World Applied Sciences Journal 27 (4): 524-532, 2013
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2013.27.04.38

Protective Effects of Kombucha Tea and Silimarin Against Thioacetamide Induced Hepatic Injuries in Wistar Rats

[1]Najmeh Kabiri, [2]Mahbubeh Setorki and [3]Mahboobeh Ahangar Darabi

[1]Shahrekord University of Medical Sciences, Shahrekord, Iran
[2]Department of Biology, Izeh Branch, Islamic Azad University, Izeh, Iran
[3]Department of Biology, Faculty of Sciences, Isfahan University, Isfahan, Iran

Abstract: Plants consumed by human contain thousands of phenolic compounds. The effects of dietary polyphenols is of great interest due to their antioxidative and possible anticarcinogenic activities. Liver is one of the organ in the body exposed to many of oxidant and carcinogen agents; therefore; the antioxidant compounds are beneficial for liver healt. In this study the hepatoprotective effects of Kombucha tea and silimarin and thioacetamide (TAA) induced liver toxicity in wistar rats are investigated and compared. In this study we used 36 male white wistar rats groups of six: (control group, group of treated with thioacetamide (TAA) for 3weeks, group treated with TAA and Kombucha tea (for 3 weeks), groups treated with Kombucha tea (for 3 weeks) and TAA, group of treated with TAA and silimarin (for 3 weeks), group of treated with TAA, silimarin and Kombucha tea (for 3 weeks). The level of serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) Lactate dehydrogenase(LDH), total bilirubin monitored to assess hepatotoxicity and hepatoprotection. The data here showed that TAA significantly has increase AST, ALT, ALP, LDH butnot the same for bilirubin. The treatment by silimarin and Kombucha tea produced a significant reduction in serum enzyme levels (AST, ALT, ALP, LDH) and reduction in bilirubin content. The results show that the protective effects of silimarin and Kombucha tea against the thioacetamide induced hepatotoxicity that may be due to the existence of polyphenol substances in the plants, these substances have an antioxidant function.

Key words: Hepatoprotective · Thioacetamide · Silimarin · Kombucha tea · Rat

INTRODUCTION

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. The liver is involved in several vital function in human metabolism. Therefore any damage to the liver indicted by hepatotoxic agents is of grave consequences [1, 2]. Liver cirrhosis associated with various pathological processes, is characterized by progressive fibrosis producing liver injury, portal hypertension and carcinoma [3]. TAA is a commonly used chemical compound that induces experimental liver fibrosis that mimics human liver cirrhosis. 4. Thioacetamide is a typical hepatotoxin causing centrilobular necrosis [5-7]. Ledda-Columbano *et al*. (1991) reported that thioacetamide induced apoptosis in the rat liver based on histochemical observations. 8 Some plants are known to possess antitumor, [9, 10] hypoglycaemic, [11] antibacterial. [12, 13,] free radical scavenging and lipid peroxidation [14] propertice. Silybum marianum belongs to, Family Asteraceae and is known as herbal medicine with hepatoprotective effects, even documented in ancient literature regarding liver disease [15, 16] Chemically.

Active constituent of milk thistle extract is basically a flavolignan, silymarin, which in itself represents the mixture of four isomeric flavonoids such as silibinin, isosilibinin, silydianin and silychristin. [17] Silibinin is

Corresponding Author: Mahbubeh Setorki, Shahrekord University of Medical Sciences, Shahrekord, Iran.
Tel: +989133121589.

the major and most active component in silymarin, [8] A number of other flavonolignans have also been found in majority of seeds. [17] Silymarin is reported to have antifbbrotic, antiinflammatory, immunomodulating and other properties. [19-22] Kombucha is a traditional fermented beverage with a history of several thousand years in the East and yet is quite popular today in the West. [23-25] Numerous studies refer to Kombucha's antimicrobial propertice and suggest that it might influence the gastro-intestinal microbial flora of human. [25-28] The beneficial propertice of kombucha tea is attributed to the presence of tea polyphenols, gluconic acid, glucuronic acid, lactic acid, vitamins, aminoacids, antibiotics and a variety of micronutrients produced during fermentation. [29] This beverage is reported to have medicinal effects against metabolic diseases, arthritis, indigestion and various types of cancer. [27] Recent studies suggeste that kombucha tea prevents paracetamol induced hepatotoxicity [30] and chromate (VI) induced oxidative stress in albino rats. [31] In this study the attempt is made to evaluate the iver injury in rats induced by thioacetamide.

MATERIALS AND METHODS

Wistar male rats (250-200 g of body weight), bred and rised in the animal house in Isfahan University were used. Animals were kept under controlled tepreture (23± 2 °C) and 12:12 –h light-dark cycle conditions.

The rats were then randomly divided into 6 groups (6 animals per group).

Group 1: Control group

Group 2: Injected (i.p.) with TAA (400 mg/kg b.w) for 2 weeks.

Group 3: Injectd with TAA (400 mg/kg b.w) and then kombucha tea (50 ml each rat, for 3 weeks)

Group 4: Kombucha tea (each rat for 3 weeks) and then injectd with TAA (400 mg/kg b.w)

Group 5: Injectd with TAA (400 mg/kg b.w) and then silimarin(200 mg/kg b.w) (for 3 weeks).

