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A CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RABBITS: EVALUATION OF CURATIVE ROLE OF TURKISH PANAX GINSENG

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ABSTRACT

Aims: The present study aimed to evaluate the effect of Turkish Panax Ginseng (TPG) on hepato-marker enzymes and biochemical parameters in animal models (rabbits) with carbon tetrachloride (CCl_4) - induced hepatotoxicity at a single dose of 1.25 ml/kg body weight as a mixture with olive oil. **Main methods:** Intoxication of rabbits by CCl_4 significantly increased ($p < 0.01$) serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), serum alkaline phosphatase (SALP) and bilirubin level, which indicate acute hepatocellular damage and biliary obstruction. Intoxicated animals were treated orally with TPG at (100, 200 and 300 mg/kg bodyweight) and Liv-52 (25 mg/kg) for 28 consecutive days. The hepatocellular damage was assessed by measuring the changes of SGOT, SGPT, SALP, total bilirubin, total protein, creatinine, blood urea nitrogen (BUN) and blood glucose level using rabbits weighing 1.2-1.5 kg. **Key findings:** In vivo results indicated oral administration with TPG could overcome CCL_4-induced immunosuppression and significantly ($p < 0.05$) exhibited protective effect. The results of TPG treated rabbits showed a significant dose-dependent reduction ($P< 0.05$) in the hepatic enzymes levels, bilirubin, BUN, blood glucose level and improvement of serum total protein when compared with that of Liv-52. Highest activity was observed for TPG at 300 mg/kg body weight dose level and the reductions of SGOT, SGPT, SALP, total bilirubin, BUN and creatinine in serum were 55.50, 52.43, 59.02, 57.32, 31.21 and 20.8%, respectively. In addition, TPG increased the serum total protein level by 3 folds, when compared with CCl_4 treated rabbits. **Significance:** TPG possess antihepatotoxic action and may be acting as a natural hepatoprotective agent against CCl_4 induced hepatocellular injury. This might be due to its active antioxidant contents and higher amount of active ginsenosides. TPG could effectively attenuate the alteration within the studied parameters in dose-dependent manner and prevent oxidative damage in immunological system.

KEYWORDS: Antihepatotoxic, Carbon tetrachloride, Turkish Panax Ginseng, Hepatoprotective, Rabbits.

INTRODUCTION

The liver is one of the important organs in the body and plays pivotal role in regulating various physiological processes. It helps to maintain the homeostasis of the body and is involved in almost all the biochemical pathways associated to growth, immunity, nutrient supply, energy provision, xenobiotic metabolism and reproduction (Ahsan MR et al. 2009). As a consequence of its role in xenobiotics metabolism, liver is not only exposed to environmental toxins in a persistent and varied manner but also is abused by poor drug habits, alcohol, prescribed and over-the-counter drugs which can eventually lead to various liver ailment like hepatitis, cirrhosis and alcoholic liver disease (Sharma et al. 1991; Subramonium and Pushpangadan 1999). Liver disease claims approximately 25000 deaths every year and, therefore, is a global public health concern (Sharma B et al. 2010). For that reason, treatment of liver diseases is extremely important. Discovery of adequate synthetic drugs for the treatment of liver diseases with minimal side effects has ever remained a real challenge. Traditional medicines, however, have sufficiently used medicinal plants to treat several human diseases over the centuries and have been very important in the health care delivery of every nation (Oluma HO et al. 2004).

Panax ginseng (C.A. Meyer) was traditionally used as a medicinal plant in Asian countries, and it has now gained worldwide popularity (Yun TK et al. 2003). Ginseng is identified to contain ginsenosides, phenolic compounds, polysaccharides, and polyacetylenes, which are known to have a chemopreventive effect through antioxidant, apoptotic, and anti- proliferative properties in various

cancers (Kim HS et al. 2004, Lee LS et al. 2013, Cheng H. et al. 2011). Red ginseng is heated *Panax ginseng* produced by steaming followed by drying, and contains higher amount of ginsenosides and polyphenolics than white ginseng (Chung et al. 2012, Yun TK et al. 2001).

The heat processing converts ginsenosides into other types of ginsenosides, including ginsenoside Rh2 and Rg3, and produces the antioxidant agents and phenolic compounds such as maltol (Nam K. et al. 2005, Kim GN et al. 2010). Since Korea red ginseng (KRG) has unique anti-carcinogenic compounds, it has been suggested that KRG has more potent chemopreventive activity than fresh and white ginseng (Wu XG et al. 2001). Also, KRG, specifically ginsenosides Rg3, Rg5 and Rh2, has been shown to increase apoptosis in human hepatocellular carcinoma cells. Ginsenoside Rh2 exhibited the apoptotic properties through caspase-3 activation in SK-HEP-1 cell lines (Kim JY et al. 2012).

