Effect on non-enzymatic antioxidants

GSH, total thiols and vitamin C levels of nitrobenzene-induced and ethanol extract treated groups (group II and group III) are shown in Table 4. The levels of GSH, total thiols and vitamin C were significantly ($P < 0.01$) decreased in the nitrobenzene-induced group (group II) as compared to the normal group (group I). On treatment with the ethanol extract of *E. hirta* (group III), GSH, total thiols and vitamin C levels were found to be enhanced significantly ($P < 0.01$). There was no significant difference between the ethanol extract alone treated group (group IV) and normal group (group I).

Histopathology

Histopathologic studies also provided a supportive evidence for biochemical analysis. In case of control (group I), the kidney section showed normal glomeruli, Bowman's tubules and blood vessels. The kidney section of the animals induced with nitrobenzene (group II) showed glomerular degeneration with loss of surrounding Bowman's capsule and tubular necrosis. Treatment with the ethanol extract of *E. hirta* (group III) showed a glomerulus with an intact Bowman's capsule, and all the renal cells which were damaged due to nitrobenzene appeared to be regenerated and almost showed normal histology. The kidney section of the animals that received the ethanol extract of *E. hirta* alone (group IV) showed normal morphology [Figure 1].

DISCUSSION

Blood urea nitrogen (BUN), serum urea, uric acid and creatinine are considered as the indicators of kidney damage, and their abnormal levels indicate marked renal injury. In our previous basic study, nitrobenzene induction produced an elevation in the concentrations of BUN, serum urea, and creatinine and significant reduction in the level of uric acid. Treatment with *E. hirta* significantly restored the levels to normalcy.[32] In the present study, nitrobenzene increased the level of renal tissue MDA production, which suggested involvement of free radicals in nitrobenzene-induced nephrotoxicity. Nitrobenzene produces a number of free radicals during its reactive metabolism in the gut as well as at the cellular level and generates superoxide anion as a by-product during oxidative metabolism. The reactive species generated during nitrobenzene metabolism are considered candidates for carcinogenicity. Furthermore, several lines of evidence suggest that nitrobenzene exerts its carcinogenicity through a non-DNA reactive (epigenetic) fashion, such as a strong temporal relationship between non-, pre- and neoplastic lesions leading to carcinogenesis.[33]

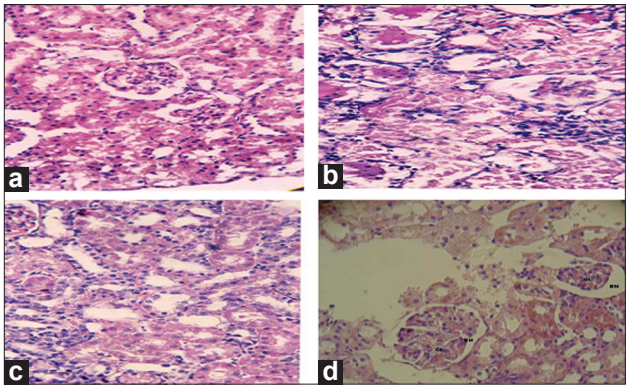

Figure 1: Histopathology of kidney. (a) Kidney section of the normal rat (group I) showing normal tubular brush borders and intact glomerulus and surrounding Bowman's capsule; (b) kidney section of the rat induced with nitrobenzene (group II) showing a glomerulus with loss of surrounding Bowman's capsule and tubular necrosis with swelling and vacuolation; (c) kidney section of the rat induced with nitrobenzene and treated with ethanol extract of *Euphorbia hirta* (group III) showing a glomerulus with an intact Bowman's capsule and mildly swollen tubules and almost showing normal morphology; (d) kidney section of rat treated with extract alone showing normal glomerulus and Bowman's capsule

The elevated levels of LPO in nitrobenzene-induced animals might be due to the renal damage caused by nitrobenzene-induced free radical generation. LPO, an index of oxidative stress, leads to deterioration of biological system[34] and is ascribed to a free radical mediated chain reaction that damages cell membrane and implicates renal oxidative stress.[35] Oxidative stress is believed to be a primary factor in various diseases.[36] Oxidative stress could also be involved in other inflammatory glomerular lesions caused by a series of mediators including cytokines and chemokines, which leads to leukocyte activation, production of reactive oxygen species (ROS) and increased glomerular damage.[37] Different studies have shown that free radicals are responsible for oxidative damage to different molecules (lipids, proteins, nucleic acid) and thus are involved in the initiation phase of some degenerative illnesses.[38] The elevated level of lipid peroxides in nitrobenzene-induced group would be a transient phenomenon of cellular damage.[39] In animals treated with ethanol extract of *E. hirta*, the rise in lipid peroxides was prevented significantly. The decrease in lipid peroxides may be due to the antioxidant effect of the extract.

The generation of ROS in kidney has been proposed as a mechanism by which many chemicals can induce nephrotoxicity.[40] The ROS generation in tissues is efficiently scavenged by the enzymatic and non-enzymatic antioxidants. The decrease in the activities of antioxidant enzymes is in close relationship with the induction of LPO.[41] Thus, the mechanism of nephrotoxicity is related to the depletion of the antioxidant defense system.[1] The reduced activity of SOD, CAT, GPx and GST could be

due to enhanced LPO or inactivation of the antioxidative enzymes.[42] The present work confirms that the observed decrease in the antioxidant activity was presumably associated with the increased LPO and oxidative stress caused by nitrobenzene. The ethanol extract administration restored the depleted renal antioxidants. This recovery to near normalcy reveals that oxidative stress induced by nitrobenzene has been nullified due to the antioxidant potential effect of *E. hirta*.

The antioxidant activity may be due to the inhibition of the formation of radicals or scavenging of the formed radical.[43] Supplementation of plant material improves all these parameters. Similar results have been reported by a number of investigators.[44,45] The biochemical findings were also supported by the histopathologic studies which showed a glomerulus with loss of surrounding Bowman's capsule and diffuse tubular necrosis in the renal section of rats induced with nitrobenzene. These histopathologic changes almost disappeared in the renal tissue of rats treated with the ethanol extract of *E. hirta*. Numerous studies have also shown that medicinal plants protect the kidney against nephrotoxicity induced by various chemicals.[46,47] Thus, the ethanol extract of *E. hirta* at a dosage of 400 mg/kg body weight showed a significant change against nitrobenzene-induced nephrotoxicity. Preliminary phytochemical screening of the ethanol extract of *E. hirta* showed a higher concentration of flavonoid. Literature has shown medicinal plants with nephroprotective properties to mediate their protection via antioxidant and/or free radical scavenging activities due to the high concentration of flavonoids and alkaloids that they contain.[48,49] Thus, in the present study, nephroprotection offered by the ethanol extract of *E. hirta* could be due to the presence of higher concentration of flavonoids present in it.

CONCLUSION

Thus, the results of this investigation suggest that the ethanol extract of *E. hirta* can prevent the toxic effects of nitrobenzene and can be used in the treatment of nephrotoxicity. The protective action of the ethanol extract of *E. hirta* was probably due its antioxidant nature which protects the kidney against nephrotoxicity induced by nitrobenzene.

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