Group 6: Injectd with TAA (400 mg/kg b.w) and then kombucha(50 ml each rat)+ silimarin (400 mg/kg b.w) for 3 weeks.

At the end of experiment, the rats are killed in order to collcte samples of serum and liver. The study was reviewed and approved by the Ethics Committee of Isfahan University, Medical Sciences.

Biochemical Examination: Serum samples from rats in groups 1 to 4 were analyzed for alanin aminotransferase (ALT), aspartataminotransferase (AST), alkalinphosphatase (ALP), Lactate dehydrogenase (LDH), total cholesterol (Ch), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C) and triglyceride (TG) and were measured applying special kits (DiaSys, Germany) which utilized the colorimetric method, in an autoanalyzer (Hitachi autoanalyzer, Hitachi Co., Tokyo) here the bilirubin was measured as well.

Histopatological Examination: Animals in each group were killed at the end of third week. The liver was removed rapidly and fixed in 10 % formalin; they were then dehydrated and paraffin embedded. Two-or-three-micron-thick tissues sectioned were cut. The sectioned tissues were routinlly stained with hematoxilen and eosin for quantitative assement of liver injuries. This assessment was performed a numerical scoring system based on injuries induced by TAA.

Statistical Analysis: All values were expressed as mean±SD. Significant differences among the groups were determined by one-way ANOVA using the SPSS 13.0 software package program.Values of $p< 0.05$ were considered as statistically significant.

RESULTS

Daily access to food and water was observed to be the same during the test period and significant deference in body weight gain was observed in control group, groups 4 and 6 in compared to the group of treated with thioacetamide at the end (Table1).

Rats treated with Thioacetamide (TAA) developed significant hepatic impairment as observed from elevated serum levels of hepatospecific enzymes AST, ALT, ALP and LDH (Table 1). Serum bilirubin level also enhanced by TAA treatment. Serum total cholesterol (Ch), HDL-cholesterol (HDL-C) and triglyceride(TG) levels were considerably reduced and LDL-cholesterol (LDL-C) increased by TAA treatment (Table 1). Plasma AST, ALT, ALP and LDH were decreased in groups 3and 4 treated with Kombucha tea compared to the group of treated with

Table 1: Effect of TAA, Kombucha tea and silimarin treatment on rat body weight, liver weight and biochemical parameters

	Groups					
Parameters	control	TAA	Kombucha+TAA	TAA+Kombucha	TAA+Silimarin	TAA+Kombucha+Silimarin
ALT(U/L)	148±26.058	767±16.971*	106.75±208.290**	126.67±3.055**	275±14.863**	125±29.103**
AST(U/L)	184.33±62.324	653±53.033*	130.75±81.2**	130.6±14.572**	354±11.899**	203.67±41.429**
ALP(U/L)	614.67±196.398	1593.50±214.253*	1261.50±261.439**	947.33±7.506**	836.50±19.122**	613.33±1.528**
LDH(U/L)	1116.33±74.272	1269.50±6.364	671.75±127.241**	808±191.909**	819.50±46.837**	741.67±133.717**
TG(mg/dl)	29.33±16.563	11±1.414	31.75±9.878	26.33±1.528**	39.50±4.123	25.67±1.155**
cho(mg/dl)	88.67±13.051	64.50±3.536*	83±21.556	113.33±1.528	89.5±2.517**	113±19.165**
LDL(mg/dl)	10±2.646	16±0.0	9.80±10.863	25.67±8.145	28±1.708**	20.33±0.577**
HDL(mg/dl)	42.33±5.859	30±5.657	41±9.933	59.33±16.258	56.25±6.551**	74.67±3.786**
Bilirobin(mg/dl)	0.4833±0.028	1.05±0.63	0.45±0.04	0.5167±0.028	0.45±0.108	0.4767±0.025
BW (g)	244.76±32.712	191.93±10.909*	208.55±26.344**	186.59±18.763	191.09±17.666	230.08±17.910**
LW (g)	8.47±0.32	7.38±0.72	9.24±0.88	7.87±0.52	7.4±0.98	8.04±0.27
LW/BW (%)	3.46±0.2	3.84±0.1	4.43±0.3	4.24±0.3	3.87±0.1	3.49±0.4