Although the beneficial effects of KRG are well documented, the administration of high dose of KRG might be detrimental through their toxicity. Several studies have reported that overdose and long-term usage of ginseng are associated with side effects such as hypertension, nausea, diarrhea, insomnia, and headache, which is known as ginseng abuse syndrome (Seely D et al. 2008). Ginsenoside Rh2, which is one of the active ginsenosides of KRG, is known to have anticancer activities, while it showed cytotoxic effects to human hepatocyte cells (Wei G et al, 2012). KRG extract may prevent hepatocarcinogenesis through modulation of the liver oxidative environment, but the chemopreventive effects may differ based on the concentrations.

According to in vitro and in vivo studies, several classical plant extracted antioxidants have been shown to protect various cells like hepatocytes and nephrocytes against lipid peroxidation or inflammation, thereby preventing the occurrence of hepatic necrosis, kidney damage and other radical associated activities (Sadiq et al. 2011, 2013). These evidences suggest that comparative studies between various kinds and amounts of ginseng for hepatoprotective and hepatocarcinogenesis need to be performed, and that the proper usage of these ginsengs on liver ailments has to be established, regardless to their sources. Therefore, the present study was undertaken to explore the hepatoprotective effects of TPG and its optimal concentration for liver treatment by measuring the serum levels of hepatic diagnostic enzyme markers, bilirubin, BUN, creatinine, total protein and glucose in rabbit models.

MATERIALS AND METHODS

The present study was conducted for 58 days in the Research station, Department of Veterinary, Faculty of Agriculture and Veterinary Medicine, Thamar University, Thamar city, Yemen.

Drugs and Chemicals

All the solvents used in the experiments were of analytical grade from Ranbaxy Chemicals Ltd. (Mumbai, India). The diagnostic hepatic enzymes (SGOT, SGPT, SALP), bilirubin, BUN, creatinine, total protein and glucose kits were procured from Span Diagnostics Ltd. (Surat, India). Liv-52 (Himalaya Drugs, India) was obtained from local supplier.

Experimental animals

Adult healthy male rabbits weighing 1200-1500 g (36 in total) and 1-1.5 years old, were used in the study. Animals were obtained from a local supplier (Thamar, Yemen), and housed in metal cages (80×80×50 cm) with soft wood shavings as bedding and all the animals were allowed to have free access to water ad libitum and fed with standard commercial food throughout the period of the experiment. The animals were acclimatized for one week before starting the experiment. The study protocol was approved by the University Ethics committee before any experiment can be conducted. The rabbits were randomly divided into five groups (n=6), every group were housed in part.

Experimental design

The experiment was conducted in two periods. The preparatory period was carried out for 30 days to acclimatize the rabbits to the experimental conditions, in the experimental period (28 days), the rabbits were divided into six groups (n=6) of equal weight means. **Group (I)** served as the normal control (Negative Control) and received only 0.5% CMC (Carboxy Methyl Cellulose) suspension by a gastric gavage throughout the experimental period. **Group (II)** served as the toxin control (Positive Control) and received a 0.5% CMC suspension by a gastric gavage. **Group (III), (IV)** and **(V)** received TPG at100, 200, and 300 mg/kg body weight /day respectively while animals in **Group (VI)** received liv-52 (5ml/kg body weight) for 28 consecutive days. On the last day, groups II, III, IV, V and VI were given single oral dose of CCl_4 (1.25 ml/kg) diluted in olive oil at rate of 1:1(v:v), sixty minutes after the last administration with their respective treatments.

Blood collection

Blood samples was collected after 24 hours of CCL_4 administration by keeping the rabbits for overnight fasting with free access only to drinking water. Blood is withdrawn from each rabbits by direct cardiac puncture under light ether anesthesia (Dongare PP et al. 2013). Blood was withdrawn from each rabbits by direct cardiac puncture under light ether anaesthesia. Blood samples were collected in previously labelled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 3000 rpm for 15 min at 25°C and the supernatant was stored in the freezer at -21^{o}C until analysis (Prochezian E et al. 2005).

