ORIGINAL ARTICLE

HEPATOPROTECTIVE EFFECT OF MINT AGAINST HEPATOTOXICITY, INDUCED BY CHLOROQUINE, IN MALE ALBINO MICE: RCT

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Background: Many drugs have been associated with hepatotoxicity worldwide. This hepatotoxicity occurs due to oxidative stress generated by those drugs. Hence, different plants, which have antioxidant effects, can be used to prevent hepatotoxicity. The objective was to find out the hepatoprotective effect of mint, against chloroquine induced hepatotoxicity, due to its antioxidant potential. **Methods:** For that randomized controlled trial study, ninety male albino mice were obtained and were divided randomly into three groups, with each group containing 30 mice. Group A was the control group. So, no intervention was done on mice of Group A. Group B and C were the experimental groups. Group B mice were given chloroquine only. The mice of Group C were given both chloroquine and mint extract. The single oral dose of chloroquine, 970 mg/ kg of body weight, was given to the mice of group B, on the 9th day of the experiment. The ethanolic extract of mint, at the dose of 1 gm/kg, was given consecutively from day 1 to day 8 of the experiment to Group C mice. Then chloroquine (970 mg/kg of the body weight) was given on nineth day to Group C. The ethanolic extract was then continuously given from day 10 to day 16 of the experiment, followed by chloroquine administration, to those mice. The blood samples were collected on 17th day by terminal intracardiac sampling technique. Data analysis was done by SPSS version 20. **Results:** Group B mice showed highly significant rise in serum ALP and significant decrease in serum albumin, as compared to those of group A. Serum AST and ALT, however, raised insignificantly. Hence, mild hepatotoxicity was induced in group B mice. On the other hand, malondialdehyde, was found to be highly significantly raised in group B mice. While, serum glutathione peroxidase was found to be declined highly significantly in group B mice, which showed oxidative stress induction. The mice of group C showed highly significant decrease in serum ALP and significant decline in serum AST. They showed significant raise in serum albumin. Serum malondialdehyde, however, declined highly significantly and serum glutathione peroxidase raised highly significantly in group C. These results in group C occur due to antioxidant action of mint. **Conclusion:** Mint revealed hepatoprotective effect due to its antioxidant potential, against chloroquine induced mild hepatotoxicity.

Keywords: Chloroquine; Mint; Hepatotoxicity; Oxidative stress; Hepatoprotective; Antioxidant

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INTRODUCTION

It has been found that about 50% of the cases of liver failure in the world, occur due to hepatotoxicity, induced by drugs.[1] Drug induced hepatotoxicity is one of the most common causes of liver failure.[2] Those drugs caused hepatotoxicity due to the induction of oxidative stress in the subjects.[3]

In oxidative stress induction, various free radicals are generated, which can attack the lipids of the membranes.[4] As a result of this attack, various secondary metabolites are formed. Malondialdehyde is one the secondarily metabolites, which is formed during lipid peroxidation process.[5] So, the increased levels of malondialdehyde are indicative of oxidative stress induction.[6] When this damage occurs in the liver cells, various enzymes, which are present inside the hepatic cells such as ALP, AST and ALT are released into the blood.[7] Raised levels of liver enzymes in the blood indicate hepatotoxicity.[8] Due to this damage, the function of the liver, such as formation of proteins, is comprised. Hence, the level of albumin is declined in hepatotoxicity.[9]

A study conducted by Edeordo Gianini *et al*, (2005) revealed that the magnitude of changes in the ALT and AST can be classified into three types i.e. mild, moderate and marked changes. Mild alteration indicates that ALT and AST changes are less than five times the upper limit of normal. Moderate alteration

means changes in the level of ALT and AST are between five to ten times the upper limit of normal. And marked changes are labelled when alteration occurs more than the ten times of the upper limit of normal. Acute liver injury is labelled when alteration of ALT and AST occurs more than the ten times of the upper limit of normal.

It has been studied previously that chloroquine, when given at the dose higher than the therapeutic dose (<2gm), can induce hepatotoxicity.[10] This hepatotoxicity occurs due to oxidative stress induction.