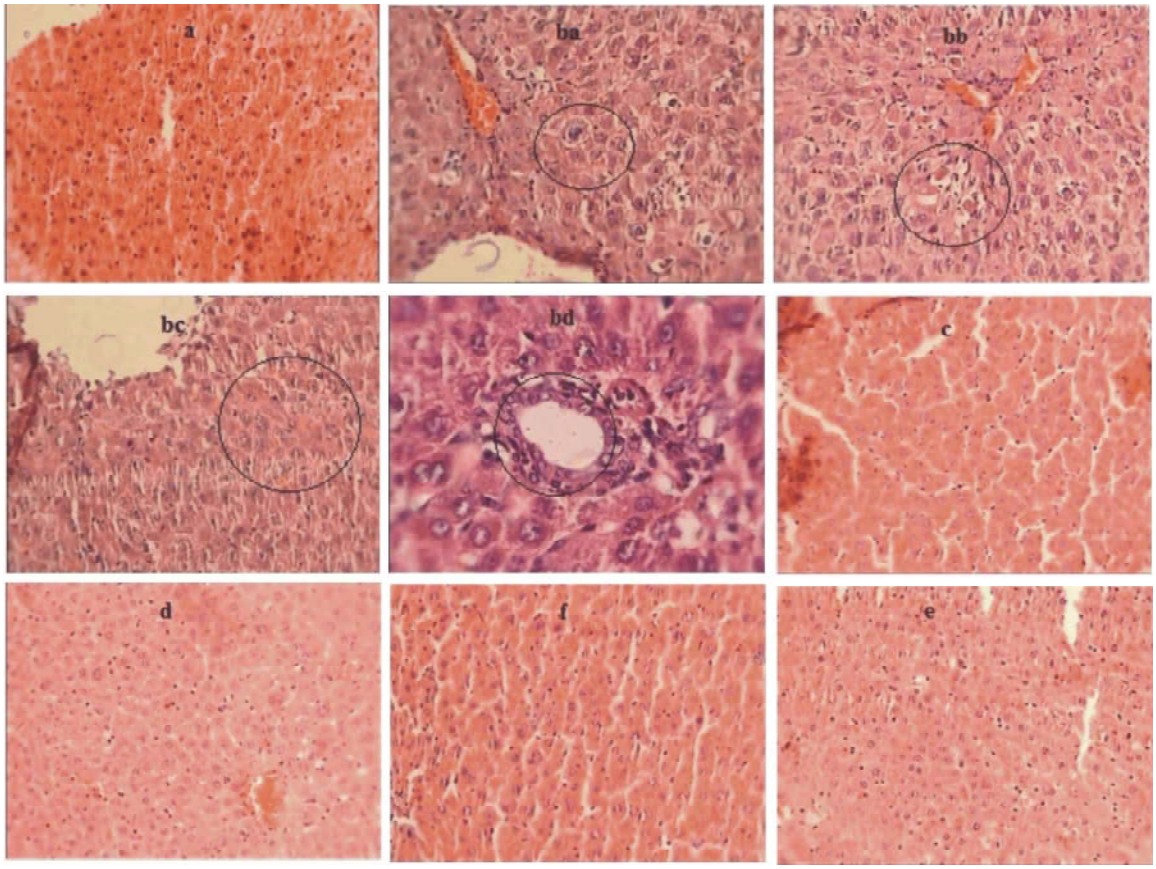

Fig. 1: Hepatoprotective e?ect Kombucha tea and silimarin against Thioacetamide induced acute hepatotoxicity in rats. Liver sections were stained with
H&E: (a) normal; (b) TTA; (c) TAA and then kombucha tea (d) : kombucha tea and then TAA (e): TAA and then silimarin(200 mg/kg b.w) (f): TAA and then kombucha+ silimarin, Magni?cation 40.
ba: Abnormal mitosis, bb: Cells with apoptosis, bc: Mitotic cells bd:Inflammation around the port area
** P > 0.05 vs. TAA

thioacetamide and were even comparable with the control group regarding a reduction in bilirubin content (Table 1). Treatment with silymarin for 3 weaks offered protection against TAA induced elevation in levels of in liver enzymes, bilirubin in serum as evidenced by a fall in its levels. The treatment by silimarin reduced serum enzyme levels (AST, ALT, ALP, LDH) and the bilirubin content(Table 1).

Histopathological Observations: Histology of the liver sections of normal control animals (Group I) showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein (Fig. 1). Histopathological studies demonstrated that that group 2 exposed to TAA, compared to the normal group causes focal necrosis, increases mitosis at cells, apoptosis, abnormally mitosis, inflammation at portal space and enlarged nucleus (Fig.1). Necrosis which is a more severe form of injury is markedly prevented by silimarin especially by 200 mg:kg body weight. In the group, treated by silimarin, the number of cells still have large nucleus but apoptosis and mitosis are not observed apoptosis and mitosis (Fig.1) and prevention groups of Kombucha tea +TTA and TTA+ Kombucha tea, showed normal histology of liver sections.