Biochemical analysis

The serum was used for analysing SGPT, SGOT, SALP, total bilirubin, total protein, creatinine, BUN, and glucose using commercially available kits.

Statistical analysis

Statistical analysis of the results was carried out using the Statistical software (SPSS Ver.21). All results were shown as the means ± SD for each group. The statistical analysis was carried out by One Way Analysis of Variance (ANOVA) followed by the Student's t-test to determine the significant differences between treatments. Difference was considered significant when $P < 0.05$.

RESULTS

Biochemical tests

Effect of TPG on biochemical parameters in CCl_4 - induced rabbits

The hepatocurative effect of TPG in CCl_4-induced hepatotoxicity in rabbits at different doses was carried out. The results showed that treatment of rabbits with CCl_4 (**Group-II**) caused a significant increase ($p < 0.01$) in levels of SGOT, SGPT, SALP, and a significant reduction in serum total protein level when compared to normal control (**Group-I**), indicating hepatocellular damage. In contrast, pre-treatment of rabbits with liv-52 (**Group-VI**) for 28 consecutive days lowered significantly ($p < 0.05$) the levels of SGOT, SGPT, SALP and restored the serum total protein.

On the other hand, the rabbits pre-treated with TPG (**Group-III, IV** and **V**) at the doses of 100, 200 and 300 mg/kg body weight produced a highly significant fall in the SGOT, SGPT, SALP levels and a significant elevation in the total protein levels in a dose dependent manner as indicated in Table 1.

Pretreatment of rabbits with TPG at 100, 200 and 300 mg/kg body weight (**Group-III, IV** and **V**) for 28 consecutive days significantly ($p < 0.05$) controlled the tested biochemical parameters which are comparable with liv-52 (**Group-VI**). Table-1 also shows the comparison of effects among the untreated (Normal Control), CCl_4 treated (Induction Control) and liv-52 treated (Standard) group with different doses of TPG treated group of rabbits. The TPG exhibited significant protection against CCl_4-induced liver injury as manifested by the reduction in toxin mediated rise in SGPT, SGOT and SALP level of rabbits.

The treatment of CCl_4 alone induced a significant rise in total bilirubin, BUN and creatinine levels compared to normal control (**Group-I**). On the other side, oral pretreatment of animals with different concentrations of TPG (**Group-III, IV** and **V**) and Liv-52 (**Group-VI**) reduced the level of total bilirubin, BUN significantly in these groups with respect to rabbits intoxicated with CCl_4. However, there is less significant decrease in creatinine level in TPG treated animals (**Group-III, IV** and **V**) as shown in Table 2.

In the **Table-2**, the level of total protein in intoxicated rabbits with CCl_4 **(Group-II)** was reduced which indicate the damage of the liver therefore, disturbing the synthesis of proteins. The groups treated with TPG **(Group-III, IV** and **V)** showed significant dose-dependent increase in total proteins and this increase is comparable to animals treated with drug standard Liv-52 **(Group-VI)**.

The **Table-2** results showed significant increase in blood sugar level in animal models treated with CCl_4 (**Group-II**) compared with normal control (**Group-I**). Similarly, there were significant dose dependent reductions in blood sugar values of animals pre-treated with TPG **(Group-III, IV** and **V)** and this reduction is comparable to Liv-52 (**Group-VI**). The increase of blood glucose level in treated group with CCl_4 (**Group-II)** indicates the hyperglycaemic effect of CCl_4 and this increase was restored dramatically in all the groups pre-treated with TPG and Liv-52 and these results is in agreement with previous studies which confirm the hypoglycaemic effect of TPG .

Table 1: Effects of TPG administration on the levels of SGOT, SGPT and SALP in serum of CCl_4 induced rabbits.

Group	Treatment	SALP U/L	SGPT U/L	SGOT U/L
I	CMC (mg/kg p.o.)	138.83±6.23 [a]	61.95±6.22 [b]	75.83±4.76 [a]
II	CCl_4 (1.25 mg/kg p.o.)	429.31±12.11 [a]	407.35±5.65 [b]	392.03±7.56 [b]
III	CCl_4+ TPG (100mg/kg p.o.)	274.55±7.02 [a]	299.02±7.21 [b]	262.85±5.56 [a]
IV	CCl_4+TPG (200mg/kg p.o.)	249.73±6.60 [a]	274.16±7.02 [a]	227.83±6.47 [a]
V	CCl_4+TPG (300mg/kg p.o.)	175.95±5.94 [b]	193.77±7.14 [b]	174.47±7.77 [b]
VI	CCl_4+LIV-52 (5mg/kg p.o.)	195.80±6.20 [a]	172.70±6.93 [b]	148.75±6.50 [a]

- Values are mean ± SD, (n=6);
- [a]$P < 0.001$ compared to respective CCl_4 treated group (II).
- [b]$P < 0.05$ compared to respective CCl_4 treated group (II).

