J Biochem Tech (2012) 3(4):339-343
ISSN: 0974-2328

Protective effect of *Solanum nigrum* fruit extract on the functional status of liver and kidney against ethanol induced toxicity

Arulmozhi V, Krishnaveni M, Mirunalini S*

Received: 14 August 2011 / Received in revised form: 11 September 2011, Accepted: 28 December 2011, Published online: 20 January 2012,

Abstract

The trend in using natural products has increased and the active plant extracts are frequently screened for new drug discoveries. The present study was aimed to investigate the effect of *Solanum nigrum* fruit extract (SNFEt) on ethanol induced toxicity in rats. Male albino wistar rats were intoxicated with ethanol and the effect of SNFEt on the levels of hepatic marker enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and bilirubin), renal markers (urea, uric acid and creatinine) and antioxidant status such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) were assessed in liver and kidney. Ethanol intoxicated rats showed significant increases in hepatic and renal markers whereas the antioxidant statuses were found to be decreased. Supplementation of SNFEt altered these changes to near normal levels and was also compared with the standard drug silymarin. This beneficial activity of the extract might be considered as an adjuvant drug in the treatment of liver disorders. Thus we propose that dietary intake of *Solanum nigrum* fruits offers protection against cellular and tissue toxicity.

Keywords*:* Ethanol, hepatic markers, renal markers, lipid peroxidation, antioxidant, *Solanum nigrum*

Introduction

Alcohol has been suspected to be a major cause of liver disease for centuries. It is a growing medical and public health issue faced by the adult and adolescent populations. Progression to an alcoholic liver disease is a multifactorial process that involves a number of genetic, nutritional and environmental factors (Day 2006). Ethanol affects almost all organs of the body because of its ability to permeate all tissues due to its water and fat soluble properties. Many studies showed a close relationship between cumulative alcohol intake and risk in the development of liver damage (Batey et al. 1992, Leiber 1994). Among various mechanisms implicated in the pathogenesis of alcoholic liver disease, free radical-mediated oxidative stress plays an important role. Oxidative stress associated with alcohol toxicity is mainly caused by reactive oxygen species (ROS) generated by the mitochondrial respiratory chain, the ethanol metabolizing cytochrome P4502E1 (CYP2E1) of hepatocytes and the NADPH oxidase of kupffer cells and liver infiltrating granulocytes. In addition, an impairment of liver antioxidant defenses and alteration of hepatic iron homeostasis may further contribute to oxidative damage (Albano 2006, Dey 2006). Furthermore these mechanisms lead to altered enzyme activity, decreased DNA repair, impaired utilization of oxygen and protein oxidation, all of which can eventually cause necrosis and subsequently lead to organ damage.

In spite of tremendous advances made in allopathic medicine, there is no rational therapy available for liver disorders. Emerging evidence demonstrates that natural products isolated from plant sources such as fruits, vegetables, cereals, pulses and herbs possess strong antioxidant activities and thus act as a remedy for various liver diseases (Lathu et al. 1999). *Solanum nigrum* Linn (Solanaceae) is an annual herb, grows as a weed and it is found on dry parts of India and other parts of the world. It has a long history of medicinal usage and has been used as a traditional folk medicine in treating various ailments such as pain, inflammation and fever (Ong 2003). It possess a variety of chemical constituents such as glycoalkoloids (solanine, solamargine, solanigrine and solasodine), steroidal glycosides (β-solamargine, solasonine & α,β-solasodamine), steroidal saponins (nigrumin I & II), pregnane saponins (solanigroside A & solanigroside B), steroidal sapogenins (diosgenin & trigogenin), steroidal genin (gitogenin), tannin and polyphenolic compounds (Potawele et al 2008).