DISCUSSION

Thioacetamide (TAA) is a classic hepatotoxic reagent used for liver cirrhosis induction. [32] TAA is hepatotoxic and effects DNA, RNA, protein synthesis and glutathione content, which, in turn, induces intrahepatic metabolic changes. [33, 34] In this study TAA administration to rats for 10 days has been observed to cause necrosis, increase mitosis at cells, apoptosis, abnormally mitosis, inflammation at portal space and enlarge nucleus as assessed histopathologically. Liver injury was also determined by biochemical parameters (plasma AST, ALT, ALP, LDH levels). Free radicals are believed to play a major role in the development of TAA induced liver cirrhosis. [35-38] TAA in reduction of the number and function of mitochondria in hepatocytes of cirrhotic rats is considered to cause uncoupling in oxidative phosphorylation leading to accumulation of NADH and lactate and diminishe energy synthesis rate. It is also suggested to decrease hepatic protein synthesis in cirrhosis, since most of the cell energy is used in the process. [39] Steinkraus *et al* and Dhanalakshmi *et al* reported that apoptosis caused by thioacetamide involving the activation of caspase-3 along with extensive necrosis. [38, 40] Different antioxidants are shown to prevent liver fibrosis and cirrhosis. [36, 41-45] Studies have shown that diets rich in fruits and vegetables are associated with lower risk of cancer, suggesting that cancer risk could be modified/prevented by making appropriate dietary manipulations. [12, 46-48] Silymarin is a popular herbal product marketed to treat liver disorders. [49] Silymarin and quercetin are polyphenolic flavonoids that increase cell resistance to lipid peroxidation. [50-52] Silymarin has been in extensive use for its hepatoprotective effects since ancient times, though other health beneficial effects of it are being recognized in recent years. Most of these effects are attributed to direct and/or indirect antioxidant property of silymarin,such as being a scavenger of reactive oxygen species, a scavenger of phenylglyoxylic ketyl radicals and a chain breaking antioxidant. [53] Silymarin is one such agent, which has been in extensive use for ages for liver treatment and thus has possibly the greatest patient acceptability. In recent years increasing body of evidence has underscored the cancer preventive efficacy of silymarin both in vitro and in vivo animal models of various epithelial cancers. [54] Posttreatment with the silymarin (50 mg/kg) orally for 30 days significantly reversed the diethylnitrosamine induced alterations in the liver tissue and offered almost complete protection. The results obtained this study indicate that silymarin exhibits good hepatoprotective and antioxidant potential against diethylnitrosamine induced hepatocellular damage in rats. [55] In recent years, findings of the studies have in part shade more light on certain aspects of hepatoprotective mechanism of silymarin. Silibinin, an active constituent of silymarin, also affords protection against T cell-dependent hepatitis through the modulation of immune response. [56] There are sufficient evidences that silymarin/silibinin possesses anticancer efficacy and additionally, as discussed further, preventive efficacy in various in vivo models of epithelial cancers. [57] The Results of the study conducted by Singh *et al* (2006) revealed the antiangiogenic potential of silibinin in animal model of lung tumorigenesis. [58] The in vivo model established that at the examined dose, silymarin exhibits a protective influence on the heart and liver tissue against toxicity induced by doxorubicin. [59] In animal experiments, silymarin and silibinin are shown to protect rat or mouse liver against hepatotoxicity induced by acute ethanol intoxication, [60] thioacetamide, [61] thallium, [62] D-galactosamine, 63 and acetaminophen, [64-66] as well as preventing a wide range of carcinogen and tumor promoter-induced cancers. [67] Silybin,

isolated from the milk thistle plant (Silybum marianum), is widely used as a therapeutic agent for a variety of disease, including human colon cancer, liver disease and prostate cancer. [68-70] Previous studies have indicated that silybin suppresses the growth of human prostate carcinoma PC-[3.71] Silybin inhibits cell proliferation, survival and angiogenesis in established prostate tumor it also, suppresses the levels of angiogenic growth factors and receptor and molecular events, driving invasion and EMT in prostate tumor. [72, 73] Kombucha converts sucrose to glucose and fructose and further to ethanol, acetic acid, lactic acid and a large number of other compounds. [74] In condueted studies it is indicated that the daily consumption of kombucha is related to an extremely high resistance to cancer. [23] The beneficial effects of kombucha tea is attributed to the presence of tea polyphenols, gluconic acid, glucuronic acid, lactic acid vitamins, aminoacids, antibiotics and a variety of micronutrients produced during fermentation. [29] Kombucha tea treatment, however, counteract to the changes in mitochondrial membrane potential and prevented apoptotic cell death of the hepatocytes. Study Bhattacharya *et al*(2011) showed that kombucha tea has the potential to ameliorate tertiary butyl hydroperoxide induced oxidative insult and cell death in murine hepatocytes more effectively than fermented black tea. [75] Kombucha is a potent antioxidant proved to reduce the damage induced by oxidative stress. 31, [76-78] The antioxidant properties, of Kombucha constituents and the protective effects of Kombucha on oxidative stress induced nephrotoxicity of Trichloroethylene treated rats (Effects of Kombucha on oxidative stress induced nephrotoxicity in rats. [77] Kombucha tea prepared by fermentation of black tea with tea fungus could be used as a preventive and urative agent against CCl4-induced hepatotoxicity. As kombucha tea is rich in compounds known to be strong antioxidants and is expected to ameliorate liver damage induced by CCl4. [79] Recent studies have suggested that kombucha tea prevents paracetamol induced hepatotoxicity [30] and chromate (VI) induced oxidative stress in albino rats. [31] Gluconic acid is considered by several reserchers to be the main therapeutic agent in kombucha, is considered as its function in the liver is considered as a detoxification agent [34, 80-81].

In this context a rise is observed in the levels of AST, ALT, ALP, LDH and bilirubin in thioacetamide treated rats. The Kombucha tea and silimarin used in this study seems to offer protection and maintain the structural integrity of hepatic cells. The protective effects are more pronounced and much better when the rats are treated with Kombucha tea and silimarin. This was evident from the significant reduction in serum AST, ALT, ALP, LDH and bilirubin content. Based on the above findings it could be concluded that Kombucha tea and silimarin proved a better antihepatotoxic activity against thioacetamide induced hepatic damage. More elaborate work is required to establish the efficiency of Kombucha tea and silimarin potent antihepatotoxic drug. Further experimental work is necessary to isolate and identify the active properties in the Kombucha tea and silimarin which are responsible for the antihepatotoxic activity.

