CHAPTER 3

EFFECT OF *PELARGONIUM RENIFORME* ROOTS ON ALCOHOL INDUCED LIVER DAMAGE AND OXIDATIVE STRESS

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Effect of *Pelargonium reniforme* Roots on Alcohol-induced Liver Damage and Oxidative Stress

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Abstract

Alcoholic liver disease is a major medical complication of drinking alcohol. Oxidative stress plays an important role in the development of the disease. The present study was carried out to evaluate the *in vitro* antioxidant properties and effect of aqueous root extract of *P. reniforme* on alcohol-induced hepatotoxicity. *In vitro* studies were carried out by determining the level of phenolic compounds (phenols, flavonols, flavonoids and proanthocyanidins), the ability of the plant extract to scavenge DPPH* and ABTS*$^{+}$ (cation radical) and its reducing power. The results showed that the plant has a significant level of phenolic compounds, the highest being the level of total phenols estimated to be 300.9 mg tannic acid/g of dry plant material. The reducing power and ability to scavenge DPPH* and ABTS*$^{+}$ radicals were found to be concentration dependent and were comparable with the reference drugs. *In vivo* administration of ethanol (5 g/kg/day) for 3 weeks resulted in liver injury. It negatively affected the serum total protein, liver marker enzymes (ALT, AST, ALP and GGT), and total bilirubin levels. Administration of the plant extract (50, 100 and 200 mg/kg), 2 h before ethanol treatment caused a significant improvement in serum protein levels and a significant decrease in the levels of the liver marker enzymes and total bilirubin. Extract administration 2 h after ethanol also gave similar results showing that the plant can enhance recovery from tissue damage. The activity of *P. reniforme* root extract compares well with silymarin, a

known hepatoprotective drug. The effect of *P. reniforme* is probably related to its antioxidant activities.

Keywords: alcohol, oxidative stress, liver damage, *Pelargonium reniforme*

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Introduction

Alcohol toxicity is one of the world's major health problems, many people are affected due to several fatal diseases caused by alcohol (Singha et al., 2007). The liver is one of the major alcoholic target organs known to be severely damaged due to chronic alcohol intake (Kundu et al., 2008). It has been observed that almost all ingested alcohol is metabolized in the liver and excessive alcohol use can lead to acute and chronic liver disease. Also, most of the consumed alcohol is eventually broken down by the liver and the products generated and accumulated during alcohol metabolism (e.g. acetaldehyde) are more toxic than alcohol itself (Kurose et al., 1996). Alcohol abuse can elicit disturbances in the delicate balance between the pro- and antioxidant system of the organism, therefore leading to oxidative stress. Increased generation of oxygen- and ethanol-derived free radicals has been observed at the microsomal level (particularly at the ethanol-inducible cytochrome *P*450 isoform), the cytosolic xanthine and aldehyde oxidase, as well as through the mitochondrial respiratory chain (Nordmann et al., 1992). Polyunsaturated fatty acids are probably the most susceptible target to free radical attack which occurs due to alcohol abuse. The reaction of free radicals with the membrane lipid components leads to lipid peroxidation. This process can eventually cause increased membrane permeability and cell death (Rakonczay et al., 2003). To counteract these oxidants, cells have several enzymatic antioxidants and nonenzymatic antioxidants, but their levels are altered in alcoholics (Saravanan & Nalini, 2007). This results in covalent modification of cellular macromolecules, morphological changes leading to tissue damage and aberrant biochemistry of liver (Lieber, 1991; McCuskey, 1991).

Although important progress has been made in understanding the pathogenesis of alcoholic liver disease, treatment strategies such as lifestyle changes, pharmacological therapy and nutrition therapy have been employed but these therapies for this disease are not very

effective (Saravanan et al., 2006), and liver transplantation is expensive and often beyond the reach of the common man (Faremi et al., 2008) A phytotherapeutic approach to modern drug development can provide many invaluable drugs from traditional medicinal plants (Saravanan et al., 2006).

Pelargonium reniforme Curtis (Geraniaceae) is a shrublet of up to 1 m height with kidney-shaped leaves and pink flowers. It is indigenous to the Eastern Cape Province in South Africa and it occurs mainly in coastal regions. It is widely used by traditional healers in areas of southern Africa for the treatment of diarrhea, dysentery, fever, respiratory tract infections, liver complaints and wounds (Watt & Breyer-Brandwijk, 1962). Information obtained from traditional healers and rural dwellers during ethnobotanical surveys carried out in the Eastern Cape Province of South Africa showed that the aqueous root extracts are commonly used in the treatment of alcohol-induced liver disorders. Infusions and decoctions of the tubers are commonly taken, while a traditional method of using the roots is to boil the tuber in milk. Also, the roots may be directly chewed or powdered and mixed with food (Latté & Kolodziej, 2004). Although the root of this plant is used in the treatment of alcohol-induced liver disorders among several ethnic groups in areas of southern Africa, there is paucity of scientific evidence regarding its usage in liver disorders. Hence, the present study was undertaken to evaluate the *in vitro* antioxidant activity as well as the protective and curative effects of *P.reniforme* root extracts against alcohol-induced damage of rat liver.

Materials and methods

Collection and identification of plant material

The plant samples were collected in December 2008 from a natural population of *P. reniforme* from Grahamstown in the Eastern Cape Province of South Africa. The plant was

identified by Prof. D.S. Grierson of the Department of Botany, University of Fort Hare, and a voucher specimen (GER 3928) was deposited at the Giffen Herbarium of the University.