Solanum nigrum elaborates a wide spectrum of pharmacological properties such as antioxidant, anticancer, hepatoprotective, neuroprotective and antiulcerogenic ones (Arulmozhi et al. 2010). Recently work has been carried on aqueous leaf extract of S*olanum nigrum* against CCL_4-induced oxidative damage in rats (Lin et al. 2008). Hsieh et al. 2006 also reported that *Solanum nigrum* exerts protection against thioacetamide induced liver fibrosis in mice.

Arulmozhi V, Krishnaveni M, Mirunalini S*

Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar, Tamil Nadu, India.

E-mail: mirunasankar@gmail.com

Moreover in India, *Solanum nigrum* has been considered an important ingredient in liv 52,a herbal formulation mainly used for treating various liver diseases (Sandhir and Gill KD 1999). These evidences suggested that *Solanum nigrum* possess a beneficial activity as an antioxidant and hepatoprotecive agent, but still there is no much knowledge of how it exerts its protective effects against liver toxicity. The present study was undertaken to investigate the underlying mechanisms of the effect of *Solanum nigrum* fruit extract on ethanol-induced toxicity in rats.

Materials and methods

Chemicals

Ethanol was obtained from E. Merck, Darmstadt, Germany and E.I.D. Parry India Ltd. (Nellikuppam, Cuddalore District, South India). All other chemicals and acids were of certified analytical grade and purchased from S.D fine chemicals, Mumbai or Himedia laboratories Pvt. Ltd., Mumbai, India.

Plant material

Solanum nigrum fruits were collected in and around Chidambaram, Cuddalore district, Tamil Nadu, India. The herbarium of the plant was identified and authenticated by the botanist Dr.V.Venkatesalu and the voucher specimen was deposited to the Department of Botany, Annamalai University, Tamil Nadu, India.

Preparation of Aqueous Fruit Extract

Solanum nigrum fruits were washed, shade dried and finely powdered. 100 gms of the powder was suspended in 250 ml of water for 2 hours and then heated at 60-65ºC for 30 min. The extract was collected separately and the processes were repeated 3 times with the residual powder, each time collecting the extract. The collected extracts were pooled and passed through fine cotton cloth. The filtrates were evaporated at 40-50ºC in a rotavapour under reduced pressure. A dark semisolid material (yield-14%) obtained was stored at 0-4ºC until use (Hossain et al. 1992). A known amount of the residual extracts were suspended in distilled water and was administered to the animals via intragastrically.

Animals and diet

Adult male albino wistar rats weighing (150-170g) were used for the study. Animals were procured from central animal house, Department of Experimental Medicine, Raja Muthiah Medical College, Annamalai University. All the animals were acclimatized for a week under standard husbandry conditions. The animals were housed in polypropylene cages (45×24×15cm), maintained under the temperature of 25 ± 2ºC and 12 h light/12 h dark condition. The animals had free access to standard pellet diet (Agro Corporation Private Limited, Bangalore, India) and water *ad libitum* was available to the animals throughout the experimental period and replenished daily. The standard pellet diet comprised of 21% protein, 5% lipids, 4% crude fiber, 8% ash, 1% calcium, 0.6% phosphorous, 3.4% glucose, 2% vitamin and 55% nitrogen free extract (carbohydrate) and it provides metabolisable energy of 3600 kcal/kg.

Animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee, Annamalai University (Reg No: 466/160/1999/CPCSEA) and experiments were performed in accordance with the "Guide for the care and use of laboratory animals" (NIH, 1985) and "Committee for the purpose of control and supervision on experimental animals" (CPCSEA).