Table 2: Effects of TPG administration on the levels of Bilirubin, BUN, creatinine, Protein and blood glucose in serum of CCl_4 induced rabbits.

Group	Treatment	T. Bilirubin mg/DL	BUN mg/DL	Creatinine mg/DL	Total Protein g/dl	Sugar mg/dl
I	CMC (mg/kg p.o.)	0.79±2.87 [b]	51.60±2.38 [a]	0.93±3.19 [a]	6.08±0.22 [a]	136.50±2.43 [a]
II	CCl_4 (1.25 mg/kg p.o.)	1.21±7.14 [a]	78.88±1.76 [a]	1.49±6.17 [b]	3.94±0.22 [a]	158.67±2.60 [a]
III	CCl_4+ TPG (100mg/kg p.o.)	0.38±4.05 [a]	64.42±2.45 [b]	1.36±7.12 [b]	4.73±0.34 [a]	94.33±2.81 [a]
IV	CCl_4+TPG (200mg/kg p.o.)	0.50±4.46 [a]	60.63±2.73 [a]	1.24±7.34 [a]	5.22±0.21 [a]	93.50±4.71 [b]
V	CCl_4+TPG (300mg/kg p.o.)	0.52±8.32 [b]	54.27±3.17 [b]	1.18±5.17 [b]	5.90±0.24 [b]	87.50±4.20 [b]
VI	CCl_4+LIV-52 (5mg/kg p.o.)	0.97±5.28 [b]	47.62±1.67 [a]	1.01±5.22 [a]	6.08±0.41 [a]	100.50±3.73 [a]

- Values are mean ± SD, (n=6);
- [a]$P < 0.001$ compared to respective CCl_4 treated group (II).
- [b]$P < 0.05$ compared to respective CCl_4 treated group (II).

DISCUSSION

The role of possible hepatoprotective activity of TPG in carbon tetrachloride induced hepatotoxicity has been demonstrated. CCl_4 is well-known and commonly used hepatotoxin in the experimental study of liver disease. The hepatotoxic effects of CCl_4 are primarily due to generation of free radicals (Shenoy et al. 2001, Sadiq et al. 2012). Therefore, it was extensively studied as a liver toxicant, and its metabolites such as trichloromethyl radical (CCl_3 S) and trichloromethyl peroxyl radical (CCl_3O_2 S) are reported to be involved in the pathogenesis of liver disease (Recknagel 1967). The biotransformation of CCl_4 by the cytochrome P450 system produces free radicals (CCl_3 S, CCl_3O_2 S), which in turn covalently bind to cell membranes and organelles to elicit lipid peroxidation (Recknagel et al. 1989, Sadiq et al. 2018). The massive generation of free radicals in CCl_4 induced liver damage provokes a sharp increase in lipid peroxidation in liver due to the increase in interaction of these free radicals with phospholipids structure and ultimately destroying the organ structure (Gil et al. 2000).

Assessment of liver function can be made by estimating the activities of the liver marker enzymes SGPT, SGOT, and SALP which are largely used as most common biochemical markers and originally present in cytoplasm with high concentration (Sadiq et al, 2011, 2018). The enzyme markers (SGOT, SGPT, SALP) and total bilirubin are the most sensitive tests employed in the diagnosis of hepatic diseases (Sathiyanarayanan et al. 2006). Because these enzymes are placed in cytoplasmic area of the cell, they get leaked into the blood stream in conformity with the extent of liver damage when there is hepatopathy (Nkosi et al. 2005). Thus, these enzymes are used as diagnostic indicators of hepatic injury. Higher release of these enzymes from the cells is indicative of cellular leakage and loss of functional integrity of the cell membrane in liver (Drotman and Lawhorn 1978).