Animals

Adult rats of Wistar strain (158.6 ± 33.18 g) were used for the study. They were obtained from the animal house of the Agricultural and Rural Development Research Institute, University of Fort Hare. They were kept in rat cages and fed on commercial rat pellets (EPOL Feeds, South Africa Ltd.) and allowed free access to fresh water *ad libitum*. The project was approved by the Ethics Committee at the University of Fort Hare.

Chemicals

The assay kits for albumin, bilirubin, total protein, alkaline phosphatase, gamma glutamyl transferase, alanine and aspartate aminotransferases were obtained from Roche Diagnostic GmbH, Mannhein, Germany. Ethanol was purchased from E. Merck, Darmstadt, Germany. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium ferricyanide, catechin, butylated hydroxytoluene (BHT), ascorbic acid, catechin, tannic acid, quercetin and $FeCl_3$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA), vanillin from BDH chemicals Ltd. (Poole, England), Folin-Ciocalteus's phenol reagent and sodium carbonate were from Merck Chemical supplies (Damstadt, Germany). All other reagents used were of analytical grade and were supplied by Merck Chemicals (Pty) Ltd., Bellville, South Africa.

Preparation of aqueous extract

The roots of *P. reniforme* were air-dried at room temperature for seven days. The dried material was then comminuted into coarse powder using the Waring commercial laboratory blender. The powder (100 g) was extracted in 1000 mL of distilled water for 48 h on an

orbital shaker (Stuart Scientific Orbital Shaker, UK). The extract was filtered using a Buchner funnel and Whatman no. 1 filter paper. The resulting filtrate was freeze-dried (Savant Refrigerated Vapour Trap, RV T41404, USA) to give a yield of 6.15 g. This was reconstituted separately in distilled water to give the required doses used in this study.

In vitro assays

Determination of total phenolics

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Wolfe et al., 2003). The extract (1 mg/mL) was mixed with 5 mL Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 mL (75 g/L) of sodium carbonate. The mixture was vortexed for 15 sec and allowed to stand for 30 min at 40^{o}C for color development. Absorbance was then measured at 765 nm using the Hewlett Packard UV-VIS spectrophotometer. Samples of extract were evaluated at a final concentration of 1 mg/mL. Total phenolic content was expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve: $y = 0.1216x$, $R^2 = 0.9365$, where x was the absorbance and y was the tannic acid equivalent (mg/g).

Determination of total flavonols

Total flavonol was estimated using the method of Kumaran and Karunakaran (2007). To 2 mL of sample (standard), 2 mL of 2% $AlCl_3$ ethanol and 3 mL (50 g/L) sodium acetate solutions were added. The absorbance at 440 nm was read after 2.5 h at 20^{o}C. Extract samples were evaluated at a final concentration of 1 mg/mL. Total flavonoid content was calculated as quercetin (mg/g) using the following equation based on the calibration curve: $y = 0.0255x$, $R^2 = 0.9812$, where x was the absorbance and y was the quercetin equivalent (mg/g).

Determination of total proanthocyanidins

The procedure reported by Sun et al. (1998) was used to determine the total proanthocyanidin. A volume of 0.5 mL of 0.1 mg/mL extract solution was mixed with 3 mL of 4% vanillin-methanol solution and 1.5 mL hydrochloric acid; the mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm. Extract was evaluated at a final concentration of 0.1 mg/mL. Total proanthocyanidin content were expressed as catechin equivalents (mg/g) using the following equation based on the calibration curve: $y = 0.5825x$, $R^2 = 0.9277$, where x was the absorbance and y is the catechin equivalent (mg/g).

Determination of total flavonoids

Total flavonoid contents were determined using the method of Ordonez et al. (2006). A volume of 0.5 mL of 2% $AlCl_3$ ethanol solution was added to 0.5 mL of sample solution. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 1 mg/mL. Total flavonoid content was calculated as quercetin (mg/g), using the following equation based on the calibration curve: $y = 0.025x$, $R^2 = 0.9812$, where x was the absorbance and was the quercetin equivalent (mg/g).

ABTS radical scavenging assay

The method of Re et al. (1999) was adopted for the ABTS radical scavenging assay. The stock solution which was allowed to stand in the dark for 16 h at room temperature contained equal volumes of 7 mM ABTS salt and 2.4 mM potassium persulfate. The resultant $ABTS^{*+}$ solution was diluted with methanol until an absorbance of about 0.70 ± 0.01 at 734 nm was reached. Varying concentrations of the plant extracts (1 mL) was reacted with 1 mL of the $ABTS^{*+}$ solution and the absorbance taken at 734 nm between 3-7 min using the

spectrophotometer. The $ABTS^{*+}$ scavenging capacity of the extract was compared with that of BHT and rutin and the percentage inhibition calculated as:

ABTS radical scavenging activity (%) = $[(Abs_{control} - Abs_{sample})]/ (Abs_{control})] \times 100$ where $Abs_{control}$ was the absorbance of ABTS radical + methanol; Abs_{sample} was the absorbance of ABTS radical + sample extract /standard.

DPPH radical scavenging assay

The effect of extracts on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi (2005). A solution of DPPH (0.135 mM) in methanol was prepared and 1 mL of this solution was mixed with 1 mL of varying concentrations of the methanol extract. The reaction mixture was vortex thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm using rutin and BHT as references. The ability to scavenge DPPH radical was calculated as:

(%) DPPH radical scavenging activity = $[(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$ where $Abs_{control}$ was the absorbance of DPPH radical + methanol; Abs_{sample} was the absorbance of DPPH radical + sample extract /standard

Determination of ferric reducing power

The ferric reducing potential of the extract was assayed as described by Duh et al. (1999). The different concentrations of the extract and the standards, rutin and BHT (0.025 - 0.5 mg/mL; 1 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1 % w/v). The mixture was incubated at 50°C for 20 min. 2.5 mL of TCA (10% w/v) was added to the mixture, which was then centrifuged for 10 min at 1000 rpm. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL distilled water

and 0.5 mL 0.1% w/v $FeCl_3$. The absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power. BHT and Vitamin C were used as standards.