ACKNOWLEDGEMENTS

The work was supported Part by a grant number 81123 from Isfahan Cardiovascular Research Center (ICRC) and part by the isfahan Universit. We would like to thank Isfahan Cardiovascular Research Center and staffs of ICRC Basic Science Laboratory.

REFERENCES

1. Shahani, S., 1999. Evaluation of hepatoprotective efficacy of APCL-A poly-herbal formulation in vivo in rats. Indian Drugs, 36: 628-631.
2. Subramoniam, A. and P. Pushpangadan, 1999. Development of phytomedicine for liver diseases. Indian Journal of Pharmacology; 31: 166-175.
3. Friedman, S.L., 2003. Liver fibrosis-frombench to bedside. J Hepatol; 38: 38-53.
4. Fatih, A., Z.K. sku-Kiraz, S. Dog ru-Abbasoglu, M. Gulluoglu, *et al*., 2010. Effect of carnosine against thioacetamide-induced liver cirrhosis in rat. Peptides, 31: 67-71.
5. Diez-Fernandez, C., L. Bosca, L. Fernandez-Simon, A. Alvarez and M. Cascales., 1993. Relationship between genomic DNA ploidy and parameters of liver damage during necrosis and regeneration induced by thioacetamide. Hepatology; 18: 912-918.
6. Diez-Fernandez, C., N. Sanz and M. Cascales, 1996. Changes in glucose-6-phosphate dehydrogenase and malic enzyme gene expression in acute hepatic injury induced by thioacetamide. Biochem. Pharmacol, 51: 1159-1163.
7. Zimmerman, H.J., 1978. Hepatotoxicity, the Adverse Effects of Drugs and Other Chemicals on the Liver. Appleton-Century-Crofts New York.

8. Ledda-Columbano, G.M., P. Coni, M. Curto, L. Giacomini, G. Faa, S. Oliverio, M. Piacentini and A. Columbano, 1991. Induction of two different modes of cell death, apoptosis and necrosis, in rat liver after a single dose of thioacetamide. *Am. J. Pathol*, 139: 1099-1109.
9. Ahmed, S., A. Rahman, M. Mathur, M. Athur and S. Sultana, 2001. Antitumor promoting activity of Asteracantha longifolia against experimental hepatocarinogenesis in rats. *Food and Chemical Toxicology*; 39: 19-28.
10. Mazumdar, U.K., M. Gupta, S. Maiti and D. Mukhejee, 1997. Antitumor activity of Hygrophila spinosa in Ehrlich ascites carcinoma and sarcoma-180 induced mice. Indian Journal of Experimental Biology 35: 473-477.
11. Fernando, M.R., 1991. Nalinie Wickramasinghe SMD., Thabrew MI, Ariyananda PL, Karunanayake EH: Effect of Artocarpus heterophyllus and Asteracanthus longifolia on glucose tolerance in normal human subjects and in maturity onset diabetic patients. Journal of Ethnopharmacology, 31: 277-282.
12. Block, G., B. Patterson and A. Subar, 199. Fruit, vegetables and cancer prevention: a review of the e Boily, Y., Vanpuyvelde, L. Screening of medicinal plants of Rwanda (Central Africa) for antimicrobial activity. Journal of Ethnopharmacology, 16: 1-13.
13. Vlietinck, A.J., L. Vanhoof, J. Totte, A. Lasure, D. Vanden Berghe, P.C. Rwangabo and J. Mvukiyumwami, 1995. Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. Journal of Ethnopharmacology, 46: 31-47.
14. Vijayakumar, M., R. Govindarajan, A. Shriwarkar, V. Kumar, A. Rawat, S. Mehrotra and P. Pushpangadan, 2005. Free radical scavenging and lipid peroxidation inhibition potential of Hygrophila auriculata. Natural Product Sciences, 11: 22-26.
15. Culpeper, N., 1650. A Physical Directory: Or a Translation of the Dispensatory Made by the College of Physicians of London. London. Peter Cole, England.
16. Foster, S., 1999. Milk Thistle: Silybum marianum. Rev. ed. Austin, Tex: American Botanical Council.
17. Lee, J.I., M. Narayan and J.S. Barrett, 2007. Analysis and comparison of active constituents in commercial standardized silymarin extracts by liquid chromatography-electrospray ionization mass spectrometry. Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences, 845: 95-103.
18. Saller, R., R. Meier and R Brignoli, 2001. The use of silymarin in the treatment of liver diseases. Drugs, 61: 2035-2063.
19. Flora, K., M. Hahn, H. Rosen and K. Benner, 1998. Milk thistle (Silybum marianum)for the therapy of liver disease. The American Journal of Gastroenterology 93: 139-143.
20. Johnson, V.J., Q. He, M.F. Osuchowski and R.P. Sharma, 2003. Physiological responses of a natural antioxidant flavonoid mixture, silymarin, in BALB/c mice: III. Silymarin inhibits T-lymphocyte function at lowdoses but stimulates in?ammatory processes at high doses. *Planta Medica,* 69: 44-49.
21. Katiyar, S.K., 2003. Silymarin and skin cancer prevention: anti-inflammatory,antioxidant and immunomodulatory effects (Review). *International Journal of Oncology* , 26: 169-176.
22. Piscitelli, S.C., E. Formentini, A.H Burstein, R. Alfaro, S. Jagannatha and J. Falloon, 2002. Effect ofmilk thistle on the pharmacokinetics of indinavir in healthy volunteers. Pharmacotherapy, 22: 551-556.
23. Dufresne, C. and E. Farnworth, 2000. Tea, kombucha and health: a review. *Food Res. Int.*, 33: 409-421.
24. Frank, G.W., 1995. Kombucha: Healthy Beverage and Natural Remedy from the Far East. *Wilhelm Ennsthaler*, Austria.
25. Greenwalt, C.J., K.H. Steinkraus and R.A. Ledford, 2000. Kombucha, the fermented tea: microbiology, composition and claimed health effects. *J. Food Prot* 63: 976-981.
26. Greenwalt, C.J., R.A. Ledford and K.H. Steinkraus, 1998. Determination and characterization of the antimicrobial activity of the fermented tea kombucha. *Lebensm.-Wiss. Technol*, 31: 291-296.
27. Sreeramulu, G., Y. Zhu and W. Knol, 2001. Characterization of antimicrobial activity in Kombucha fermentation. *Acta Biotechnol*; 21: 49- 56.
28. Steinkraus, K.H., K.B. Shapiro, J.H. Hotchkiss and R.P. Mortlock, 1996. Examinations on antibiotic activity of tea fungus/Kombucha beverage. Acta Biotechnologica; 16: 199-205.

