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Hepatoprotective and antioxidant properties of methanolic extract of *Piper nigrum Linn.* in rats

Ramnik Singh[1], Narinder Singh[2] B.S. Saini[3], H.S. Rao[3]

[1]*SSS College of Pharmacy, Gurdaspur, Punjab, India*

[2]*Department of Pharmacology, SGRD Medical Institute, Amritsar, Punjab, India*

[3]*Institute of Pharmaceutical Sciences and Engineering, Amritsar, Punjab, India*

ABSTRACT

Hepatoprotective and antioxidant effects of methanolic extracts of *Piper nigrum* Linn. (MPN) were studied in sodium oxalate treated rats. Lipid peroxidation was induced in rats by administration of 100 mg/kg body weight sodium oxalate. The protective effect of MPN was assessed by monitoring the serum and tissue levels of malondialdehyde, catalase activity, aspartate transaminase (AST) and alanine transaminase (ALT) as well as serum vitamin C content in the normal, control and experimental rats after 10 and 20 days of MPN administration. It was observed that MPN administration lowers significantly ($p<0.05$) the serum and tissue levels of malondialdehyde, as well as AST and ALT activities in a dose dependent manner. The serum level of vitamin C and activity of catalase in the serum and tissues were however shown to be significantly elevated ($p<0.05$). After 10 days of administration of 200 mg/kg body weight of MPN extract, serum level of malondialdehyde was reduced from 46.856±1.050 to 32.166±0.884 nm/h, AST activity from 58±2.85 to 31±1.42 IU and ALT activity from 39±2.52 to 25±1.28 IU. Moreover, administration of 200 mg/Kg body weight of MPN for 10 days caused an increase in serum catalase activity and serum vitamin C level was increased from 46.52±7.75 to 80.24±5.02 mg/100 ml. In the tissues, the same trend was observed. The result also indicated that prolonged MPN administration (for 20 days) significantly increased serum vitamin C level and the activity of catalase in both the serum, liver and the kidney ($p<0.05$). Also, the serum and tissue levels of malondialdehyde and transaminase activities (AST and ALT) were significantly reduced ($p<0.05$).

KEYWORDS: Antioxidant, Hepatoprotective, lipid peroxidation, *Piper nigrum*, sodium oxalate, transaminases.

INTRODUCTION

The liver is the key organ of metabolism, secretion and excretion and it is continuously and widely exposed to xenobiotics, environmental pollutants and chemotherapeutic agents because of its strategic location in the body. Liver disease is a worldwide problem (1-4). Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. It is, therefore, necessary to search for alternative drugs for the treatment of liver disease to replace currently used drugs of doubtful efficacy and safety.

Piper nigrum Linn. (Piperaceae) has long been used in folk medicine as stomachic aromatic stimulant, diaphoretic and hepatoprotective. Black pepper stimulates taste buds causing reflex stimulation gastric secretions, improving digestion and treating in gastro-intestinal upsets and flatulence. *Piper longum* is included in herbal formulation which marketed under the trade name of Livomyn. Hence present was conducted to investigate the hepatoprotective and antioxidant effects of *Piper nigrum*.

MATERIALS AND METHODS

Preparation of MPN

The *Piper nigrum* fruits used were purchased from a local market of Kottayam, kerala, India in the month of March, 2006. A voucher specimen (B-05) has been kept in our laboratory for future reference. The collected materials were washed thoroughly in water, air dried for a week at 35-40°C and pulverized in electric grinder. The powder obtained was extracted in methanol. The extracts were then made to powder by using rotary evaporator under reduced pressure.

Experimental animals

Albino rats (Wistar strain) of either sex weighing 150-200 gram were used for this study. The protocol was approved by Institutional Animal Ethics Committee. Experimental rats were allowed to stabilize for one week before the commencement of the experiment. They were kept in cages in a room maintained at room

temperature and were allowed feed and water *ad libitum*.

Chemicals

Assay kits for the estimation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were purchased from Randox, UK. Sodium oxalate was purchased from Sigma Chemical Co., USA. All other chemicals used were of analytical grade.