Alcohol-induced hepatotoxicity studies

The animals were randomly divided into 10 groups, each comprising six and were orally administered as follows:

- Group 1 (alcohol control) received 0.5 mL of 20% ethanol (5 g/kg body weight) (Faremi et al., 2008).
- Group 2 (normal control) received 0.5 mL of distilled water.
- Groups 3, 4 and 5 received aqueous extract of *P. reniforme* at 50, 100 and 200 mg/kg respectively 2 h before administration of 20% ethanol.
- Groups 6, 7 and 8 received aqueous extract of *P. reniforme* at 50, 100 and 200 mg/kg respectively 2 h after administration of 20% ethanol.
- Group 9 (positive control) received silymarin, the known hepatoprotective compound (Sigma Chemical Company, USA) at 25 mg/kg, 2 h before administration of 20% ethanol
- Group 10 (positive control) received silymarin (25 mg/kg) 2 h after administration of 20% ethanol.

The administration was done repeatedly on a daily basis for three weeks using metal oropharyngeal cannula. All rats from each group were sacrificed 24 h after their respective 21 daily doses. Blood samples were collected for evaluating the biochemical parameters. The

study was carried out following the approval from the Ethical Committee on Animal Use and Care of the University of Fort Hare, South Africa.

Biochemical estimations

The activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel (1957). Serum bilirubin level was estimated by the method of Malloy and Evelyn (1937). Serum total protein, albumin and globulin levels were estimated by Biuret method (Reinhold, 1953). Gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) activities were determined by the method of Rosalki and Rau (1972).

Statistical analysis

The data for the various biochemical parameters were analyzed by one way analysis of variance (ANOVA) followed by Student's *t*-test using SAS. *P*-values <0.05 were considered statistically significant. The experimental results for the *in vitro* assays were expressed as mean ± standard deviation of three replicates.

Results

The aqueous extract of *P. reniforme* root was characterized by the presence of phenolic compounds. The levels of these compounds are shown in Table 1. The plant extract has a high level of total phenols (300.9 ± 0.1 mg tannic acid/g extract) and a low level of total flavonols (12.13 ± 0.01 mg quercetin/g extract). The plant extract also exhibited a significant inhibition of DPPH* and $ABTS^{*+}$ radicals in a dose dependent manner (figures 1 and 2). Significant DPPH* and $ABTS^{*+}$ radical scavenging activity was evident at all the tested concentrations of the extract and compared well with the standards - butylated

hydroxytoluene (BHT) and rutin. As illustrated in figure 3, Fe^{3+} was transformed to Fe^{2+} in the presence of the plant extract and the reference compounds, BHT and Vitamin C, to measure the reductive capability. The results indicated that the reductive capability of the plant extract was dose dependent. At 0.5 mg/ml, the absorbance was 1.48 while those of BHT and Vitamin C were 1.97 and 2.6 respectively.

Table 2 shows the effect of pre-treatment and post-treatment of *P. reniforme* extract on alcohol induced liver damage based on the activities of some liver marker enzymes (ALT, AST, ALP and GGT), and levels of total, conjugated and unconjugated bilirubin. Animals in group 1 (alcohol only) developed hepatic damage when compared with animals in group 2 (distilled water only). This was evidenced by a significant elevation in the levels of the hepatic enzyme markers studied. Pre-treatment of the animals with three different doses of the plant extract before alcohol administration (groups 3, 4 and 5) ensured protection of the liver as shown by the significant decrease in the levels of all the hepatic enzyme markers studied. The effects of post treatment of *P. reniforme* extract after alcohol administration (groups 6, 7 and 8) showed that the plant can enhance recovery from tissue damage as shown by the significant decrease in levels of the liver marker enzymes under consideration. The levels of total and conjugated bilirubin are also good indices for hepatotoxicity. The levels of total and unconjugated bilirubin increased significantly in group 1 in comparison with group 2 which is an evidence of hepatic damage while it reduced significantly in both the pre and post treated groups. Also, the levels of conjugated bilirubin reduced significantly in group 1, while a significant increase was observed in the treated groups. The effects of pre-treatment and post-treatment with silymarin, a known hepatoprotective compound (groups 9 and 10), on all parameters are also shown in Table 2. The activities of the liver marker enzymes and the levels of total and unconjugated bilirubin were reduced significantly while a significant

increase was observed in the level of conjugated bilirubin, in all groups treated with silymarin and this compares well with the results obtained for the plant extracts.

The levels of serum total protein decreased significantly in ethanol alone-fed rats compared with those in control animals (Table 3). There was an improvement in the levels of these proteins in rats pre-treated and post-treated with the plant extract (groups 3-8). However, no significant changes were observed in the levels of albumin and globulin, though their levels increased slightly in the normal control and treated groups in comparison with the alcohol control group. The results obtained from the treated animals also compares well with that obtained from animals treated with silymarin (groups 9 and 10), as a significant increase in the levels of the serum total proteins was also observed.

Discussion

There is increasing evidence that oxidative stress plays a vital role in the pathogenesis of alcohol liver disease (Lindros, 1995; Zima et al., 2001). Alcohol-induced hepatic tissue damage is mediated by acetaldehyde and reactive oxygen species (Zima et al., 2001). The removal and neutralization of these noxious toxic metabolites are considered to be vital initial steps in the prevention of alcohol-related liver diseases (Ozaras et al., 2003).