There are various antioxidants, enzymatic and non- enzymatic, which are produced in our bodies.[11] These antioxidants scavange free radicals, that are generated during lipid peroxidation process. They have the ability to lose electrons due to which they are able to stop free radical chain reactions. The phenolic acids inhibit the conversion of Fe^{+3} into Fe^{+2}. Hence, they end non-enzymatic lipid peroxidation process. Antioxidants balance the increased formation of free radicals.[12] Due to the excessive utilization of antioxidants, their levels are declined in the body.[13]

Worldwide, a lot of research is going on to find such herbs and plants, which contain antioxidants.[14] For that purpose, mint (mentha) is being widely studied.[15] Mint (mentha) contains abundant number of antioxidants.[16] Flavonoids and polyphenols are the two important antioxidants, which are present in the mint. These antioxidants have the ability to make further new antioxidants and even, they can activate the existing antioxidants.[17] They can scavenge free radicals generated in lipid peroxidation process. [18] Due to their antioxidant potential, they can be used for prevention of hepatotoxicity.

About 20 different species of mentha has been discovered so far in the world.[19] Various species of mentha have been found widely in Pakistan. Mentha arvensis is one of them, which has been used in the current study.[20] So, the current study has been conducted to determine antioxidant and hepatoprotective potential of mentha arvensis. Mentha arvensis can be used for therapeutic purposes against hepatotoxicity induced by different drugs.

MATERIAL AND METHODS

For this randomized controlled study, ninety male albino mice were included. Those mice were bought from University of Veterinary and Animal Sciences, Lahore. The selection of Mice for the study was done by non- probability consecutive sampling method. Those 90 mice were divided into three groups of 30 each. Group A was selected as control, in which no intervention was done. The mice of group B were given chloroquine orally only, at the dose of 970 mg/kg of body weight on the 9th day of the experiment. The mice of Group C were given both chloroquine and mint. The ethanolic extract of the mint, at the dose of 1 gm/kg of the body, was given from day 1 of experiment to day 8 of experiment. Then, on nineth day of the experiment, the chloroquine, at the dose of 970 mg/kg of body weight was given. The ethanolic extract of mint was continued to be given to the mice of that group from day 10 to day 16 of the experiment, followed by chloroquine. Blood samples of the mice were collected on the 17th day of experiment by terminal intracardiac sampling technique. Data analysis was done by SPSS version 20.

RESULTS

The mean values of ALT, AST, ALP, albumin, total proteins, malondialdehyde and glutathione peroxidase, in controlled group A mice, are 9.73±4.35, 57.20±22.50, 106.47±10.87, 2.70±0.75, 6.21±1.79, 0.12±0.37 and 1.03±0.13 respectively.

One way ANOVA was applied to compare the mean values of those three groups. One way ANOVA showed that the difference of serum ALP was highly significant (p=0.000) among all the three groups. While significant difference (p=0.05) was found in the mean values of serum AST and albumin. However, serum ALT and total proteins depicted no significant difference (p>0.05) among the three groups (Table 1).

While table 2 shows highly significant (p=0.000) difference in the mean values of serum malondialdehyde and serum glutathione peroxidase among all the three groups, when one way ANOVA was applied.

Post hoc Tukey's test was then applied to compare significant results of the two groups. Post hoc Tukey's test depicts that when results were compared between group B, in which hepatotoxicity was induced by chloroquine with group A, in which no intervention was done, they showed highly significant (p<0.000) rise in the mean value of serum ALP (234.77±100.14) in group B as compared to that in group A (106.47±10.87. While significant (p=0.05) decline in serum level of albumin (2.30±0.28) observed in group B as compared to that in group A (2.70±0.75). But, serum AST level (61.13±14.10) did not rise significantly (p>0.05) in group B as compared to that in group A (57.20±22.50).

Table 4, however, revealed that serum level of malondialdehyde (0.22±0.12) rose highly significantly (p<0.000) in group B as compared to that in group A (0.12±0.037). While, serum glutathione peroxidase level declined highly significantly (p<0.000) in group B than in group A (0.12±0.037).

Similarly, results of group C, in which mint along with chloroquine was given, were compared by Post hoc Tukey's test with those of group B, in which only choroquine was administered. The comparison

revealed highly significant (p<0.000) decline in the value of ALP (104.57±20.34) in group C as compared to that in group B (234.77±100.14). Serum level of AST (43.63±25.74) declined significantly (p=0.05) in group C as compared to that in group B (61.13±14.10). Serum level of albumin (2.65±0.39) raised significantly (p=0.05) in group C as compared to that in group B (2.30±0.28).