29. Vijayaraghava, R., M. Singh, P.V.L. Rao, R. Bhattacharya, P. Kumar, K. Sugendran, O. Kumar, S.C. Pant and R. Singh, 2000. Subacute (90 days) oral toxicity studies of Kombucha Tea. *Biomedical and Environmental Sciences*; 13: 293-299.
30. Pauline, T., P. Dipti, B. Anju, S. Kavimani, S.K. Sharma, A.K. Kain, S.K.S. Sarada, M. Sairam, G. Ilavazhagan, D. Kumar and W. Selvamurthy, 2001. Studies on toxicity; anti-stress and hepatoprotective properties of kombucha tea. *Biomedical and Environmental Science*; 14: 207-213.
31. Sai Ram, M., B. Anju, T. Pauline, P. Dipti, A.K. Kain, S.S. Mongia, S.K. Sharma, B. Singh, R. Singh, G. Ilavazhagan, D. Kumar and W. Selvamurthy, 2000. E?ect of Kombucha tea on chromate (VI)induced oxidative stress in albino rats. *Journal of Ethnopharmacology,* 71: 235-240.
32. Nozu, F., N. Takeyama and T. Tanaka, 1992. Changes of hepatic fatty acid metabolism produced by chronic thioacetamide administration in rats. *Hepatology*, 15: 1099-1106.
33. Dashti, H., B. Jeppson, I. Haggerstrand, *et al.*, 1989. Thioacetamide- and carbon tetrachloride-induced liver cirrhosis. *Eur Surg Res*; 21: 83-91.
34. Zimmermann, T., A. Muller, G. Machmik, *et al.*, 1987. Biochemical and morphological studies on production and regression of experimental liver cirrhosis induced by thioacetamide in Uie: WIST rats. *Z Versuchstierkd*, 30: 165-180.
35. Abul, H., T.C. Mathew, H.M. Dashti and A. Al-Bader, 2002 Level of superoxide dismutase,glutathione peroxidase and uric acid in thioacetamide-induced cirrhotic rats.Anat *Histol Embryo;* 31: 66-71.
36. Balkan, J., S. Dog ru-Abbasog lu, Kanbag l1 OC, U. evikbas, G. Aykac-Toker and M. Uysal, 2001. Taurine has protective effect against thioacetamide-induced liver cirrhosis by decreasing oxidative stress. *Hum Exp Toxicol*; 20: 251-254.
37. Lukivskaya, O., E. Patsenker, R. Lis, V.U. Buko, 2008. Inhibition of inducible nitric oxide synthase activity prevents liver recovery in rat thioacetamide-induced fibrosis reversal. *Eur J Clin Invest;* 38: 317-325.
38. Sun, F., S. Hayami, Y. Ogiri, S. Haruna, K. Tanaka, Y. Yamada, S. Tokumaru and S. Kojo, 2000. Evaluation of oxidative stress based on lipid hydroperoxide, vitamin C and vitamin E during apoptosis and necrosis caused by thioacetamide in rat liver. *Biochim. Biophys. Acta;* 1500: 181-185.
39. Perez, M.J., F. Sanchez-Medina, M. Torres, A. Gil, A. Suarez, 2004. Dietary nucleotides enhance the liver redox state and protein synthesis in cirrhotic rats. *J Nutr*; 134: 2504-2508.
40. Hayami, S., K. Ikeda, F. Sun, K. Tanaka and S. Kojo, 1999. Increase of caspase-3 activity in rat liver and plasma by thioacetamide. *Biochem. Pharmacol*; 58: 1941-1943.
41. Bruck, R., H. Shirin, H. Aeed, Z. Matas, A. Hochman, M. Pines, *et al.*, 2001. Prevention of hepatic cirrhosis in rats by hydroxyl radical scavengers. *J Hepatol*; 35: 457-464.
42. Bruck, R., M. Ashkenazi, S. Weiss, I. Goldiner, H. Shapiro, H. Aeed, *et al.*, 2007. Prevention of liver cirrhosis in rats by curcumin. Liver Int; 27: 373-383.
43. Cruz, A., F.J. Padillo, E. Torres, C.M. Navarrete, J.R. Munoz-Castaneda, F.J. Caballero, *et al*., 2005. Melatonin prevents experimental liver cirrhosis induced by thioaceta- mide in rats. *J Pineal Res;* 39: 143-150.
44. Hsieh, C.C., H.L. Fang and W.C. Lina, 2008. Inhibitory effect of solanum nigrum on thioacetamide-induced liver fibrosis in mice. *J Ethnopharm*; 119: 117-121.
45. Rai, M., M.R.S.M. Pai, P. Sabitha and P. Pai, 2001. Hepatoprotective in?uence of selenium in experimental liver cirrhosis. *Kuwait Med J, ;* 33: 333-336.
46. Kelemen, L.E, J.R. Cerhan, U. Lim, S. Davis, W. Cozen, M. Schenk, J. Colt, P. Hartge and M.H. Ward, Vegetables, fruit and antioxidant-related nutrients and risk of non-Hodgkin lymphoma: a National Cancer Institute Surveillance, Epidemiology and End Results population-based casecontrol study. Am. J. Clin. Nutr 2006;83:1401–1410.
47. Pavia, M., C. Pileggi, C.G. Nobile and I.F. Angelillo, 2006. Association between fruit and vegetable consumption and oral cancer: a meta-analysis of observational studies. *Am. J. Clin. Nutr;* 83: 1126-1134.
48. Steinmetz, K.A. and J.D. Potter, 1991. Vegetables, fruit and cancer. I. *Epidemiol. Cancer Causes Control*; 2: 325-357.
49. Wua, J.W., L. Lin and T. Tsai, 2009. Drug-drug interactions of silymarin on the perspective of pharmacokinetics *Journal of Ethnopharmacology*; 121: 185-193.