In the current study, various concentrations of TPG extract were utilized to investigate whether TPG extract may play an important role in modulating redox status, and the optimum intake of TPG may suppress hepatocarcinogenesis CCl_4-treated rabbits. Ginseng is a traditional medicine to treat a variety of disorders, including cancers (Yun TK et al. 2003). Hyemee Kim et al, 2015 study were a pre-clinical model of hepatocellular cancer that exhibits many phenotypic characteristics relevant to the liver cancer (Ito N et al. 1988). It was hypothesized that TPG may prevent hepatocarcinogenesis through modulation of the liver redox environment and oxidative stress, but that hepatoprotective effect may differ based on the concentration. The significant increase in SGPT, SGOT, and SALP levels in the serum after oral administration of CCl_4 indicated that CCl_4 intoxication compromised the integrity of the hepatic cell membranes (Sadiq et al. 2013, 2018).

Previous studies showed that red ginseng at dose of 3-6 g/day for eight weeks improved the antioxidant enzymes and oxidative stress markers in healthy human [Kim JY, et al, 2012]. And, the 8 g/day intake of red ginsengs may not improve the redox status of glutathione in human (Hyemee Kim et al, 2015). Oxidative stress represents an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system (Valko et al, 2006, Sadiq et al. 2011).

The influence of several doses of TPG has been investigated for their efficacy in controlling the CCl_4 induced liver damage. The findings of this study proved that the treatment of CCl_4 intoxicated rabbits with TPG restored the depleted studied biochemical parameters. CCl_4-treated rabbits experience extensive liver damage induced by toxin. The elevated levels of these marker enzymes in the current study were observed in Group II rabbits. However, during the investigation, reduction of SGOT, SGPT and SALP concentrations were observed due to the influence of TPG as shown in Table-1. Pre-treatment of rabbits with TPG and Liv-52 significantly decreased levels of SGPT, SGOT, and SALP towards the normal value. These findings are in agreement with the commonly accepted view that serum levels of transaminases enzymes go back to normal level due to stabilization of plasma membrane in addition to repair of hepatic tissue damages caused by CCl_4 (Aniya Y et al. 2005).

Bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte. TPG treatment decreased serum bilirubin level in liver damage induced by CCl_4, indicating the effectiveness of TPG in normalizing the functions of the liver. In the present study, significant increases in total bilirubin content in the CCl_4 treated group are the indicatives of liver damage. TPG treatment inhibited the CCl_4 induced increase in the all the three groups as shown in Table-2.

The kidney supports in keeping homeostasis of the body by reabsorbing important material and evacuating waste yields. Creatinine is a commonly used as measure of kidney function (Rincón AR et al. 1999). Rahmat A et al. 2014 suggested that, the increase in the level of creatinine in the blood considered an indicator to the kidney damage. Therefore, oral administration of CCl_4 may result in a significant increase in creatinine and blood urea nitrogen concentrations and the increase is indicative of cellular leakage and loss of functional integrity of cell membrane in renal tissue (Preethi KC et al. 2009). In the current study, the pretreatment of rabbits with TPG significantly reduced serum BUN levels in a dose dependently manner, compared to CCl_4 intoxicated group (Group-II) by enhancing the renal function that is generally impaired in CCl_4–induced rabbits. On other hand, elevated serum level of creatinine in the CCl_4 intoxicated group (Group-II) was found to reduce insignificantly consequence to administration of TPG in a dose-dependent manner. But the dosage of 200 mg/kg body weight of TPG (Group-IV) reduced serum creatinine level significantly compared to Liv-52 (Group-VI).

Previous studies have showed that administration of CCl_4 to a diversity of animal types result in a rapid reduction in protein synthesis in the liver (Soni B et al, 2008, Gowda S t al, 2010). In the current study, CCl_4 intoxication reduced the serum total protein level by three folds which is attributed to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of cytochrome P-450 enzymes leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver (Suresh K et al, 2007). The study findings suggested that pre-treatment of TPG restored the total protein level that proposes the stabilization of endoplasmic reticulum leading to protein synthesis.

The serum glucose level in this study was higher in CCl_4 treated rabbits (Group-II) compared to Group I (normal control). The elevation of glucose level could be recognized to destruction of hepatocytes brought by CCl_4 intoxication (Muriel Pet al. 2001, Sadiq et al. 2013) or due to the reduction of glycogen contents as a result of degradation of glycogen to glucose in hepatocytes after treatment with CCl_4 which leads to increase in glucose level in the blood (Gaw A et al. 1998). The pretreatment of TPG in groups (III, IV, V) reduced the serum glucose level in a dose-dependent manner compared to Group II and this reduction by TPG could be attributable to the antioxidant activity of TPG, which attenuated the oxidative threat caused by CCl_4 and restored normal physiological functions.