Table-4, showed highly significant (p<0.000) fall in the serum level of malondialdehyde (0.18±0.05) in group C as compared to that in group B (0.79±0.12). While, serum glutathione peroxidase level (0.94±0.17) rose highly significantly (p<0.000) in group C than that in group B (0.79±0.12).

Table-1: Comparison of serum ALP AST, ALT, albumin and total proteins among groups A, B and C by one way ANOVA test. Values are given out here as mean±SD

Values are given out here as mean±SD Parameters	Group A (n= 30)	Group B (n=30)	Group C (n=30)	*p*-value
ALT (U/L)	9.73±4.35	11.47±5.96	10.00±2 63	0.285
AST (U/L)	57.20±22.50	61.13±14. 20	43.63±25.74	0.005
ALP (U/L)	106.47±10.87	234.77±100.14	104.57±20.34	0.000*
Albumin (g/dl)	2.70±0.75	2.30±0.28	2.65±0.39	0.007
Total proteins (g/dl)	6.21±1.79	6.10±0.37	6.41±0.37	0.530

Values are given out here as mean±SD, *p<0.001= highly significant

Table-2: Comparison of serum malondialdehyde and glutathione peroxidase among groups A, B and C by one way ANOVA test. Values are given out here as mean±SD

Parameters	Group A	Group B	Group C	*p*-value
Serum malondialdehyde (ng/ml)	0.12±0.37	0.22±0.12	0.18±0.05	0.000*
Serum glutathione peroxidase (ng/dl)	1.03±0.13	0.79±0.12	0.94±0.17	0.000*

Values are given out here as mean±SD, *p<0.001= highly significant

Table-3: Comparison of serum ALP, AST, ALT, albumin and total proteins among groups A, B and C by Post hoc Tuckey's test. Values are given out here as mean ± SD

Group comparisons	AST(U/L)	ALP (U/L)	Albumin (g/dl)
Between group B and A	0.756	0.000*	0.10
Between group C and B	0.006	0.000¨	0.27

Values are given out here as mean±SD, *p<0.001= highly significant

Table-4: Comparison of serum malondialdehyde and glutathione peroxidase among groups A, B and C by Post hoc Tukey's test. Values are given out here as mean ± SD

Group comparisons	Serum malondialdehyde (ng/ml)	Serum glutathione peroxidase (ng/dl)
Group B and A	0.000*	0.000*
Group C and B	0.000*	0.000*

Values are given out here as mean±SD, *p<0.001= highly significant

DISCUSSION

The above data suggest that hepatotoxicity induced in the present study was mild in nature. So, in the present study minor elevation of ALT and AST indicate mild damage of hepatocytes. While, for declaring acute liver injury, marked elevation of ALT and AST was required.

Raised in the levels of serum alanine aminotransferase and aspartate aminotransferase was found due to their release into the circulation, after the damage of the hepatocytes. Lipid peroxidation caused the cell membrane to lose its integrity. ALT and AST, which were present in the cytoplasm of hepatocytes, released into the circulation. The magnitude of elevation of serum ALT and AST was directly correlated to the number of the hepatocytes that were damaged.

Alkaline phosphatase was present in the cytoplasm of canalicular or biliary cells. So, when damage occurred to cell membrane due to lipid peroxidation, ALP was released into the circulation. In the present study, highly significant elevation of serum ALP indicates damage to biliary cells.

Most of the plasma proteins are formed by the liver, including albumin. Albumin makes up the major bulk of plasma proteins. About 60% of plasma proteins are albumin.[21] In the present study, damage to hepatocytes resulted in reduced synthesis of total proteins and albumin. Serum albumin depicted highly significant decline but the total proteins did not decline significantly in group B when results were compared with those of control group (group A).

These results are contradictory to those obtained by Pari L *et al*, (2005), who had used the same dose of chloroquine, i.e., 970 mg/kg in female wister rats. Significant elevation of ALT, AST and ALP was noticed in their study. Chloroquine caused damage to hepatocytes. As a result, enzymes were released into

the circulation. But, compared to that study, mild hepatotoxicity has been induced in the current study with the use of same dose of chloroquine, i.e., 970 mg/kg. Which shows that higher doses of chloroquine must be tested to induce hepatotoxicity in mice.