Study design

Animals were randomized into six groups of six animals each. The mode of administration to all the groups was given through gastric intubation. Group I control rats received 0.2 ml of gum accacia, group II rats received 20% ethanol (3.95 g/kg b.wt twice a day i.e 7.9 g/kg/day) (Rajakrishnan et al. 1997), group III rats received 20% ethanol along with SNFEt (250 mg/kg b.wt) on a daily basis for 30 days (Raju et al. 2003), group IV rats received 20% ethanol along with the standard drug silymarin (25 mg/kg b.wt), group V rats received SNFEt and group VI rats received silymarin alone.At the end of the experimental period (30 days), all the rats were kept overnight fast and anesthetized using ketamine chloride (24 mg/kg body weight) by intramuscular injection and sacrificed by cervical decapitation between 8.00 am to 10.00 am. Blood was collected in clean dry test tubes with few drops of heparin and plasma obtained was used for various biochemical estimations. Tissues such as liver and kidney were removed, cleared off blood and immediately transferred to ice-cold containers containing 0.9% Nacl. Tissues were homogenized in 5ml of the buffer and used for the estimation of various biochemical parameters.

Biochemical Estimations

The activity of both serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by using the diagnostic kit based on the method of Reitman's and Frankel 1997. Serum alkaline phosphatase (ALP) was estimated using kind and king 1954. The serum gamma glutamyl transferase (GGT) was assayed according to the method Rosalki and Rau 1972. Serum bilirubin was estimated by the method of Malloy and Evelyn 1937. Estimation of renal functional markers was determined by urea, uric acid and creatinine. Serum urea was estimated according to the method of Fawcett and Scott 1960, Serum uric acid was estimated based on the enzymic method described by caraway 1955 and Serum creatinine was determined by Jaffe 1886 color reaction. Antioxidants such as Superoxide dismutase (SOD) in tissues was assayed by the method of Kakkar et al. 1984, The activity of Catalase (CAT) in tissues was determined by the method of Sinha 1972, The activity of Glutathione peroxidase (GPx) in tissues was measured by the method of Rotruck et al 1973. Reduced glutathione in tissues was estimated by the method of Ellman 1959.

Statistical analysis

All quantitative measurements were expressed as means ± SD for control and experimental animals. The data were analyzed using one way analysis of variance (ANOVA) on SPSS/PC (statistical package for social sciences, personal computer) and the group means were compared by Duncan's Multiple Range Test (DMRT). The results were considered statistically significant if the p value were less than 0.05.

Results

The body and organ weights of control and experimental rats were shown in Table 1. The body weight was found to be significantly reduced in ethanol treated rats whereas the liver-body weight ratio was found to be increased in animals fed with ethanol as compared to control. Supplementation of SNFEt (250 mg/kg b.wt) and silymarin (25 mg/kg b.wt) reversed the weight loss during the experimental period.

The activities of hepatic marker enzymes in control and experimental groups were shown in Table 2. Hepatic markers such as AST, ALT, ALP, GGT and serum bilirubin were significantly

Table 1: Effect of SNFEt on body weight and liver weight to body weight ratio of control and ethanol administered rats

Groups	Body weight (g)			(Liver wt / body wt) × 100
	Initial (0 day)	Final (30 day)	Net gain (g)	
Control	152.78 ± 4.31	176.50 ± 8.41[a]	23.72 ± 4.10[a]	1.98 ± 0.62[a]
Ethanol	160.83 ± 7.02	167.00 ± 7.49[b]	6.17 ± 0.47[b]	3.94 ± 1.78[b]
Ethanol+SNFEt (250mg/kg b.wt)	157.11 ± 6.26	171.00 ± 5.04[c]	13.89 ± 1.22[c]	2.28 ± 1.05[c]
Ethanol+Silymarin (25mg/kg b.wt)	156.21 ± 3.84	175.00 ± 5.66[c]	18.79 ± 1.82[d]	2.17± 0.87[c]
Control+SNFEt	153.63 ± 5.77	173.60 ± 7.54[ac]	19.97 ± 1.77[d]	2.07 ± 0.68[a]
Control+Silymarin	155.00 ± 3.51	175.00 ± 7.96[ac]	20.00 ± 4.45[d]	2.00 ± 0.53[a]

Values are expressed as mean ± SD, n=6.
Values not sharing a common superscript ([a,b,c,d]) differ significantly at p<0.05 (DMRT).