Treatment of animals

Rats were divided randomly into five groups with 10 rats in each group. Rats in Group A served as control normal and were given distilled water (DW) orally. Rats in Group B, the control experimental group, were given 100 mg/kg body weight of sodium oxalate intraperitoneally and sacrificed 12 h later. Rats in Group C were fed with 50 mg/kg body weight of MPN extract with DW daily. After the first 10 days, half of the rats in the group were given 100 mg/kg body weight of sodium oxalate and sacrificed 12 h later. The remaining half was fed with the same concentration of MPN for a total period of 20 days before intraperitoneal administration of sodium oxalate and sacrificed 12 h later. Rats in Group D were fed with 100 mg/kg body weight of MPN extract with DW daily. After the first 10 days, half of the rats in the group were given 100 mg/kg body weight of sodium oxalate and sacrificed 12 h later. The remaining half was fed with the same concentration of MPN for a total period of 20 days before intraperitoneal administration of sodium oxalate and sacrificed 12 h later. Rats in Group E were fed with 200 mg/kg body weight of MPN extract with DW daily. After the first 10 days, half of the rats in the group were given 100 mg/kg body weight of sodium oxalate and sacrificed 12 h later. The remaining half was fed with the same concentration of MPN for a total period of 20 days before intraperitoneal administration of sodium oxalate and sacrifice 12 h later. Blood samples were drawn from ether anaesthetized rats by cardiac puncture. The liver and kidney of rats were removed immediately after rats were killed by administering sodium pentobarbitone (100mg/kg) intravenously. 0.5 g of each tissue was homogenized in 10-fold weight of ice cold 0.15 M KCl solution. The preparation was centrifuged at 1500 rpm for 15 min. The supernatant was decanted and stored frozen until required.

Measurement of biochemical parameters

Assay for malondyaldehyde was done through the method of Janeiro (5) in which MDA reacted with thiobarbituric acid reactive substances produced during lipid peroxidation to produce a red pigment. This pigment was then analyzed using ultraviolet spectrophotometer at 534 nm. Transaminases (SGOT, SGPT) produces oxaloactate and pyruvate which in turn coupled with 2,4-dinitrophenyl hydrazine to give corresponding hydrazone and was measured colormetrically (6). Catalase activity was estimated by the procedure of Sinha (7). In this method dichromate in acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide, per chromic acid is formed which is an unstable intermediate. The chromic acid produced is measured colorimetrically at 570 nm and the quantity of hydrogen peroxide remaining was calculated from standard curve. Serum reduced ascorbic acid level was determined by method of Urbach et al. (8) in which red bishydrazone was formed.

Statistical analysis

Values are expressed as Mean ± S.E.M. Randomized Complete Block Design Analysis of Variance was used for statistical analysis. *P* Values less than 0.05 were considered significant.

RESULT AND DISCUSSION

Effect of MPN consumption on sodium oxalate-induced malondialdehyde formation in rats

The effect of 10 and 20 days consumption of MPN extract on the level of malondialdehyde in sodium oxalate treated rats is presented in Tables 1a and b. After 10 days consumption of MPN, control normal group of rats had a serum malondialdehyde level of 30.295±0.365 nM/h. Administration of sodium oxalate (100 mg/kg body weight) produced a statistically significant increase in serum level of malondialdehyde ($p<0.05$). Consumption of low concentration of MPN (50 mg/kg body weight) did not seem to protect the rats. However, increasing the concentration of MPN consumed to 200 mg/Kg body weight produced a statistically significant decrease in the level of malondialdehyde. A similar trend was observed in the liver and kidney.

In the liver, the initial level of malondialdehyde was 5.923±0.465 nM/h/g of tissue. Administration of sodium oxalate (100 mg/kg body weight) produced a level of 9.12¤0±0.850 nM/h/g, the difference between the control and experimental rats was significant ($p<0.05$). The level of malondialdehyde in the kidney of untreated rat was 4.062±¤0.298 nM/h/g. Treatment of rats with sodium oxalate (100 mg/kg body weight) increased this level to 7.634±0.068 nM/h/g, while administration of 200 mg MPN/kg body weight offered about 60% protection. After 20 days consumption of

MPN, the level of malondialdehyde in control normal group of rats was significantly lower ($p<0.05$) compared to control experiment group of rats, which were treated with sodium oxalate. Consumption of low concentration of MPN (50 mg/Kg body weight) for 20 days did not seem to protect the rats but consumption at higher concentration of MPN produced a statistically significant lowering of malondialdehyde level ($p<0.05$). Moderate increase in malondialdehyde level was observed in rats treated in the liver and kidney. Prolonged MPN consumption at higher concentration produced about 20% protection from sodium oxalate effect in either the liver or the kidney. There was a significantly pronounced increase in the rate of production of malondialdehyde in both the serum and liver and kidney homogenates of the rats treated with sodium oxalate. The induction of lipid peroxidation in the test animals may be a consequence of the direct influence of oxalate. This observation is quite interesting because of the fact that calcium oxalate is the major constituent of most renal stones implicated to cause the release of peroxides. Since calcium chloride has no effect on lipid peroxidation, it is oxalate that induces this action (9).