50. Bongiovanni, G.A., E.A. Soria, A.R. Eynard, 2007 Effects of the plant ?avonoids silymarin and quercetin on arsenite-induced oxidative stress in CHO-K1 cells. *Food and Chemical Toxicology*; 45: 971-976.
51. Kravchenko, L.V., S.V. Morozov, V.A. Tutel'yan, 2003. Effects of ?avonoids on the resistance of microsomes to lipid peroxidation in vitro and ex vivo. *Bull. Exp. Biol. Med*; 136: 572-575.
52. Valko, M., C.J. Rhodes, J. Moncol, M. Izakovic and M. Mazur, 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem.-Biol. Interact*; 160: 1-140.
53. Luper, S., 1998. A review of plants used in the treatment of liver disease: part 1,.*Altern. Med. Rev*; 3: 410-421.
54. Kaur, M., 2007. Silymarin and epithelial cancer chemoprevention: How close we are to bedside? *Rajes Toxicology and Applied Pharmacology*; 224: 350-359.
55. Pradeep, K., C.V. Raj Mohan, K. Gobianand and S. Karthikeyan, 2007. Silymarin modulates the oxidant–antioxidant imbalance during diethylnitrosamine induced oxidative stress in rats. *European Journal of Pharmacology*; 560: 110-116.
56. Schumann, J., J. Prockl, A.K. Kiemer, A.M. Vollmar, R. Bang and G. Tiegs, 2003. Silibinin protects mice from T cell-dependent liver injury. *J. Hepatol*; 39: 333-340.
57. Sharma, G., R.P. Singh, D.C. Chan and R. Agarwal, 2003. Silibinin induces growth inhibition and apoptotic cell death in human lung carcinoma cells. *Anticancer Res*; 23: 2649-2655.
58. Singh, R.P., G. Deep, M. Chittezhath, M. Kaur, L.D. Dwyer-Nield, A.M. Malkinson and R. Agarwal, 2006. Effect of silibinin on the growth and progression of primary lung tumors in mice. *J. Natl. Cancer Inst*; 98: 846-855.
59. Rašković, A., N. Stilinović, J. Kolarović, V. Vasović, S. Vukmirović and M. Mikov, 2011. The protective effects of silymarin against doxorubicin-induced cardiotoxicity and hepatotoxicity in rats. *Molecules*.; 16: 8601-8613.
60. Valenzuela, A., C. Lagos and L.A. SchmidtK, 1985. Videla, Silymarin protection against hepatic lipid peroxidation induced by acute ethanol intoxication in the rat. Biochemical Pharmacology; 34: 2209-2212.
61. Schriewer, H. And J. Lohmann, 1976. [Disturbances in the regulation of phospholipid metabolism of the whole liver, mitochondria and microsomes in acute thioacetamide poisoning and the influence of silymarin]. *Arzneimittelforschung*; 26: 65-69.
62. Mourelle, M., L. Favari and J.L. Amezcua, 1988. Protection against thallium hepatotoxicity by silymarin. Journal of Applied Toxicology; 8: 351-354.
63. Chrungoo, V.J, K. Singh and J. Singh, 1997. Silymarinmediated differentialmodulation of toxicity induced by carbon tetrachloride, paracetamol and D-galactosamine in freshly isolated rat hepatocytes. *Indian Journal of Experimental Biology*; 35: 611-617.
64. Campos, R., A. Garrido, R. Guerra and A. Valenzuela, 1989 Silybin dihemisuccinate protects against glutathione depletion and lipid peroxidation induced by acetaminophen on rat liver. *Planta Medica*; 55: 417-419.
65. Muriel, P., T. Garciapina, V. Perez-Alvarez and M. Mourelle, 1992. Silymarin protects against paracetamol-induced lipid peroxidation and liver damage. *Journal of Applied Toxicology*; 12: 439-442.
66. Nencini, C., G. Giorgi and L. Micheli, 2007. Protective effect of silymarin on oxidative stress in rat brain. *Phytomedicine*; 14: 129-135.
67. Zhao, J., M. Lahiri-Chatterjee, Y. Sharma and R. Agarwal, 2000. Inhibitory effect of a ?avonoid antioxidant silymarin on benzoyl peroxide-induced tumor promotion, oxidative stress and inflammatory responses in SENCAR mouse skin. *Carcinogenesis*; 21: 811-816.
68. Dhanalakshmi, S., P. Agarwal, L.M. Glode and R. Agarwa, 2003. Silibinin sensitizes human prostate carcinoma DU145 cells to cisplatin- and carboplatin-induced growth inhibition and apoptotic death. *International Journal of Cancer;* 106: 699-705.
69. Hogan, F.S., N.K. Krishnegowda, M. Mikhailova and M.S. Kahlenberg, 2007. Flavonoid, silibinin, inhibits proliferation and promotes cell-cycle arrest of human colon cancer. *Journal of Surgical Research*; 143: 58-65.
70. Velebnı, S., G. Hrèkova and A. Königová, 2010. Reduction of oxidative stress and liver injury following silymarin and praziquantel treatment in mice with Mesocestoides vogae (Cestoda) infection. *Parasitology International*; 59: 524-531.