In the present study, the hepatoprotective activity of TPG was compared with Liv-52 which is widely used in treatment of liver diseases of varying origins. It enhances tocopherol levels, which inhibits lipid peroxidation and scavenges free radicals (Saini MR et al. 1984). Liv-52 (Group-VI) caused a significant decrease in serum enzymes activity and restored the tested parameters in a dose-dependent manner induced by CCl_4 in rabbits.

It is believed that high ROS production has been shown to lead to DNA damage, mutations of tumour suppressors, gene instability, and carcinogenesis, and damage other molecules including the fatty acid side chains of lipids in the membranes of the cell (Bartsch H et al, 2006, Sadiq et al. 2016). However, direct measurement of ROS has not established well due to their instability, ROS generation is usually indirectly assayed by detecting specific biomarkers, such as lipid peroxidation (TBARS analysis) (Samoylenko A, et al. 2013). Previous study of Bak MJ, et al. 2012 showed that red ginseng oil has a protective effect on liver damages by inducing the antioxidant enzymes activity and by inhibiting lipid peroxidation in vitro and in vivo. Similarly, Hyemee Kim et al. 2015 demonstrated that red ginsengs suppress the level of TBARS, a lipid peroxidation biomarker compared to control using rat models. It suggests that red ginsengs have an antioxidant property, which is in an agreement to the results of the present study irrespective to the source of the ginseng.

Based on the current study and the previous studies, it is suggested that, the mechanism by which TPG offers protection against CCl_4-induced hepatocellular metabolic alterations could be due to inducing microsomal enzymes either by accelerating the detoxification and excretion of CCl_4 or by inhibition of lipid peroxidation through inhibition of cytochrome P-450 aromatase favoring liver regeneration. In addition, the therapeutic effect of TPG can be explained by the higher content of active ginsenosides.

CONCLUSION

The present study was aimed to evaluate the efficacy of TPG on the liver functions in CCl_4 induced injuries. Activities of SGPT, SGOT and SALP in serum were increased in CCl_4-intoxicated rabbits. A marked elevation in the concentration of total bilirubin, BUN, creatinine, and glucose level was also observed in the hepatotoxin-treated rabbits and high reduction of total protein. The findings of the study have shown the ability of TPG to recover these parameters from the CCl_4 damage to almost normal levels. It is believed that, the restoration of all the biochemical parameters was in a

dose-dependent manner and we believe that the optimal concentrations showed high activity is 300 mg and this activity is regardless to the country source of cultivation of ginseng. Results of the study provide scientific bases to the use of TPG in liver ailments and can be used to compensate the declining activities of antioxidant enzymes and thereby reduce the risks of lipid peroxide. The present study recommends that TPG could be given as a new formulating drug to reduce the chance of patient with the most chronic dangerous disease.