Consumption of MPN produced a dose dependent decrease in the rate of formation of malondialdehyde in the serum, liver and the kidney. Prolonged MPN consumption has been shown to have a significant inhibitory effect on sodium oxalate-induced lipid peroxidation in rats. This is evident in the decrease in the resultant rate of formation of Thiobarbituric Reactive Substances (TBA-RS) observed in rats fed with MPN extracts for 10 days and 20 days accordingly. This corroborates the findings of Sano et al. (10) that MPN has an antioxidant property against lipid peroxidation in rats.

Table 1a. Effect of 10 days consumption of MPN on the level of malondialdehyde in sodium oxalate treated rats.

Groups	Serum (nM/h)	Liver (nM/h/g)	Kidney (nM/h/g)
Normal (No treatment)	30.295 ± 0.365	5.923 ±0.465	4.062±0.298
Control (Normal + 100 mg/kg of Sodium Oxalate)	46.856±1.050[a]	9.120±0.850 [a]	7.634±1.068 [a]
Control + 50 mg/kg of MPN	47.427±3.082 [a]	8.323±1.136 [ab]	6.091±0.368 [ab]
Control + 100 mg/kg of MPN	37.294 ±2.068[abc]	7.142±1.150 [abc]	5.788±0.350 [abc]
Control + 200 mg/kg of MPN	32.166±0.884[abcd]	6.193±0.750 [abcd]	4.522±0.950 [bcd]

Values are mean ± S.E.M. for five determinations. a = significantly different from normal at P<0.05; b = significantly different from control at P<0.05; c = significantly different from group fed with 50 mg/kg of MPN at P<0.05; d = significantly different from group fed with 100 mg/kg of MPN at P<0.05.

Table 1b. Effect of 20 days consumption of MPN on the level of malondialdehyde in sodium oxalate treated rats.

Groups	Serum (nM/h)	Liver (nM/h/g)	Kidney (nM/h/g)
Normal (No treatment)	26.523 ± 2.365	5.223 ±0.465	3.062±0.598
Control (Normal + 100 mg/kg of Sodium Oxalate)	42.856±3.050 [a]	7.420±0.850 [a]	6.634±1.008 [a]
Control + 50 mg/kg of MPN	39.727±0.982 [ab]	7.023±0.513 [a]	5.509±1.368 [ab]
Control + 100 mg/kg of MPN	34.094 ±2.068 [abd]	6.143±0.615 [abc]	5.188±0.950 [ab]
Control + 200 mg/kg of MPN	31.122±1.088 [abcd]	5.893±1.750 [abcd]	3.922±0.550 [abcd]

Values are mean + S.E.M for five determinations. a=significantly different from normal at P<0.05; b=significantly different from control at P<0.05; c =significantly different from group fed with 50 mg/kg of MPN at P<0.05; d =significantly different from group fed with 100mg/kg of MPN at P<0.05.

Table 2a. Effect of 10 days consumption of MPN on catalase activity in sodium oxalate treated rats.

Groups	Serum		Liver		Kidney	
	Vol. of H_2O_2 (ml)	%A	Vol. of H_2O_2 (ml)	%A	Vol. of H_2O_2 (ml)	%A
Normal (No treatment)	0.295 ±0.005	8.61	0.235 ±0.005	10.25	0.215 ±0.015	11.15
Control (Normal + 100 mg/kg of Sodium Oxalate)	0.333±0.004	7.00 [a]	0.353±0.004	5.65 [a]	0.323±0.014	7.05 [a]
Control + 50 mg/kg of MPN	0.325±0.025	6.97 [a]	0.259±0.015	9.66 [ab]	0.225±0.025	11.18 [b]
Control + 100 mg/kg of MPN	0.226±0.004	00 [abc]	0.196±0.014	9.23 [ab]	0.226±0.024	10.52 [b]
Control + 200 mg/kg of MPN	0.250±0.035	00 [abc]	0.350±0.025	6.50 [abcd]	0.290±0.025	8.55 [abcd]

Values are mean+ S.E.M for five determinations. a=significantly different from normal at P<0.05 b=significantly different from control at P<0.05, c =significantly different from group fed with 50 mg/kg of MPN at P<0.05. , d =significantly different from group fed with 100 mg/kg of MPN at P<0.05. Note: %A = Percentage Activity. Values are mean+ S.E.M for five determinations. a=significantly different from normal at P<0.05 b=significantly different from control at P<0.05, c =significantly different from group fed with 50 mg/kg of MPN at P<0.05. , d =significantly different from group fed with 100 mg/kg of MPN at P<0.05.