Table 2: Effect of SNFEt on hepatic marker enzymes and bilirubin in serum of control and experimental animals

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)	Bilirubin (mg/dl)
Control	74.87 ± 6.56[a]	23.94 ± 1.59[a]	86.19 ± 5.88[a]	2.40 ± 0.21[a]	0.59 ± 0.06[a]
Ethanol	146.98 ± 10.47[b]	68.15 ± 4.61[b]	167.40 ± 14.63[b]	7.87 ± 1.48[b]	1.72 ± 0.12[b]
Ethanol+SNFEt (250mg/kg b.wt)	108.83 ± 8.43[c]	31.62 ± 3.14[c]	121.02 ± 7.64[c]	3.62 ± 0.48[c]	1.03 ± 0.06[c]
Ethanol+Silymarin (25mg/kg b.wt)	90.46 ± 7.83[d]	25.06 ± 2.11[a]	114.08 ± 9.23[c]	2.91 ± 0.27[ac]	0.85 ± 0.08[d]
Control+SNFEt	76.07 ± 6.02[a]	20.66 ± 1.15[d]	88.62 ± 3.95[a]	2.34 ± 0.19[a]	0.58 ± 0.04[a]
Control+Silymarin	75.63 ± 5.33[a]	23.55 ± 1.28[ad]	86.73 ± 6.90[a]	2.80 ± 0.25[ac]	0.60 ± 0.05[a]

Values are expressed as mean ± SD, n=6.
Values not sharing a common superscript ([a,b,c,d]) differ significantly at p<0.05 (DMRT).

elevated in ethanol administered rats. Co-administration of SNFEt significantly reduced the above-mentioned markers to near normal levels. This effect was comparable to that observed with the standard drug silymarin. Moreover, there were no significant changes observed in control and treated groups.

Table 3 shows the effect of SNFEt on renal functional markers. The levels of urea, uric acid and creatinine were significantly higher in ethanol-induced rats whereas treatment with SNFEt and silymarin significantly decreased the levels to near normal values. No significant changes were observed between control and treated groups.

Table 3: Effect of SNFEt on renal function markers in the serum of control and ethanol treated rats

Groups	Urea (mg/dL)	Uricacid (mg/dL)	Creatinine (mg/dL)
Control	25.38 ± 1.69[ad]	1.30 ± 0.06[a]	0.86 ± 0.06[a]
Ethanol	45.26 ± 2.96[b]	2.63 ± 0.35[b]	1.88 ± 0.07[b]
Ethanol+SNFEt (250mg/kg b.wt)	30.51 ± 2.13[c]	2.01 ± 0.16[c]	0.99 ± 0.08[c]
Ethanol+Silymarin (25mg/kg b.wt)	27.86 ± 2.01[d]	1.93 ± 0.13[c]	0.89 ± 0.06[d]
Control+SNFEt	23.65 ± 1.19[a]	1.39 ± 0.08[a]	0.80 ± 0.01[d]
Control+Silymarin	25.74 ± 1.72[ad]	1.35 ± 0.07[a]	0.67 ± 0.03[e]

Values are expressed as mean ± SD, n=6.
Values not sharing a common superscript ([a,b,c,d]) differ significantly at p<0.05 (DMRT).

Finally, SOD, CAT, GPx and GSH were measured as an index of tissue antioxidant status (Tables 4 and 5). The activity of these antioxidants was remarkably declined in rats administered ethanol when compared to the other experimental groups. In response to SNFEt and silymarin treatment, the activities were brought back to near normal levels whereas in control and treated groups, no significant changes were indicated.