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REFERENCES

1. Ahsan MR, Islam KM, Bulbul IJ. Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. Eur J Sci Res. 2009; 37(2): 302-310.
2. Aniya Y, KoyamaT, Miyagi C. Free radical scavenging and hepatoprotective actions of the medicinal herb, *Crossocephalum crepidioides* from the Okinawa Islands. Biol Pharm Bull, 2005; 28(1): 19–23.
3. Bak MJ, Jun M, Jeong WS. Antioxidant and Hepatoprotective Effects of the Red Ginseng Essential Oil in H(2)O(2)-Treated HepG2 Cells and CCl(4)-Treated Mice International journal of molecular sciences. 2012; 13: 2314-30. doi: 10.3390/ijms13022314.
4. Bartsch H, Nair J. Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer: role of lipid peroxidation, DNA damage, and repair. Langenbeck's archives of surgery / Deutsche Gesellschaft fur Chirurgie, 2006; 391: 499-510. doi:10.1007/s00423-006-0073-1.
5. Cheng H, Li S, Fan Y, et al. Comparative studies of the antiproliferative effects of ginseng polysaccharides on HT-29 human colon cancer cells. Medical oncology, 2011; 28: 175-81.
6. Chung IM, Kim J-W, Seguin P, et al. Ginsenosides and phenolics in fresh and processed Korean ginseng (*Panax ginseng* CA Meyer): Effects of cultivation location, year, and storage period. Food chemistry, 2012; 130: 73-83.
7. Dongare PP, Dhande SR, Kadam VJ. Standardization of Carbon Tetrachloride-Induced Hepatotoxicity In the Rat. AJPTR, 2013; 3(5): 2249-3387.
8. Drotman RB, Lawhorn GT. Serum enzymes as indicators of chemical induced liver damage. Drug Chem Toxicol, 1978; 1: 163–71.
9. Gaw A, Cowan RA, O'Reill DS, Stewart MJ, Shepherd J. Clinical Biochemistry-An Illustrated Color Text, Churchill Livingstone, Oxford, 1998; 56.
10. Gil MI, Tomas-Barberan FA, Hesspierce B, Holecroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J Agric Food Chem, 2000; 48: 4581–4589.
11. Gowda S, Desai PB, Kulkarni SS, Hull VV, Math AA, Vernekar SN. Markers of renal function tests.J Med Sci., 2010; 2(4): 170-173.
12. Hyemee K, Mi-Kyung H, Haymie C, Hyun-Seuk M, Hae-Jeung L. Chemopreventive Effects of Korean Red Ginseng Extract on Rat Hepatocarcinogenesis. Journal of Cancer, 2015; 6(1): 1-8. doi: 10.7150/jca.10353.
13. Ito N, Tsuda H, Tatematsu M, et al. Enhancing effect of various hepatocarcinogens on induction of preneoplastic glutathione S-transferase placental form positive foci in rats--an approach for a new medium-term bioassay system. Carcinogenesis, 1988; 9: 387-94.
14. Kim GN, Lee JS, Song JH, et al. Heat processing decreases Amadori products and increases total phenolic content and antioxidant activity of Korean red ginseng Journal of medicinal food, 2010; 13: 1478-84. doi:10.1089/jmf.2010.1076.
15. Kim HS, Lee EH, Ko SR, et al. Effects of ginsenosides Rg3 and Rh2 on the proliferation of prostate cancer cells. Archives of pharmacal research, 2004; 27: 429-35.
16. Kim JY, Park JY, Kang HJ, et al. Beneficial effects of Korean red ginseng on lymphocyte DNA damage, antioxidant enzyme activity, and LDL oxidation in healthy participants: a randomized, double-blind, placebo-controlled trial. Nutrition journal, 2012; 11: 47. doi:10.1186/1475-2891-11-47.
17. Lee L-S, Cho C-W, Hong H-D, et al. Hypolipidemic and antioxidant properties of phenolic compound-rich extracts from white ginseng (Panax ginseng) in cholesterol-fed rabbits. Molecules, 2013; 18: 12548-60.
18. Muriel P, Alba N, Perez-Alvarez VM, Shibayama M, Tsutsumi VM. Kupffer cells inhibition prevents hepatic lipid peroxidation and damage induced by carbon tetrachloride. Comp Biochem Physiol C Toxicol Pharmacol, 2001; 130(2): 219-226.
19. Nam K. The comparative understanding between red ginseng and white ginsengs, processed ginsengs (Panax ginseng C.A. Meyer). J Ginseng Res., 2005; 29: 1-18.
20. Nkosi CZ, Opoku AR, Terblanche SE. Effect of pumpkin seed (Cucurbita pepo) protein isolate on the activity levels of certain plasma enzymes in CCl_4-induced liver injury in lowprotein fed rats. Phy. The. Res., 2005; 19: 341–345.
21. Oluma HO, Umoh EU, Onekutu A, Okolo J. Antibacterial potentials of eight medicinal plants