Table 2b. Effect of 20 days consumption of MPN on catalase activity in sodium oxalate treated rats.

Groups	Serum		Liver		Kidney	
	Vol. of H_2O_2 (ml)	%A	Vol. of H_2O_2 (ml)	%A	Vol. of H_2O_2 (ml)	%A
Normal (No treatment)	0.288 ±0.025	8.45	0.135 ±0.025	14.55	0.175 ±0.015	12.86
Control (Normal + 100 mg/kg of Sodium Oxalate)	0.323±0.034	7.00 [a]	0.313±0.084	7.25 [a]	0.283±0.016	8.51 [a]
Control + 50 mg/kg of MPN	0.245±0.035	10.05 [ab]	0.299±0.055	8.29 [ab]	0.115±0.025	15.35 [ab]
Control + 100 mg/kg of MPN	0.286±0.024	8.55 [abc]	0.065±0.004	17.35 [abc]	0.106±0.024	15.67 [ab]
Control + 200 mg/kg of MPN	0.197±0.025	12.15 [abcd]	0.075±0.025	19.01 [abcd]	0.099±0.015	16.15 [ab]

Note: %A = Percentage Activity. Values are mean + S.E.M for three determinations. a=significantly different from normal at P<0.05 b=significantly different from control at P<0.05, c =significantly different from rats fed with 50 mg/kg of MPN at P<0.05. , d =significantly different from rats fed with 100 mg/kg of MPN at P<0.05.

Effect of MPN consumption on catalase activity of sodium oxalate treated rats

The catalase activity in the serum, liver and kidney of rats consuming MPN after sodium oxalate treatment is presented in Tables 2a and b. The initial serum catalase activity for control rats at 10 and 20 days was identical (8.6%). Rats which received sodium oxalate had a slightly lower catalase activity. Consumption of MPN produced a higher level of catalase at 10 and 20 days of treatment; the difference between the experimental control and treated rats was significant ($p<0.05$). However, in the liver and kidney, catalase activity was significantly higher in rats treated with MPN ($p<0.05$). The difference was highly significant at 20 days. There was a decreased level in catalase activity in the serum as well as in the tissues. Catalase catalyses the conversion of hydrogen peroxide, a powerful and potentially harmful oxidizing agent, to water and molecular oxygen. This decrease is thought to occur as a result of accumulation of hydrogen peroxide, which is a product of peroxidation in tissues. As hydrogen peroxide concentration increases, more and more of the enzyme will be used up in an attempt to clear off the accumulating hydrogen peroxide. This result agrees with the findings of Selvam and Kurien (9) who reported a significant decrease in catalase activities in both the liver and kidney 12 h after the administration of sodium oxalate in rats. The mechanism of induction of lipid peroxidation by sodium oxalate may involve inhibition of catalase activity *in vivo* since *in vitro* studies have revealed progressive inhibition of catalase activity and increase in lipid peroxidation with increasing oxalate concentration (9). However, the control groups showed a significant increase in catalase activity as a result of MPN consumption. The increase observed also followed a dose-dependent pattern with the most pronounced increase found when rats were fed for a period of 20 days. This result also indicates that prolonged MPN consumption could be chemo-preventive by gradually causing an increase in the activity of catalase. This result, coupled with the finding that MPN consumption of MPN lowers the rate of production of TBARS in the serum and the tissues, could also suggest that the increase in catalase activity observed may be as a result of reduced extent of lipid peroxidation which in turn reduces the concentration of hydrogen peroxide that accumulates in the tissues and in the serum. This therefore means lesser amount of catalase enzyme will be used up at any given time.