When the results of serum malondialdehyde and glutathione peroxidase were compared between group A and group B, highly significant elevation in the level of serum malondialdehyde observed in group B as compared to those in group A. While, highly significant decline in the level of glutathione peroxidase was observed in group B as compared to that in group A. Highly significant elevation of malondialdehyde indicates lipid peroxidation, as malondialdehyde is one of the secondary metabolites of lipid peroxidation. Hence, it indicates the induction of oxidative stress by chloroquine. The large number of free radicals, generated in lipid peroxidation process, were scavenged by glutathione peroxidase. Hence, due to excessive utilization of glutathione peroxidase for scavenging purpose, its serum level declined. These results are consistent with those determined by Pari, (2005). They had used chloroquine to induce hepatotoxicity in female wister rats. Single oral dose of 970mg/kg of chloroquine was used. They observed significant elevation of hydroperoxides and thiobarbituric acid reactive substances. On the other hand, reduced serum level of glutathione peroxidase indicated its utilization in scavenging the free radicals which were generated in lipid peroxidation process.

The ethanolic extract of mentha arvensis was used in the current study because the previous studies had revealed that ethanolic extract exhibited most significant hepatoprotective and antioxidant effects due to the greatest concentration of antioxidants, i.e., flavonoids and phenolic acids, present in the ethanolic extract. In the current study, when the results were compared between group C (mint and chloroquine) and group B (chloroquine only), significant reduction of AST (43.63±25.74) and highly significant reduction of ALP (104.57±20.34) occurred in group C (mint+chloroquine). Serum albumin levels were raised (2.65±0.39) significantly in group C. These findings suggest that ethanolic extract of mint (mentha) possess hepatoprotective effect. This hepatoprotective effect seems to be due to the antioxidant effects.

When the serum levels of malondialdehyde and glutathione peroxidase were compared between group C and group B, highly significant decline in malondialdehyde (0.18±0.05) and highly significant elevation of glutathione peroxidase (0.94±0.17) was observed in group C as compared to that in group B. Reduction of serum levels of malondialdehyde, which is a secondary metabolite of lipid peroxidation process, indicates decline in lipid peroxidation process by the ethanolic extract of mint. While the increased serum level of glutathione peroxidase, which had declined in group B due to its utilization in scavenging the increase number of free radicals, indicated that antioxidants in the ethanolic extract of mint had also increased their formation and activity. Hence, these findings indicate that mint (mentha) possessed antioxidant effects, which is responsible for its hepatoprotective effect.

These findings are comparable with the findings obtained by Patil *et al*, (2012). They studied the hepatoprotective effect of mint (mentha). For which, they induced hepatotoxicity by administering CCl4. ALT, AST and ALP levels were raised. They used three different extracts i.e. aqueous, methanolic and ethanolic extracts. Pretreatment with ethanolic extract showed most significant decrease in serum ALT, AST and ALP levels. They depicted that hepatoprotective effect was most likely due to the presence of antioxidants such as flavonoids.

Dar *et al*, (2014) revealed the antioxidant role of mentha arvensis. Their results showed that antioxidants, such as phenolic acids, possessed hydroxyl group, due to which they were able to scavenge free radicals. The antioxidants also inhibited non-enzymatic lipid peroxidation process by converting Fe^{+3} into $Fe^{+2.}$

Similar findings were obtained by Wani *et al*, (2018). Their results also showed that ethanolic extract of mentha arvensis possessed highest concentration of flavonoids and phenolic acids.

Polyphenols in mentha arvensis, have been found to possess various antioxidant roles. They inhibit lipid peroxidation by iron chelation. They inhibit enzymes, NADPH oxidase and xanthine oxidase, which are involved in generation of reactive oxygen species. They suppress formation of malondialdehyde by inhibiting cyclooxygenase and lipoxygenase. They also increase the formation of antioxidant enzymes.

CONCLUSION

Ethanolic extract of the mint has hepatoprotective effect due to its antioxidant potential against chloroquine induced hepatotoxicity in mice.

AUTHORS' CONTRIBUTION

SK: Literature search, conceptualization of study design, data collection, data analysis, data interpretation, write-up, proof reading. AF: Data collection, data analysis, dta interpretation. SK: Write-up

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