In the present study, we found that the aqueous root extract of *P. reniforme* had significant antioxidant activity. The extract was able to scavenge DPPH* and $ABTS^{*+}$ free radicals in a concentration dependent manner. It was also observed to have a significant level of phenolic compounds which are very important because of their free radical scavenging ability due to their hydroxyl groups (Hatano et al., 1989). Phenolics are the major plant compounds with antioxidant activity. This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and

triplet oxygen, or decomposing peroxides (Zheng & Wang, 2001). Flavonoids are included among the several phenolic compounds present in the aqueous root extracts of the plant. The hydrogen donating substituents (hydroxyl groups) attached to the aromatic ring structures of flavonoids, enable them to undergo a redox reaction which in turn, helps them scavenge free radicals (Brand-Williams et al., 1995). In addition, the plant extract showed very good reducing capacity, which may also serve as a significant indicator of its potential antioxidant activity (Hazra et al., 2008). The observed antioxidant property of *P. reniforme* may enable it mop up noxious toxic metabolites released when alcohol is abused and this may explain the observed protection of the liver cells from damage and the improvement in the functional status of the cells after damage.

Excess alcohol consumption has been linked with altered liver metabolism and liver damage, with leakage of cytoplasmic liver enzymes into the blood (James, 1993). AST and ALT are considered among the most sensitive markers of hepatocellular injury. ALP, which is secreted from the lysosomes, is also a marker enzyme for assessing liver damage (Singha et al., 2007). When the integrity of the lysosomal membrane changes and/or the membrane of the lysosome is ruptured by deleterious influences, this acid hydrolase enters the blood stream, producing transient increase in the activity of lysosomal enzymes in the serum. GGT index has been reported high in alcoholic liver disease and measurement of GGT has been claimed to be an extremely sensitive test and marker of ethanol-induced hepatic damage (Sandhir & Gill, 1999). The increased levels of these enzymes (AST, ALT ALP and GGT) in the serum have been observed in alcohol administered rats, which indicate increased permeability, damage and necrosis of hepatocytes (Goldberg & Watts, 1965). Pretreatment with the extract of *P. reniforme* significantly decreased levels of serum enzyme markers, thus suggesting that the extract possessed compounds that protected the hepatocytes from alcohol-

induced liver injury and subsequent leakage of the enzymes into the circulation. Decreased levels of the enzyme markers in the post-treated group compared to control were an indication that the extract also possessed a curative effect.

Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. It provides useful information on how well the liver is functioning (Saravanan et al., 2006). Bilirubin, a chemical breakdown product of hemoglobin, is conjugated with glucuronic acid in hepatocytes to increase its water solubility. Unconjugated hyperbilirubinemia was observed in alcohol-fed rats, which may be as a result of mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes. Decrease in serum bilirubin after treatment with *P. reniforme* indicated the effectiveness of the drug in the maintenance of normal functional status of the liver.

Hypoalbuminaemia is most frequent in the presence of advanced chronic liver diseases. Hence the decline in total protein content can be deemed useful index of the severity of cellular dysfunction in chronic liver diseases. The lowered level of total proteins recorded in alcohol-treated rats reveals the severity of hepatopathy. Stabilization of serum protein levels in the pre and post-treatment groups administered with *P. reniforme* is further a clear indication of the improvement of the functional status of the liver cells.

Conclusion

On the basis of the results obtained in the present study, it is concluded that the aqueous extract of the roots of *Pelargonium reniforme*, which contains significant amounts of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. It also has a very good reducing power. These *in vitro* assays indicate that this plant extract is a significant source of natural antioxidants which might be helpful in removing and

neutralizing noxious toxic metabolites released when alcohol is abused. Also, the results show that the plant extract has protective and curative effects on alcohol-induced liver damage as it restored the liver marker enzymes, serum bilirubin and protein to normal levels.

The potency of *P. reniforme* root extract compares well with silymarin with respect to the hepatic markers observed. Multiple mechanisms may interplay in its protective and curative effects on the liver. Hence, it merits further development for exploiting it as a therapeutic agent.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free-radical method to evaluate antioxidant activity. Food Sci Technol-Leb 28, 25-30.

Duh, P.D., Tu, Y.Y., Yen, G.C., 1999. Antioxidant activity of water extract of Harng Jyur (*Chrysanthemum morifolium* Ramat). Lebensmittel Wissenschaft und Technol 32, 269-277.

Faremi, T.Y., Suru, S.M., Fafunso, M.A., Obioha, U.E., 2008. Hepatoprotective potentials of *Phyllanthus amarus* against ethanol-induced oxidative stress in rats. Food and Chemical Toxicology 46, 2658-2664.

Goldberg, D.M., Watts, C., 1965. Serum enzyme changes as evidence of liver reaction to oral alcohol. Gastroenterology 49 (3), 256-261.

Hatano, T., Edamaysu, R., Mori, A., Fujita, Y., Yasuhara, E., 1989. Chemical and Pharmaceutical Bulletin 37, 2016-2021.

Hazra, B., Biswas, S., Mandal, N., 2008. Antioxidant and free radical scavenging activity of *Spondias pinnata*. BMC Complementary and Alternative Medicine 8, 63.

James, W.P.T., 1993. Alcohol: Its metabolism and effects. In: Garrow, J.S., James, W.P.T., eds., Human Nutrition and Dietetics. Churchill Livingstone, London, pp. 103-118.