Discussion

Throughout our study, it was apparent that chronic ethanol administration produced toxicity in rats as examined by weight loss and increased organ weight. Excessive alcohol ingestion disturbs the metabolism of most nutrients in the diet resulting in malnutrition (Leiber 2000). This may be one of the reasons for decreased bodyweight in ethanol- administered rats. A weight gain was observed after supplementation with SNFEt characteristic of its protective effect against ethanol. Moreover, the ratio between the liver weight and total body weight showed significant decrease in ethanol-fed rats treated with SNFEt as compared with those of control rats. This protective effect may be due to the presence of nutrients and polyphenols present in *Solanum nigrum* fruits (Rastogi and Mahrota 1998) which might have detoxified the liver and improved the body weight to near normal levels.

Generally, hepatic cells take part in a variety of metabolic actions and restrain a host of enzymes. During liver injury, transport function of the hepatocytes is disturbed which leads to leakage of plasma membrane, thereby causing an increased enzyme level in serum (Jadon et al. 2007). In our study, we observed a significant increase in the activities of liver marker enzymes such as AST,

Table 4: Effect of SNFEt on the activities of SOD, CAT, GPx and GSH in the liver of control and experimental rats

Groups	Liver			
	SOD (U*/mg protein)	CAT (U#/mg protein)	GPx (U$/mg protein)	GSH (mg/100 g wet tissue)
Control	7.48 ± 0.60[a]	74.12 ± 4.15[a]	11.17 ± 2.08[a]	136.57±9.86[a]
Ethanol	4.12 ± 0.40[b]	46.32 ± 3.11[b]	5.23 ± 0.16[b]	78.21±6.50[b]
Ethanol+SNFEt (250mg/kg b.wt)	6.20 ± 0.45[c]	66.71 ± 4.01[c]	9.09 ± 0.59[c]	95.31±7.92[c]
Ethanol+Silymarin(25mg/kg b.wt)	6.43 ± 0.50[c]	67.92 ± 5.60[cd]	10.38 ± 1.08[ac]	108.87±8.94[d]
Control+SNFEt	7.91 ± 0.66[a]	73.46 ± 5.11[ad]	10.88 ± 1.29[a]	131.42±9.44[a]
Control+Silymarin	7.26 ± 0.59[a]	71.58 ± 4.57[acd]	11.57 ± 1.69[a]	134.26±9.67[a]

U* = enzyme concentration required to inhibit the chromogen produced by 50% in one minute under standard condition.
U# = µmole of H_2O_2 consumed/minute. U$ = µg of GSH utilized/minute. Values are given as means ± SD for six rats in each group. Values not sharing a common superscript ([a,b,c,d]) differ significantly at p < 0.05 (DMRT).

Table 5: Effect of SNFEt on the activities of SOD, CAT, GPx and GSH in the kidney of control and ethanol administered rats

Groups	Kidney			
	SOD (U*/mg protein)	CAT ($U^{\#}$/mg protein)	GPx ($U^{\$}$/mg protein)	GSH (mg/100 g wet tissue)
Control	11.30 ± 1.36^{a}	39.82 ± 2.88^{ad}	8.50 ± 0.50^{ae}	113.52 ± 9.14^{a}
Ethanol	7.51 ± 0.74^{b}	23.93 ± 2.03^{b}	4.77 ± 0.21^{b}	63.87 ± 5.57^{b}
Ethanol+SNFEt (250mg/kg b.wt)	9.77 ± 0.94^{c}	34.56 ± 2.28^{c}	6.43 ± 0.29^{c}	96.41 ± 7.81^{c}
Ethanol+Silymarin(25mg/kg b.wt)	10.02 ± 0.96^{ac}	37.41 ± 2.42^{cd}	7.05 ± 0.38^{d}	103.62 ± 8.87^{ac}
Control+SNFEt	11.21 ± 1.25^{a}	42.29 ± 3.25^{a}	9.01 ± 0.68^{a}	113.21 ± 9.05^{a}
Control+Silymarin	11.42 ± 1.45^{a}	39.98 ± 3.16^{ad}	8.14 ± 0.47^{e}	109.43 ± 8.99^{a}

U* = enzyme concentration required to inhibit the chromogen produced by 50% in one minute under standard condition.
$U^{\#}$ = μmole of H_2O_2 consumed/minute. $U^{\$}$ = μg of GSH utilized/minute. Values are given as means ± SD, n=6.
Values not sharing a common superscript (^{a,b,c,d}) differ significantly at $p < 0.05$ (DMRT).