Aspartate and Alanine transaminases activity in the serum, liver and kidney of sodium oxalate treated rats

AST activity in the serum, liver and kidney of rats consuming MPN after sodium oxalate treatment is presented in Tables 3a and b. Sodium oxalate treatment produced a statistically significant increase in the AST level in the serum, liver and kidney. Consumption of MPN within 10 days produced a significant decrease in AST activity in the serum, kidney and liver. The kidney has almost 60% protection. After 20 days of consuming the MPN extracts, though there was a decrease in AST level in the serum, liver and kidney, the difference was not statistically significant compared to the experimental control group. Effects of 10 and 20 days consumption of MPN extract, the serum and tissue levels of ALT activity in sodium oxalate treated rats is presented in Tables 4a and b. Administration of 100 mg/kg body weight of sodium oxalate to experimental rats produced a statistically significant rise in the level of ALT as compared with untreated rats ($p<0.05$). Consumption of MPN produced a concentration dependent decrease in the activity of ALT. Prolonged consumption of MPN (for 20 days) slightly decreased the level of ALT. The difference between the untreated and experimental rats was not significant. One of the mechanisms in this model is considered to be initiated by the accumulation of oxalate which causes consecutive lipid peroxidation of the cell membranes and endoplasmic reticulum. The peroxidative products caused the cell membrane to become leaky with the consequent release of these enzymes into the blood. This agrees with the work of Masayuki et al. (11) who reported that lipid peroxidation is recognized to be a major factor in the liver injury model. The decreased level of AST and ALT observed after consumption of the MPN extract would suggest that the release of these enzymes had been inhibited. Probably, a chemical component in the MPN is stabilizing the integrity of the cell membrane, keeping the membrane intact and the enzymes enclosed.

Effect of MPN consumption on Vitamin C depletion by sodium oxalate

The concentration of vitamin C in the serum on control rats and rats consuming MPN after sodium oxalate treatment is presented in Table 5. Concentration of Vitamin C in the control rats was 93.2 mg/100 ml; administration of sodium oxalate decreased this to about 50%. Consumption of MPN for either 10 or 20

days produced an increase in the serum level of vitamin C. The difference in the vitamin C content between 10 and 20 days and between normal and control rats were statistically significant ($p<0.05$). There was a significant decrease in the serum concentration of reduced ascorbic acid in rats treated with sodium oxalate. This depletion may be a direct consequence of the accumulation of oxalate in the system of those experimental animals. The antioxidant effect of Vitamin C is well established and it is also known to have protective functions against oxidative damage to lipid membranes. This protective ability may involve degradation of some of the vitamin C molecules present in the blood. Animals fed with MPN were able to conserve the amount of vitamin C in the serum. There is also a marked difference between the serum concentrations of reduced ascorbic acid in the control and experimental groups. This conservation could be explained to be as a result of the fact that certain constituents of MPN extract are capable of free radical quenching just as vitamin C. As these constituents of MPN extract accumulates in the blood, more vitamin C molecules are freed from the free radical quenching responsibility, hence leading to the conservation of ascorbic acid in the serum. This ascorbic acid conservation characteristic of MPN extract, coupled with its ability to modulate catalase activity positively and impede lipid peroxidation, is an indication of the fact that MPN extract has the capacity to boost body's antioxidant activity.

Table 3a. Effect of 10 days consumption of MPN on AST activity in sodium oxalate treated rats in IU.

Groups	Serum	Liver	Kidney
Normal (No treatment)	13± 0.55	18±0.72	13±0.65
Control (Normal + 100 mg/kg of Sodium Oxalate)	58±2.85 ^{a}	75±4.55 ^{a}	51±2.50 ^{a}
Control + 50 mg/kg of MPN	41±2.56 ^{ab}	47±2.25 ^{ab}	31±1.62 ^{ab}
Control + 100 mg/kg of MPN	35±1.90 ^{abc}	41±2.57 ^{abc}	23±1.22 ^{abc}
Control + 200 mg/kg of MPN	31±1.42 ^{abcd}	35±1.55 ^{abcd}	18±1.20 ^{abcd}

Values are mean + S.E.M for three determinations. a=significantly different from normal at P<0.05 b=significantly different from control at P<0.05, c =significantly different from group fed with 50 mg/kg of MPN at P<0.05. , d =significantly different from group fed with 100 mg/kg of MPN at P<0.05.

Table 3b. Effect of 20 days consumption of MPN on AST activity in sodium oxalate treated rats in IU.