Kumaran, A., Karunakaran, R.J., 2007. *In vitro* antioxidant activities of methanol extracts of *Phyllanthus* species from India. Lebens-Wiss Technologie 40: 344-352.

Kundu, R., Dasgupta, S., Biswas, A., Bhattacharya, A., Pal, B.C., Bandyopadhyay, D., Bhattacharya, S., Bhattacharya, S., 2008. *Cajanus cajan* Linn. (Leguminosae) prevents

alcohol-induced rat liver damage and augments cytoprotective function. Journal of Ethnopharmacology 118: 440-447.

Kurose, I., Higuchi, H., Kato, S., Miura, S., Ishii, H., 1996. Ethanol-induced oxidative stress in the liver. Alcoholism: Clinical and Experimental Research 20, 77A-85A.

Latté, K.P., Kolodziej, H., 2004. Antioxidant Properties of Phenolic Compounds from *Pelargonium reniforme*. Journal of Agricultural and Food Chemistry 52: 4899-4902.

Lieber, C.S., 1991. Hepatic, metabolic and toxic effects of ethanol. Alcoholism: Clinical Experimental Research 15: 573-592.

Lindros KO (1995): Alcoholic liver disease: pathobiological aspects. Journal of Hepatology 23: 7-15.

Liyana-Pathiranan, C.M., Shahidi, F., 2005. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L) as affected by gastric pH conditions. Journal of Agricultural and Food Chemistry 53: 2433-2440.

Malloy, H.T., Evelyn, K.A., 1937. The determination of bilirubin with the photoelectric colorimeter. The Journal of Biological Chemistry 119 (112), 481-490.

McCuskey, R.S., 1991: *In vivo* microscopy of the effects of ethanol on the liver. In: Liver Pathology and Alcohol. Humana Press, Totowa, NJ, pp. 563-570.

Nordmann, R., Ribiere, C., Rouach, H., 1992. Implication of free radical mechanisms in ethanol-induced cellular injury. Free Radical Biology & Medicine 12 (3), 219-240.

Ordonez, A.A.L., Gomez, J.D., Vattuone, M.A., Isla, M.I. 2006. Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. Food Chemistry 97, 452-458.

Ozaras, R., Tahan, V., Aydin, S., Uzun, H., Kaya, S., Senturk, H., 2003. N-Acetylcysteine attenuates alcohol-induced oxidative stress in animals. World Journal of Gastroenterology 9 (4), 791-794.

Rakonczay, Z., Boros, I., Jarmay, K., Hegyi, P., Lonovics, J., Takacs, T., 2003. Ethanol administration generates oxidative stress in the pancreas and liver, but fails to induce heat-shock proteins in rats. Journal of Gastroenterology and Hepatology 18 (7), 858-867.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26, 1231-1237.

Reinhold, J.G., 1953. Manual determination of serum total protein, albumin and globulin fractions by Biuret method. In: Reiner M, ed., Standard Methods in Clinical Chemistry. Academic Press, New York, p. 88.

Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology 28, 56-63.

Rosalki, S.B., Rau, D., 1972. Serum gamma-glutamyl transpeptidase activity in alcoholism. Clinical Chimica Acta 39, 41-47.

Sandhir, R., Gill, K.D., 1999. Hepatoprotective effects of Liv-52 on ethanol induced liver damage in rats. Indian Journal of Experimental Biology 37, 762-766.

Saravanan, N., Nalini, N., 2007. Antioxidant Effect of *Hemidesmus indicus* on Ethanol-Induced Hepatotoxicity in Rats. Journal of Medicinal Food 10 (4), 675-682.

Saravanan, R., Viswanathan, P., Pugalendi, K.V., 2006. Protective effect of ursolic acid on ethanol-mediated experimental liver damage in rats. Life Sciences 78, 713-718.

Singha, P.K., Roy, S., Dey, S., 2007. Protective activity of andrographolide and arabinogalactan proteins from *Andrographis paniculata* Nees against ethanol-induced toxicity in mice. Journal of Ethnopharmacology 111, 13-21.

Sun, J.S., Tsuang, Y.W., Chen, I.J., Huang, W.C., Hang, Y.S., Lu, F.J., 1998. An ultra-weak chemiluminescence study on oxidative stress in rabbits following acute thermal injury. Burns 24, 225-231.

Watt, C., Breyer-Brandwijk, M.G., 1962. The medicinal and poisonous plants of southern and eastern Africa. Livingstone, Edinburgh, London, Great Britain, pp. 449-455.

Wolfe, K., Wu, X., Liu, R.H., 2003. Antioxidant activity of apple peels. Journal of Agricultural and Food Chemistry 51, 609-614.

Zheng W, Wang SY (2001): Antioxidant activity and phenolic compounds in selected herbs. Journal of Agricultural and Food Chemistry 49: 5165-5170.

Zima, T., Fialova, L., Mestek, O., Janebova, M., Crkovska, J., Malbohan, I., Stipek, S., Mikulikova, L., Popov, P., 2001. Oxidative stress, metabolism of ethanol and alcohol-related diseases. Journal of Biomedical Science 8, 59-70.

Table 1: Polyphenolic contents of the aqueous root extract of *P. reniforme*

Phenolic compounds	*P. reniforme* root extract
Total Phenol[a]	300.9 ± 0.1
Total Flavonoid[b]	16.6 ± 0.1
Total Flavonol[c]	12.13 ± 0.01
Total Proanthocyanidin[d]	271.3 ± 0.2

(n = 3, X ± S.D.).

[a]Expressed as mg tannic acid/g of dry plant material.

[b]Expressed as mg quercetin/g of dry plant material.