71. Singh, R.P., K. Raina, G. Deep, D. Chan and R. Agarwal, 2009., Silibinin suppresses growth of human prostate carcinoma PC-3 orthotopic xenograft via activation of extracellular signal-regulated kinase 1/2 and inhibition of signal transducers and activators of transcription signaling. *Clinical Cancer Researc ;* 15: 613-621.
72. Singh, R.P., K. Raina, G. Sharma and R. Agarwal, 2008. Silibinin inhibits established prostate tumor growth, progression, invasion and metastasis and suppresses tumor angiogenesis and epithelial–mesenchymal transition in transgenic adenocarcinoma of the mouse prostate model mice. *Clinical Cancer Research*; 14: 7773-7780.
73. Zhenga, D., Y. Wanga, D. Zhanga, Z. Liub, C. Duana, L. Jiaa, F. Wanga, Y. Liua, G. Liua, L. Haoa and Zhangc, 2011. In vitro antitumor activity of silybin nanosuspension in PC-3 cells.*Cancer Lett* 2011*;* 28 307: 158-164.
74. Malbas, R., E. Loncar, M. Djuric and I. Dosenovic, 2008. Effect of sucrose concentration on the productsof Kombucha fermentation on molasses.´*Food Chemistry*; 108: 926-932.
75. Bhattacharya, S., P. Manna, R. Gachhui, P.C. Sil, 2011. Protective effect of kombucha tea against tertiary butyl hydroperoxide induced cytotoxicity and cell death in murine hepatocytes. *Indian J Exp Biol*; 9: 511-524.
76. Anand, S.S., 2005. Protective effect of vitamin B6 in chromium-induced oxidative stress in liver. *J Appl Toxicol*; 25: 440-443.
77. Gharib, O.A., 2007. Does kombucha tea reduce the damage induced by radiation exposure? *Egypt J Sci Applic ;* 0: 141-157.
78. Yoshioka, T., K. Kawada, T. Shimada and M. Mori, 1979. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. *Am J Obstet Gynecol;* 135: 372-376.
79. Murugesan, G.S., M. Sathishkumar, R. Jayabalan, A.R. Binupriya, K. Swaminathan and S.E. Yun, 2009. Hepatoprotective and Curative Properties of Kombucha Tea Against Carbon Tetrachloride-Induced Toxicity *J. Microbiol. Biotechnol*; 19: 397-402.
80. Loncar, E.S., S.E. Petrovic, R.V. Malbasa, R.M. Verac, 2000. Biosynthesis of glucuronic acid by means of tea fungus. *Nahrung*; 44: 138-139.
81. Tietze, H.W., 1995. Kombucha. The Miracle Fungus, 6th ed. Phree Books, Australia.