previously mentioned regimen.

At the end of the experiment all animals were fasted for 12 hours, anesthetized by ether, and blood samples were collected from retro-orbital sinus for estimation of:

- Marker enzymes for liver function like Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) , Alkaline phosphatase (ALP) and λ glutamic transpeptidase (λGTP).[11]
- Serum glutathione peroxidase (GPX) .[12]
- Serum TNF-α level by ELISA.[13]

Statistical Analysis: All statistical analysis was computed by SPSS version 14.

The values obtained were revealed as mean ± S.E. Data were analyzed using student's'‘t’-test and results were considered significant at $P < 0.05$.

RESULTS

Serum AST, ALT , λ glutamic trans-peptidase (λGTP) and alkaline phosphatase (ALP) were insignificantly decreased , in rat group supplemented by ginseng and significantly increased in both rat groups injected by methotrexate or supplemented by ginseng with methotrexate compared to control group (Table 1).

The antioxidant enzyme glutathione peroxidase (GPx) activity in serum was significantly increased in rats groups which either supplemented by ginseng or ginseng with methotrexate . On the other hand , rats received methotrexate had significantly decreased in GPx activity in serum compared to control group (Table 1).

The serum TNF-α were significantly increased in methotrexate group , while their levels were insignificantly changed in ginseng group and in ginseng methotrexate group $p>0.05$ compared to control group (Table 1).

Comparing Ginseng MTX group with MTX group, the levels of serum AST, ALT , λ glutamic trans-peptidase (λGTP) , alkaline phosphatase (ALP), and TNF-α were significantly decreased while serum glutathione peroxidase enzyme was significantly increased . On the other hand, the levels of serum AST, ALT , λ glutamic trans- peptidase (λGTP) , alkaline phosphatase, and TNF-α were significantly increased while serum glutathione peroxidase enzyme was significantly decreased in Ginseng MTX group compared to Ginseng group (Table 1).

DISCUSSION

In this study protective effects of ginseng on MTX-induced liver damage in rats was investigated. We found that the mean levels of ALT, AST, ALP and λGTP in rats that received MTX plus ginseng were significantly lower than those animals received only MTX. Difference of studied parameters between MTX plus ginseng and control group was not significant. Up to now, various studies revealed protective effects of ginseng in hepatic damages .[14] In these studies, it was shown that extract of ginseng reduced treatment period of acute and chronic hepatitis.[15]

The results of the present study showed that **(**GPx **)** was significantly increased in rats that received MTX plus ginseng than those animals received only MTX. Difference of studied parameters between MTX plus ginseng and control group was significant.

The mechanisms which provide ginseng’s hepatoprotective effects are closely attributed to antioxidation properties. Ginseng enhanced the antioxidant defense mechanism and increased self-antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GSH), and hemeoxygenase-1 in the aged-rat liver and hepatotoxins-induced liver damages in rats.[18] Ginseng treatments inhibited oxidative stress damage such as lipid peroxidation , malondialdehyde , thiobarbituric acid reactive substance , alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH).[16,17,19,20]

The profective effects have been histologically and histochemically monitored, recently, further molecular mechanism studies found that ginseng suppresses mitogen-Activated protein kinase (MAPK)signals **,** nuclear factor-kappa B (NF-KB), and inducible nitric oxide synthase (INOS) protein expression.[21,19]

The results of the present study showed that **(**TNF-α **)** was significantly decreased in rats that received MTX plus ginseng than those animals received only MTX. Difference of studied parameters between MTX plus ginseng and control group was significant.

Inflammatory effects of ginseng have been responsible for the liver protection. Ginseng suppressed the production of inflammatory cytokines (IL-1β, IFN-γ) and chemokines (MCP-1, MIP-2β, KC) in CCl_4-treated mice.[18] Recently, ginseng was found to inhibit tumor necrosis factor alpha (TNF-α)-stimulated NF-kB activation and further suppressed the gene expression of iNOS and cyclooxygenase-2 (COX-2) in HepG-2 cells.[22]

Ginseng has a protective effect against many toxicants in human and experimental animals and can increase body resistance to many harmful factors and can protect tissues from damage when an organism is in stress. It has antioxidant activity as it contains ginsenosides, phenolic acids, flavonoids, and saponins. These properties of the ginseng are thought to provide many beneficial preventative effects against org an damage.[23]

CONCLUSION

Ginseng and its principal components, ginsenoisdes, have shown a wide array of pharmacological activities including beneficial role in the regulation of liver functions and the treatment of liver disorders of acute/chronic hepatotoxicity.

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Table (1) Liver function, antioxidant and anti-inflammatory markers in all groups.

Groups / *Parameters*	*Control*	*Ginseng*		*Methotrexate*		*Ginseng Methotrexate*		
	Mean ±S.E.M	***Mean ±S.E.M***	**P value Vs control**	***Mean ±S.E.M***	**P value Vs control**	***Mean ±S.E.M***	**P value Vs Methot.**	**P value Vs Ginseng**
AST (U/L)	106.6 ±5.33	105.8 ±4.4	0.5	327.4 ±23.6	0.001	104.6 ±3.7	0.001	0.8
ALT (U/L)	32.42 ±0.43	33.41 ±7.36	0.3	83.63 ±6.13	0.001	26.49 ±1.9	0.001	0.9
ALP (U/L)	206.9 ±2.3	209.23 ±3.5	0.6	315.1 ±18.6	0.001	227.25 ±9.9	0.001	0.3
(λGTP) (IU/L)	40.83 ±1.1	37.9 ±2.9	0.09	83.3 ±1.2	0.001	42.4 ±2.9	0.001	0.01
GPx (U/g)	18.3 ±0.3	20.6 ±0.4	0.2	11.85 ±5.2	0.02	19.6 ±0.03	0.01	0.5
TNF-α (pg/ml)	1.07±0.26	0.52±0.1	P>0.05†	1.93±0.08	P<0.05*	0.84±0.1	P>0.05†	P>0.05†