[c]Expressed as mg quercetin/g of dry plant material.

[d]Expressed as mg catechin/g of dry plant material.

Table 2: Effect of aqueous extract of *P. reniforme* roots on hepatic markers in the serum of control and ethanol-administered rats

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)	Total bilirubin (µmol/l)	Unconjugated bilirubin (µmol/l	Conjugated bilirubin (µmol/l
Group 1	356 ± 0^{a}	66.5 ± 19.21^{a}	394.5 ± 5^{a}	9.25 ± 1.5^{a}	11.5 ± 5.2^{a}	10.5 ± 5.2^{a}	1 ± 0^{c}
Group 2	117 ± 20^{e}	42.75 ± 6.9^{c}	309 ± 0^{b}	7 ± 1.83^{ba}	5.75 ± 1.5^{b}	3.25 ± 1.3^{c}	2.5 ± 0.58^{a}
Group 3	140.75 ± 12.3^{cbd}	60.75 ± 5.25^{ba}	211 ± 76.81^{cd}	7.75 ± 0.96^{ba}	8 ± 2.16^{b}	6.75 ± 2.4^{b}	1.25 ± 0.5^{bc}
Group 4	141.75 ± 10.2^{cbd}	55.25 ± 4.5^{bac}	260 ± 41.7^{cb}	7.25 ± 1.71^{ba}	7.75 ± 2.75^{b}	6 ± 0.6^{b}	1.75 ± 1.5^{bac}
Group 5	145 ± 8.98^{cb}	54.75 ± 4.35^{bac}	244.75 ± 63.8^{cbd}	6 ± 1.15^{b}	7 ± 1.41^{b}	5.5 ± 0.6^{b}	1.5 ± 0.58^{bac}
Group 6	123.75 ± 7.5^{ed}	62.75 ± 15.11^{a}	285.5 ± 43^{cb}	7.67 ± 0.58^{ba}	7.25 ± 3.3^{b}	5.25 ± 1.9^{b}	2 ± 1.41^{bac}
Group 7	134.5 ± 12.01^{ced}	58.75 ± 5.25^{ba}	285.75 ± 82.1^{cb}	8.5 ± 1^{a}	6.75 ± 0.5^{b}	5.5 ± 2.7^{b}	1.25 ± 0.5^{bc}
Group 8	126 ± 19.34^{ced}	54.5 ± 10.25^{bac}	240 ± 77.13^{cbd}	7.25 ± 0.96^{ba}	7 ± 1.41^{b}	5.33 ± 1.29^{b}	1.67 ± 1.15^{bac}
Group 9	159.25 ± 25.73^{b}	47.25 ± 8.3^{bc}	174 ± 86.12^{d}	7.5 ± 3^{ba}	7.5 ± 0.58^{b}	5.25 ± 0^{b}	2.25 ± 1.26^{ba}
Group 10	141.75 ± 10.9^{cbd}	56.25 ± 6.6^{bac}	272 ± 9.24^{cb}	5.5 ± 1^{b}	7.25 ± 0.5^{b}	6 ± 0.96^{b}	1.25 ± 0.5^{bc}

Values are expressed as means ± S.D. for six rats in each group. Values not sharing a common superscript differ significantly at $p < 0.05$

AST- Aspartate aminotransferase; ALT- Alanine aminotransferase; ALP- Alkaline phosphatase; GGT- Gamma glutamyl transferase

Table 3: Effect of aqueous extract of *P. reniforme* roots on the serum protein components of control and ethanol-administered rats.

Groups	Total proteins (g/l)	Albumin (g/l)	Globulin (g/l)
Group 1	71 ± 0^{b}	18 ± 0^{a}	53 ± 0^{ba}
Group 2	73.25 ± 1.5^{ba}	19.5 ± 1^{a}	53.75 ± 2.06^{ba}
Group 3	76.25 ± 6.6^{ba}	19 ± 2.16^{a}	57.25 ± 5.97^{ba}
Group 4	79 ± 3.56^{a}	20 ± 1.15^{a}	59 ± 3.16^{a}
Group 5	77.25 ± 2.36^{ba}	19 ± 4.83^{a}	58.25 ± 2.63^{a}
Group 6	76.5 ± 5.92^{ba}	20.5 ± 1^{a}	56 ± 5.35^{ba}
Group 7	74.5 ± 5.26^{ba}	20 ± 0.82^{a}	54.5 ± 4.51^{ba}
Group 8	74 ± 4.24^{ba}	18.75 ± 2.36^{a}	55.25 ± 2.5^{ba}
Group 9	75 ± 2.45^{ba}	20 ± 3.16^{a}	56.75 ± 1.5^{ba}
Group 10	75.75 ± 3.59^{ba}	19 ± 1.15^{a}	55.75 ± 3.59^{ba}

Values are expressed as means ± S.D. for six rats in each group. Values not sharing a common superscript differ significantly at $p < 0.05$