Groups	Serum	Liver	Kidney
Normal (No treatment)	10± 0.55	07±0.22	07±0.24
Control (Normal + 100 mg/kg of Sodium Oxalate)	40±3.23 ^{a}	52±3.11 ^{a}	41±3.25 ^{a}
Control + 50 mg/kg of MPN	31±1.30 ^{ab}	41±1.58 ^{ab}	31±2.32 ^{ab}
Control + 100 mg/kg of MPN	27±1.32 ^{abc}	31±1.25 ^{abc}	27±1.28 ^{abc}
Control + 200 mg/kg of MPN	35±2.05 ^{abcd}	41±2.55 ^{abcd}	36±2.55 ^{abcd}

Values are mean + S.E.M for three determinations. a=significantly different from normal at P<0.05 b=significantly different from control at P<0.05, c =significantly different from group fed with 50 mg/kg of MPN at P<0.05. , d =significantly different from group fed with 100 mg/kg of MPN at P<0.05.

Table 4a. Effect of 10 days consumption of tea extract on ALT activity in sodium oxalate treated rats in IU.

Groups	Serum	Liver	Kidney
Normal (No treatment)	19± 0.88	18±0.79	18±0.25
Control (Normal + 100 mg/kg of Sodium Oxalate)	39±2.52 ^{a}	29±2.23 ^{a}	25±1.28 ^{a}
Control + 50 mg/kg of MPN	34±2.16 ^{ab}	25±2.52 ^{ab}	21±1.83 ^{ab}
Control + 100 mg/kg of MPN	28±3.22 ^{abc}	21±1.55 ^{abc}	17±0.85 ^{bc}
Control + 200 mg/kg of MPN	25±1.28 ^{abcd}	17±092 ^{bcd}	17±0.92 ^{bc}

Values are mean+ S.E.M for three determinations. a=significantly different from normal at P<0.05 b=significantly different from control at P<0.05, c =significantly different from group fed with 50 mg/kg of MPN P<0.05. , d =significantly different from group fed with 100 mg/kg of MPN at P<0.05.

Table 4b. Effect of 20 days consumption of tea extract on ALT activity in sodium oxalate treated rats in IU.

Groups	Serum	Liver	Kidney
Normal (No treatment)	22± 1.55	18±0.67	19±1.25
Control (Normal + 100 mg/kg of Sodium Oxalate)	37±2.35 [a]	32±1.29 [a]	31±2.32 [a]
Control + 50 mg/kg of MPN	35±2.58 [a]	26±2.15 [ab]	21±1.77 [ab]
Control + 100 mg/kg of MPN	30±1.62 [ab]	26±1.95 [ab]	17±1.02 [bc]
Control + 200 mg/kg of MPN	44±2.05 [ab]	35±2.65 [abcd]	34±2.56 [abcd]

Values are mean+ S.E.M for three determinations. a=significantly different from normal at P<0.05 b=significantly different from control at P<0.05, c =significantly different from group fed with 50 mg/kg of MPN at P<0.05. , d =significantly different from group fed with 100 mg/kg of MPN at P<0.05.

Table 5. Effect of 10 and 20 days consumption of tea extract on serum vitamin C content of sodium oxalate treated rats in mg/100ml.

Groups	After 10 days	After 20 days
Normal (No treatment)	93.20±8.35	88.42±5.21
Control (Normal + 100 mg/kg of Sodium Oxalate)	46.52±7.75 [a]	56.59±18.25 [a]
Control + 50 mg/kg of MPN	54.28±5.95 [ab]	67.54±4.05 [ab]
Control + 100 mg/kg of MPN	73.62±2.13 [abc]	83.08±2.55 [abc]
Control + 200 mg/kg of MPN	80.24±5.02 [abcd]	101.65±6.98 [abcd]

Values are mean + S.E.M for three determinations. a =significantly different from normal at P<0.05 b=significantly different from control at P<0.05, c =significantly different from group fed with 50 mg/kg of MPN at P<0.05. , d =significantly different from group fed with 100 mg/kg of MPN at P<0.05.

The observation that prolonged consumption of MPN enhances vitamin C conservation and prevented vitamin C depletion by sodium oxalate (100 mg/kg body weight) would suggest the antioxidant properties of vitamin C in protecting the cell against oxidative stress. Black pepper was shown to enhanced the absorption of orally administered curcumin. But no study has been reported so far for the effects of black pepper on oral absorption of vitamin C. This study therefore provided an evidence for a possible role of MPN as antioxidant and hepatoprotective agent. However further studies on free radical scavenging effects and clinical investigations of black pepper.

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