from the lower Benue valley of Nigeria against *Salmonella typhi*., Niger. J. Bot., 2004; 17: 1-11.
22. Preethi KC, Kuttan R. Hepato and reno protective action of *Calendula Officinalis L*.flower extract.Indian J Exp Biol., 2009; 47: 163-168.
23. Prochezian E, Ansari SH. Hepatoprotective activity of Abutilon indicum on experimental liver damage in rats. Phytomedicine, 2005; 12: 62-64.
24. Rahmat AA, Dar FA, Choudhary IM. Protection of CCl 4-Induced Liver and Kidney Damage by Phenolic Compounds in Leaf Extracts of *Cnestis ferruginea* (de Candolle). Pharmacognosy Res., 2014; 6(1): 19-28. doi:10.4103/0974-8490.122913.
25. Recknagael R. Carbontetrachloride hepatotoxicity. Pharmacol Rev., 1967; 19: 145-196.
26. Recknagel RO, Glende EA, Dolak JA, Waller RLC. Mechanism of carbon tetrachloride toxicity. Pharmacol. Ther, 1989; 43: 139–154.
27. Rincón AR, Covarrubias A, Pedraza-Chaverrí J, Poo JL, Armendáriz-Borunda J, Panduro A. Differential effect of CCl 4 on renal function in cirrhotic and non-cirrhotic rats. Exp Toxicol Pathol, 1999; 51(3): 199-205.
28. Sadiq SM, Kavishankar GB, Rajesha J. Antidiabetic effect of secoisolariciresinol diglucoside in streptozotocin-induced diabetic rats. Phytomedicine, 2013; 20; 237–245.
29. Sadiq SM, Rajesh J. Investigation of in vitro and in vivo antioxidant potential of Secoisolariciresinol diglucoside. Molecular and Cellular Biochemistry, 2012; 012-1487-4. http://dx.doi.org/10.1007/s11010.
30. Sadiq SM, Rajesha J. Secoisolariciresinol diglucoside - a potential flaxseed bioactive: Investigation of in vitro and in vivo antioxidant potential. Mol Cell Biochem, 2013; 373: 179–87.
31. Sadiq SM, Shaukath AK, Rajesha J. Secoisolariciresinol diglucoside– a phytoestrogen nutraceutical of flaxseed: synthesis and evaluation of antioxidant potency. Free Rad Antiox, 2011; 1: 31–38.
32. Sadiq SM, Shaukath AK, Rajesha J. Synthesis and Evaluation of *in vitro* Antibacterial Properties of Secoisolariciresinol Diglucoside. International Journal of Biochemistry Research & Review, 2016; 9(2): 1-10.
33. Saini MR, Kumar S, Jagetia GC, Navita S. Effectiveness of Liv-52 against radiation sickness and dermatitis. Indian Pract, 1984; 11-33.
34. Samoylenko A, Hossain JA, Mennerich D, et al. Nutritional countermeasures targeting reactive oxygen species in cancer: From mechanisms to biomarkers and clinical evidence. Antioxidants & redox signalling, 2013; 19: 2157-96.
35. Sathiyanarayanan L, Arulmozhi S, Chidam baranathan N. Anticholesterolemic, hepatoprotective and antioxidant activity of Glinus lotoides Linn. against ethanol induced liver damage in rats. Phcog Mag, 2006; 2: 160–62.
36. Seely D, Dugoua JJ, Perri D, et al. Safety and efficacy of panax ginseng during pregnancy and lactation. The Canadian journal of clinical pharmacology Journal canadien de pharmacologie clinique. 2008; 15: e87-94.
37. Sharma A, Chakraborti KK, Handa SS. Anti-hepatotoxic activity of some Indian herbal formulations as compared to silymarin. Fitoterapia 1991; 62: 229–235.
38. Sharma B, Sharma UK. Hepatoprotective activity of some indigenous plants, Int J Pharma. Tech. Res., 2010; 2: 568-572.
39. Shenoy KA, Somayaji SN, Bairy KL. Hepatoprotective effects of Ginkgo biloba against carbon tetrachloride induced hepatic injury in rats. Indian J. Pharmacol, 2001; 33: 260–266.
40. Soni B, Visavadiya N, Madamwar D. Ameliorative action of *cyanobacterial phycoerythrin* on CCl4-induced toxicity in rats. Toxicol, 2008; 248: 59–65.
41. Subramonium A, Pushpangadan P. Development of phytomedicines for liver diseases. Indian J. Pharmacol, 1999; 31: 166–175.
42. Suresh Kumar SV, Sujatha C, Syamala J, Nagasudha B, Mishra SH. Hepatoprotective activity of extracts from *Pergulari adaemia Forsk* against Carbon tetrachloride induced toxicity in rats.Phcog Mag., 2007; 3: 11.
43. Valko M, Rhodes CJ, Moncol J, et al. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-biological interactions, 2006; 160: 1-40. doi:10.1016/j.cbi.2005.12.009.
44. Wu XG, Zhu DH, Li X. Anticarcinogenic effect of red ginseng on the development of liver cancer induced by diethylnitrosamine in rats. Journal of Korean medical science, 2001; 16: S61-5.
45. Yun TK, Lee YS, Lee YH, et al. Anticarcinogenic effect of Panax ginseng C.A. Meyer and identification of active compounds. Journal of Korean medical science, 2001; 16: S6-18.
46. Yun TK. Experimental and epidemiological evidence on non-organ specific cancer preventive effect of Korean ginseng and identification of active compounds. Mutation research, 2003; 523-524: 63-74.