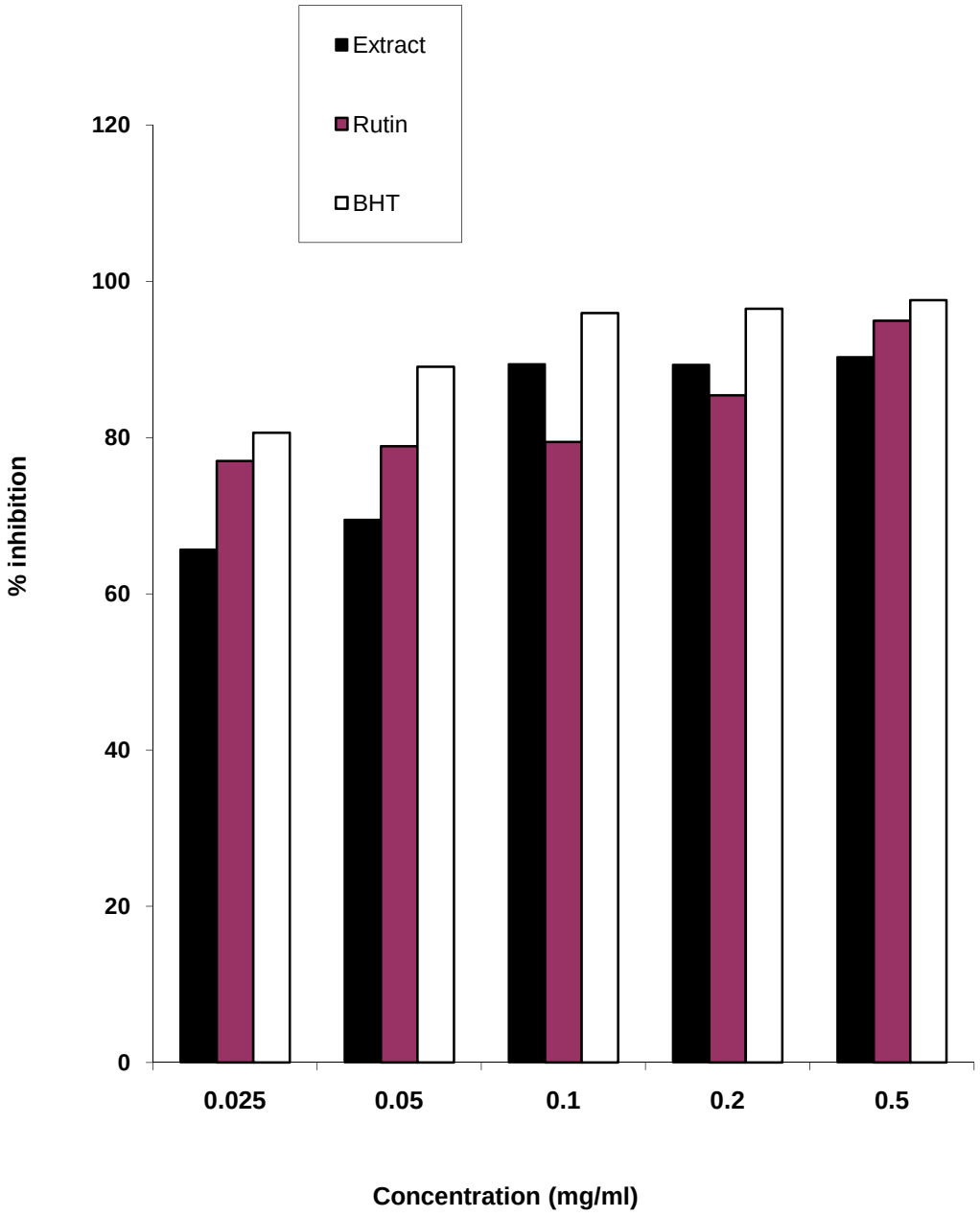

Figure 1: DPPH radical scavenging activity of the aqueous extract of *P. reniforme* roots