ALT, ALP, GGT and bilirubin on ethanol intoxicated rats, which indicates increased permeability, damage and/or necrosis of hepatocytes (Sathiavelu et al. 2009). Treatment with SNFEt significantly alleviates the increased activities of liver marker enzymes to near normal levels, which may be a consequence of stabilization of plasma membrane and maintaining the functional status of the liver from ethanol toxicity. Reports also show that *Solanum nigrum* extract has the ability to maintain the structural integrity of hepatocytic cell membrane or involved in the regeneration of damaged liver cells (Lin et al. 2008, Hsieh et al. 2008). Our result was also compared with the standard drug silymarin, which is widely used for over 20 years in clinical practice for the treatment of various toxic liver diseases (Messener and Brissot 1990). Thus, from the above findings it is evident that SNFEt has a remarkable hepatoprotective effect against liver damage.

Alcohol abuse may result in a range of serious disorders in different organs, including liver and kidney. Kidney is an important organ actively involved in maintaining homeostasis of the body by reabsorbing important material and excreting waste products. It has been reported that habitual consumption of large amount of alcohol was associated with an increased risk of kidney failure in the general populations (Parekh and Klag 2001). Kidney functional markers such as urea, uric acid and creatinine are the main indicators of renal dysfunction. In our result ethanol administrated rats showed significant increase in the levels of urea, uric acid and creatinine. This increase is mainly caused by increased production of ROS and acetaldehyde which are the mediators of tissue damage and finally leads to altered kidney function and renal failure (Freund and Ballinger 1988). In contrast to ethanol fed rats, we found that renal markers were brought back to normal on treatment with SNFEt. Thus it is inferred that SNFEt preserves the functional capacity of the kidney against ethanol toxicity.

Antioxidants represent the cellular defense against free radicals. Enzymatic antioxidants such as SOD, CAT and GPx are the initial line of defense against oxidative injury. In the present study, we observed a significant decrease in SOD activity in the erythrocytes and tissues of ethanol-treated rats. This decrease could be due to inefficient scavenging of ROS which might be implicated to oxidative inactivation of enzymes (Jayaraman et al. 2009). CAT acts as a preventive antioxidant and plays an important role in protection against the deleterious effects of reactive oxygen species (ROS). Recently, reports have shown that significant decrease in the activity of catalase during ethanol ingestion indicates inefficient scavenging of H_2O_2 (Mallikarjuna et al. 2008). Our results were found to be in parallel with the above observations in ethanol-fed groups. GPx has a role in defending cells against oxidative stress and this in turn involves GSH as a cofactor. GPx catalyses the oxidation of GSH to GSSG at the expense of H_2O_2 (Cerutti et al. 1994). Decreased GPx activity was observed in the alcoholic group. This decreased activity may be implicated to either free radical dependent inactivation of enzyme or depletion of its co-substrate (i.e.GSH) or NADPH on ethanol treatment (Chandra et al. 2000).

GSH an important non-enzymatic antioxidant tripeptide which is thought to be an important endogenous defense molecule against peroxidative destruction of cellular membranes. It reacts directly with ROS and electrophilic metabolites, protects essential thiol groups from oxidation and serves as a substrate for several enzymes including GPx. In our study, the concentration of GSH was significantly reduced in alcoholic-treated rats which were in support with other reports (Frenandez et al. 1997). The reduced levels may be due to ROS being generated during alcohol metabolism that lead to GSH oxidation and lipid peroxidation.

