HEPATOPROTECTIVE ACTIVITY OF *DAUCUS CAROTA L.* AQUEOUS EXTRACT AGAINST PARACETAMOL, ISONIAZID AND ALCOHOL INDUCED HEPATOTOXICITY IN MALE WISTAR RATS.

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Summary

Daucus carota L. commonly known as carrot, very widely consumed vegetable in south asia, been shown to provide protection against hepatotoxicity, thus its regular consumption during the exposure to hepatotoxins in clinical setup (INH in treatment of tuberculosis) may reduce their toxic effects. The effect of *Daucus carota L.* (DCE) on paracetamol, isoniazid (INH) and alcohol induced liver damage was evaluated. Hepatotoxicity were induced in different group of Wistar rats, with paracetamol (4 mg/kg), INH (100 mg/kg) and alcohol (20 mg/kg of 40% v/v). The biochemical markers *viz.* Alanine transaminase(ALT), Aspartate transaminase (AST), bilirubin (both total and direct) and prothrombin time (PT) were estimated and compared with histopathological study. The paracetamol, INH and alcohol-induced increase in biochemical markers mentioned above were significantly lowered by pretreatment with the DCE. The histopathologic studies showed that pretreatment with DCE restored the hepatic histology to normal in INH and alcohol challenged groups, but failed to do so in paracetamol group. The carrot extract could provide a significant protection against paracetamol, INH and alcohol induced hepatocellular injury in Wistar rats.

Keywords: Alcohol; Carrot extract; Hepatic enzymes; Hepatoprotection; Isoniazid; Paracetamol.

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Introduction

Liver is one of the important organs concerned with metabolism of endogenous substances as well as xenobiotics and is the first target for various toxic insults.[1] Hepatotoxic agents include not only some chemicals but also some drugs that are used in clinical practice.[2] A routinely used analgesic, paracetamol[3]; primary anti tubercular agent, isoniazid (INH)[4] and commonly abused alcoholic beverages are implicated with hepatic injury. Such drug induced hepatotoxicity not only limits their further use but might interfere with essential metabolic functions leading to consequences of altered carbohydrate, protein and fat metabolism.

The treatment of drug induced hepatotoxicity involves withdrawl of causative agent and administration of specific antidotes (such as N-acetyl cystein, in paracetamol toxicity) if available. Discontinuation of therapy with INH due to hepatotoxic adverse effect may compromise the efficacy of anti-tubercular treatment, since INH is a key-primary anti-tubercular drug. In majority occasions the treatment of hepatotoxicity is empirical and includes multivitamins as well as some marketed herbal preparations.[5] Plant preparations of *Ocimum sanctum*[6] *, Azadirachta indica*[7]*, etc* have been reported to be hepatoprotectives in paracetamol induced hepatotoxicity in rats but their efficacy in other models of hepatotoxicity is not reported. *Daucus carota L.* of the Apiaceae family is an annual or biannual herb mostly confined to the temperate regions of Europe, Asia and Africa. Its decoction has been reported to be a popular remedy for jaundice[8] in addition to its traditional uses for treating kidney, respiratory, cardiovascular disorders, *etc*.[9] *Daucus carota* (carrot) a commonly consumed vegetable in Indian subcontinent has been reported to be hepatoprotective against injury induced by carbon tetrachloride (CCl_4) as described by Bishayee (1995)[10] and lindane as described by Balasubramaniam (1998)[11]. However its efficacy against paracetamol, isoniazid and alcohol induced hepatotoxicity is not well documented. The present study was therefore, planned to investigate hepatoprotective activity of *Daucus carota* in male Wistar rats subjected to paracetamol, isoniazid and alcohol induced hepatic injury.

Materials and methods

Preparation of carrot extract

Extraction was carried out by the method described earlier with modification, that no water was added nor the extract was centrifuged. Fresh roots of *Daucus carota L* commonly known as carrot was purchased from local market and authenticated by Agrkar Research Institute, Pune. Two hundred and fifty grams of carrot was homogenized (without adding water) and squeezed through cheese cloth to obtain the extract (juice). Each day freshly prepared extract was administered orally at dose of 25ml/kg in two divided doses with 4 hours interval as described by Bishayee (1995)[10].

Animals

Wistar rats of either sex weighing between 170 ± 20 grams procured from Venkateshwara Enterprises, Bangalore were acclimatized to the laboratory under 12;12 light dark cycle for a week with free access to standard diet (Amrut Brand) and water. Ethical clearance was obtained from Institutional Animal Ethical Committee constituteded as per CPCSEA guidelines.