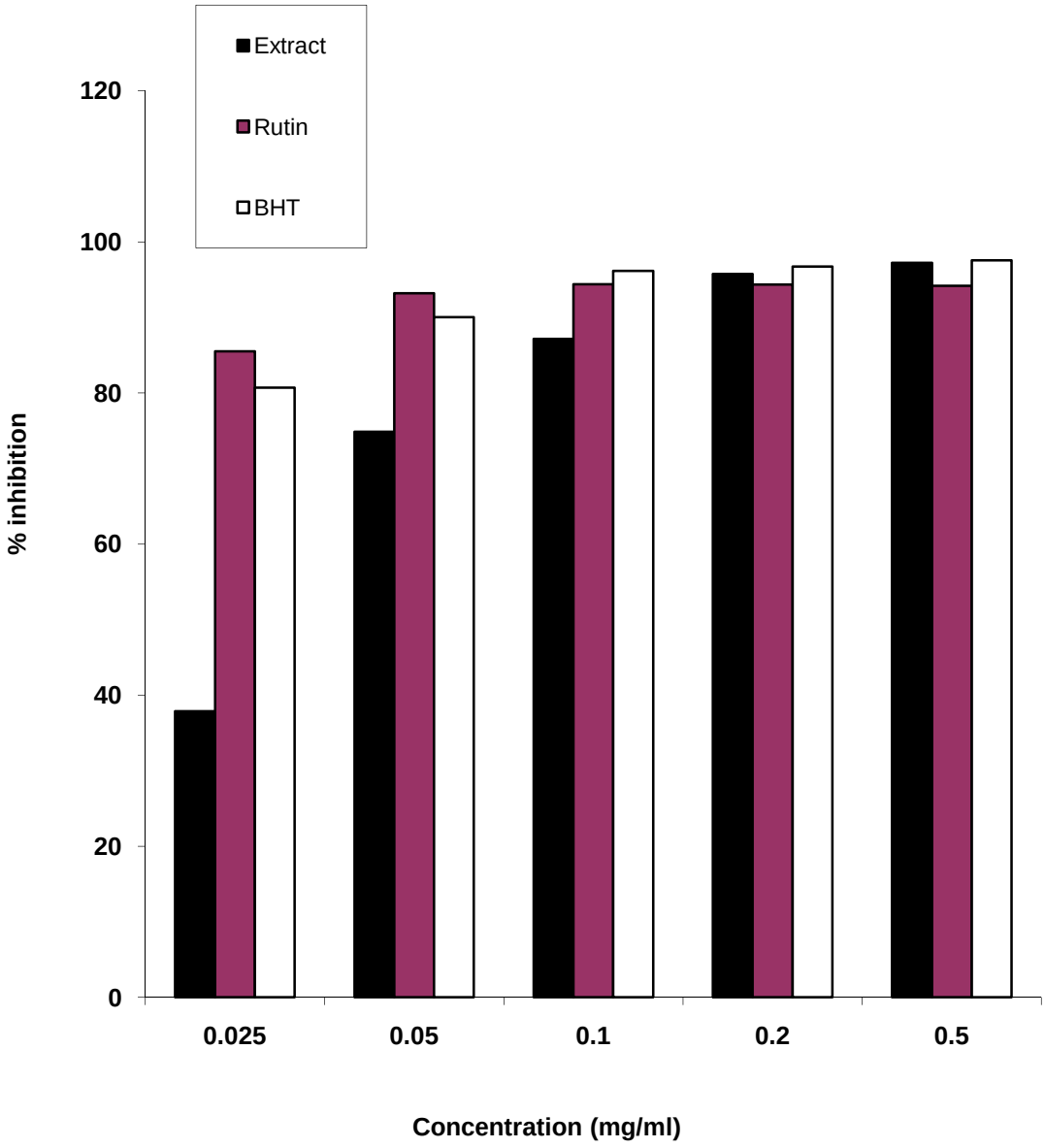

Figure 2: ABTS radical scavenging activity of the aqueous extract of *P. reniforme* roots

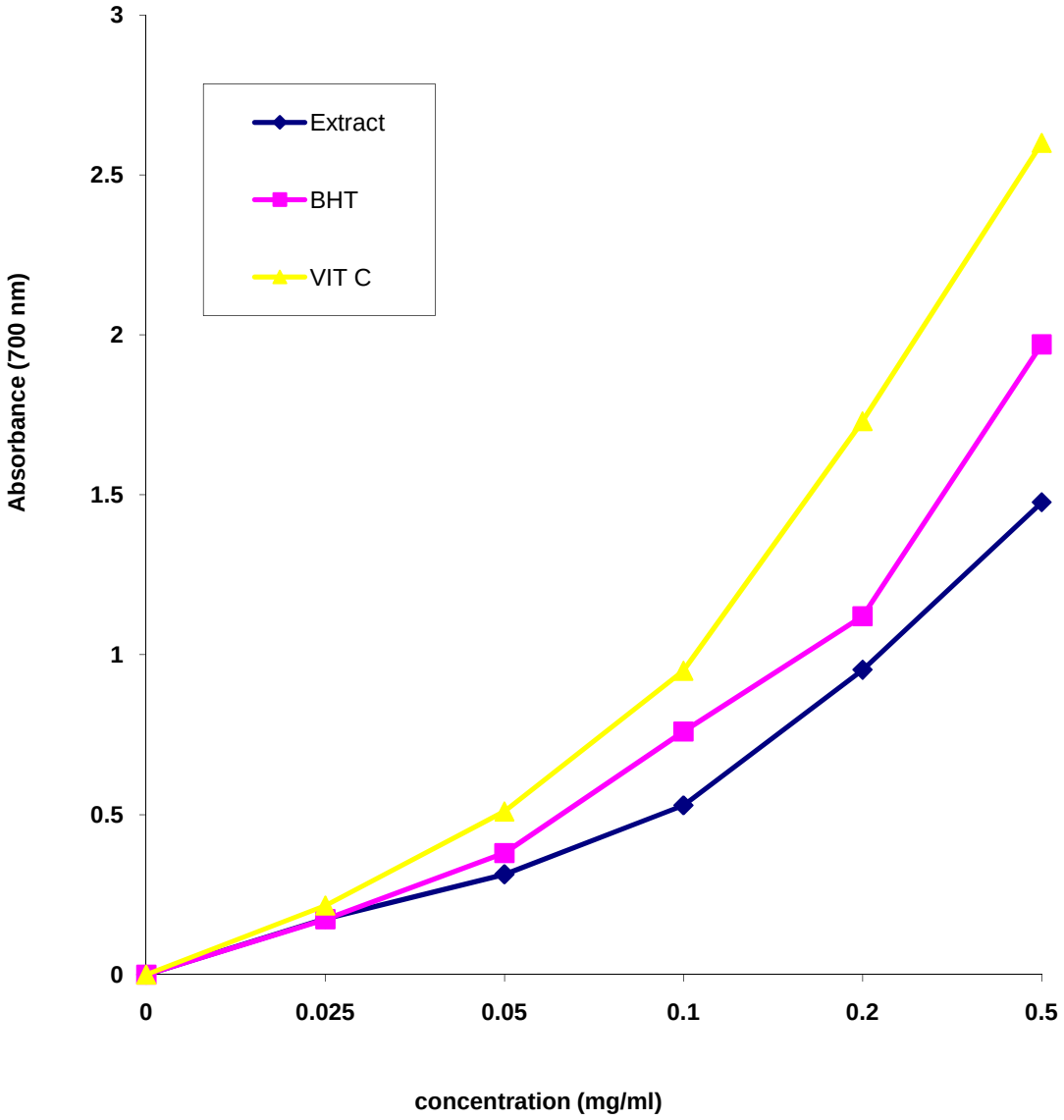

Figure 3: Total ferric reductive potential of the aqueous extract of *P. reniforme* roots