Co-administration of SNFEt with ethanol significantly altered the activities of both the enzymatic and non-enzymatic antioxidants to near normal levels, which proves to be a potent antioxidant, a finding that correlates with recent reports (Lin et al 2008, Hsieh et al 2008). Furthermore, the antioxidant property of the extract may therefore be due to the presence of high content of polyphenolic compounds such as flavonoids and steroids, vitamin C and β-carotene. In addition, saponins (nigrumin I & II) have also been identified as the active ingredient of *Solanum nigrum* for hepatoprotective activity (Rastogi and Mahrota 1988).

Conclusion

The findings of our study suggest that *Solanum nigrum* fruit extract detoxifies the toxic effects of acetaldehyde and that it is also involved in regeneration processes. This protective effect is mainly due to its free radical scavenging activity which has been suggested as a possible mechanism of action of SNFEt against cellular toxicity. Furthermore, the high content of polyphenols, steroids and saponins present in SNFEt efficiently retards the liver injury by blocking oxidative stress and enhancing antioxidant status and thus preventing from organ damage and necrosis. Therefore, dietary supplementation of *Solanum nigrum* fruits offers protection against liver and kidney toxicity. Further studies are warranted to elucidate the mechanisms of action and indentifying the active compounds from *Solanum nigrum* fruits against cellular and organ toxicity.

References

Albano E (2006) Alcohol, oxidative stress and free radical damage. Proc Nutr Soc 65:278-290

Arulmozhi V, Krishnaveni M, Karthishwaran K et al (2010) Antioxidant and antihyperlipidemic effect of *Solanum nigrum* fruit extract on the experimental model against chronic ethanol toxicity 6:42-50

Batey RG, Burns T, Benson RJ et al (1992) Alcohol consumption and the risk of cirrhoisis. Med J Aust 156:413-416

Caraway WT (1955) Determination of uric acid in serum by carbonate method. Am J Clin Path 25:840-845

Cerutti P, Ghosh R, Oya Y et al (1994) The role of cellular antioxidants defence in oxidant carcinogenesis. Environ. Health Perspect 102:123-29

Chandra R, Aneja R, Rewal C et al (2000). An opium alkaloid papaverine ameliorates ethanol induced hepatotoxicity: diminution of oxidative stress. Ind J Clin Biochem 155:160

Day CP (2006) Genes or environment to determine alcoholic liver disease and non alcoholic fatty liver disease. Liver Int 26:1021-1028

Dey A, Cederbaum AI (2006) Alcohol and oxidative liver injury. Hepatol 43:63-74

Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82:70-77

Fawcett JK and Scott JE (1960) A rapid and precise method for the determination of urea. J Clin Path 3:156-159

Frenandez-Checa JC, Kaplowitz N, Garcia Ruiz C et al (1997) GSH transport in mitochondria: defense against TNF-induced oxidative stress and alcohol induced defect. Am J Physiol 273:7-17

Freund G and Ballinger WE (1988) Decrease of benzodiazepine receptors in frontal cortex of alcoholics. Alcohol 5: 275-282

Hossain MZ, Shibib BH, Rahman R (1992) Hypoglycemic effects of *Coccina indica* inhibition of key glycogenic enzyme, glucose-6-phosphatase. Ind J Exp Biol 30:418-420

Hsieh CC, Fang HL, Lina W C (2008) Inhibitory effect of *Solanum nigrum* on thioacetamide-induced liver fibrosis in mice. J Ethanopharmacol 119:117-121

Jadon A, Bhadauria M, Shukla S (2007) Protective effect of *Terminalia belerica* Roxb. and gallic acid against CCL_4 induced damage in albino rats. J Ethnopharmacol 109:214-218

Jaffe M (1886) Concerning the precipitate produced in normal urine by picric acid and a new reaction of creatinine. Physiol Chem 10:91-400

Jayaraman J, Muniappan V, Nalini N (2009) Potential beneficial effect of naringenin on lipid peroxidation and antioxidant status in rats with ethanol induced hepatotoxicity. J Pharm Pharmacol 61:1383-1390

Kakkar P, Das B, Viswanathan P N (1984) A modified spectrophotometric assay of superoxide dismutase (SOD). Ind J Biochem Biophys 21:130-32

Kind PRN and King EJ (1954) Estimation of plasma phosphatases by determination of hydrolysed phenol with amino-antipyrine. J Clin Path 7:330-332

Lathu U, Ramesh MG, Latha MS (1999) Hepatoprotective effect of an ayurvedic medicine. Indian Drug 36:470-473

Leiber CS (1994) Alcohol and the liver. Gastroenterol 106:1085-1105

Leiber CS (2000) Alcohol and the liver: metabolism of alcohol and its role in hepatic and extrahepatic disease. Mount Sinai J Med 64:84-94

Lin HM, Tseng HC, Wang CJ et al (2008) Hepatoprotective effect of *Solanum nigrum* Linn extract against CCl_4 induced oxidative damage in rats. Chem Biol Int 171:283-293

Mallikarjuna K, Sahitya P, Sathyavelu K et al (2008) Ethanol toxicity: Rehabilitation of hepatic antioxidant defense system with dietary ginger. Fitoterapia 79:174-178

Malloy HT and Evelyn KA (1973) The determination of bilirubin with the photoelectric colorimeter. J Biol Chem 119:481-490

Mendenhall C, Roselle GA, Garside P et al (1995) Relationship of protein calorie malnutrition to alcoholic liver disease: A re-examination of data from two veteran's administration co-operative studies. Alcoholism: Clinical and experimental research 19:635-641

Messner M and Brissot P (1990) Traditional management of liver disorders. Drugs 40:45-57

Ong HC (2003) Sayuran Khasiat Makanan Dan Ubatan, Utusan Publication Distributors. Snd Bhd. Fakulti Sains: Universiti Malaya 92-93

Parekh RS and Klag MJ (2001) Alcohol: role in the development of hypertension and end-stage renal disease. Curr Opin Nephrol Hypertens 10:385-390

Potawele SE, Sinha SD, Shroff KK et al (2008) *Solanum nigrum* Linn: A phytochemical review. Pharmacol Online 3:140-163

Rajakrishnan V, Vishvanathan P, Menon VP (1997) Hepatotoxic effect of alcohol on female rats and sibilings: Effect of N-acetylcysteine. Histology Res 9:37-50

Raju K, Anbuganapathy G, Gokulakrishnan V et al (2003) Effects of dried fruits of *Solanum nigrum* Linn against CCl_4 induced hepatic damage in rats. Biol pharm Bull 26:1618-1619

Rastogi RP and Mahrota BN (1988) Compendium of medicinal plants. 5th edn. Publication and information directorate, Newdelhi

Reitman S and Frankel S (1957) A caloriemetric method for the determination of serum glutamate oxaloacetic and glutamate pyruvic transaminases. Am J Clin Pathol 28:56-63

Rosalki SB and Rau D (1972) Serum gamma-glutamyl transpeptidase activity in alcoholism. Clin Chim Acta 39:41-47

Rotruck JT, Pop AL, Ganther HF et al (1973) Selenium: biochemical role as a component of glutathione peroxidase. Science 179:588-590

Sandhir R and Gill KD (1999) Hepatoprotective effects of liv-52 on ethanol induced liver damage in rats. Ind J Exp Biol 37:762-766

Sathiavelu J, Senapathy GJ, Devaraj R et al (2009) Hepatoprotective effect of chrysin on prooxidant-antioxidant status during ethanol induced toxicity in female albino rats. J Phar Pharmacol 61:809-817

Sinha KA (1972) Colorimetric assay of catalase. Anal Biochem 47:389-94

Sultana S, Parvez M, Iqbal M et al (1995) Crude extracts of hepatoprotectve plants, *Solanum nigrum* and *Cichorium intybus* inhibit free radical-mediated DNA damage. J Ethnopharmacol 45:189-192