The animals were divided into 8 groups (n=6, in each). Four of them were treated with saline and the other four groups with *Daucus carota L.* extract (DCE) throughout the study. On day 8, three groups from each of the pretreatments (saline and DCE) were fasted overnight and challenged with either paracetamol/ INH/ alcohol. Remaining two groups continued to receive saline/DCE throughout the study.

Drug induced hepatotoxicity

As reported earlier hepatotoxic dose 2mg/kg[3] of paracetamol was found to be ineffective while, the dose of 7mg/kg used to produce hepatotoxicity by Dass and Shah (2000)[12] was highly toxic. Preliminiary experiments were conducted to determine the oral hepatotoxic dose of paracetamol and was found to be 4 mg/kg.

Similarly, INH in the dose of 50 mg/kg as reported by Nimbkar[13] failed to induce hepatotoxicity. Through preliminary studies 100 mg/kg was found to induce hepatotoxicity and in the present study the same dose was administered orally in single dose for 30days using intragastric tube.

As reported by Kapoor (1994)[14] 40% *v/v* alcohol in the dose of 20mg/kg orally was used in two divided doses one hour apart every day for 21 days to induce hepatotoxicity in the present study.

At the end of specified treatment period the animals were anaesthetized to collect the cardiac blood for ALT, AST, bilirubin and PT estimation. Later the animals were sacrificed humanely by over anesthesia to dissect out the liver for histopathological investigations.

Liver function tests

Serum AST and ALT were estimated by the method described by Reitman and Frankel[15], while bilirubin by the method described by Malloy and Evelyn[16] using biochemical estimation kits procured from Beacon Diagnostics Pvt. Ltd., Navsari. Prothrombin Time (PT) was estimated as described by Hull et.al.[17] using Thromboplastin reagent procured from Tulip Diagnostic (P) Ltd, Goa. AST and ALT were expressed in U/ml and bilirubin (total & direct) were expressed in mg%. and PT was expressed in seconds.

Histopathological studies

The dissected out liver tissues were processed and embedded in paraffin wax to take sections. The sections taken were stained with hematoxylin and eosin (H&E) for histopathological studies.

Statistical analysis

The results were analysed by ANOVA followed by post hoc Dunnet's test and $p \leq 0.05$ was considered as significant.

Results

Paracetamol induced hepatotoxicity:

ALT, AST, total as well as direct bilirubin and PT were significantly ($p<0.001$) elevated in saline pretreated group as compared to control group. Pre-treatment with DCE though failed to restore the values to normal, lowered significantly ($p<0.01$) the elevated enzyme (ALT & AST) (Fig 1&2), bilirubin (total and direct) (Fig 3&4) and PT (Fig 5). (Table I)

INH induced hepatotoxicity:

ALT, AST, total as well as direct bilirubin and PT level were significantly ($p<0.001$) elevated in saline pretreated group as compared to that of control group. Pre-treatment with DCE restored the elevated enzyme (ALT & AST) (Fig 1&2) levels to normal and significantly ($p<0.05$) reduced bilirubin (total and direct) (Fig 3&4) and PT (Fig 5). (Table I)

Fig 1. Effect of DCE on paracetamol, INH and alcohol induced changes in serum AST.

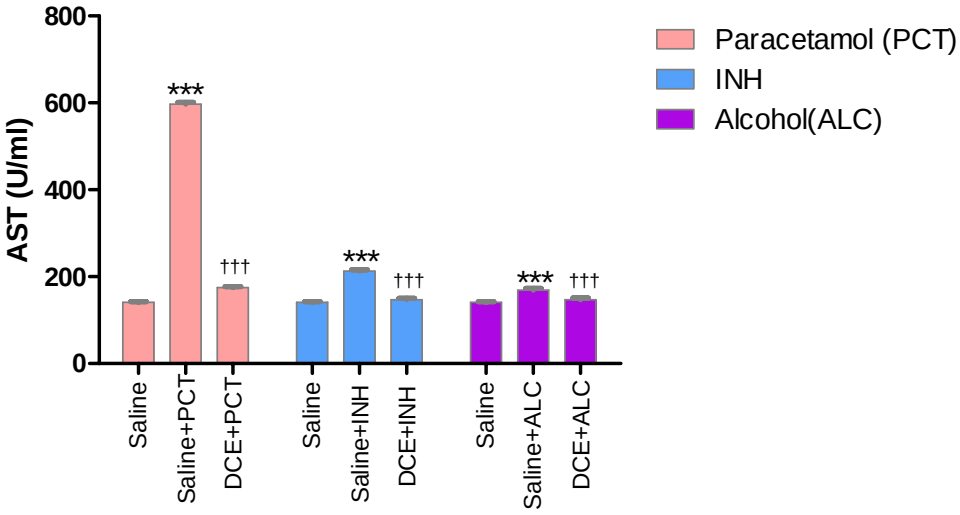
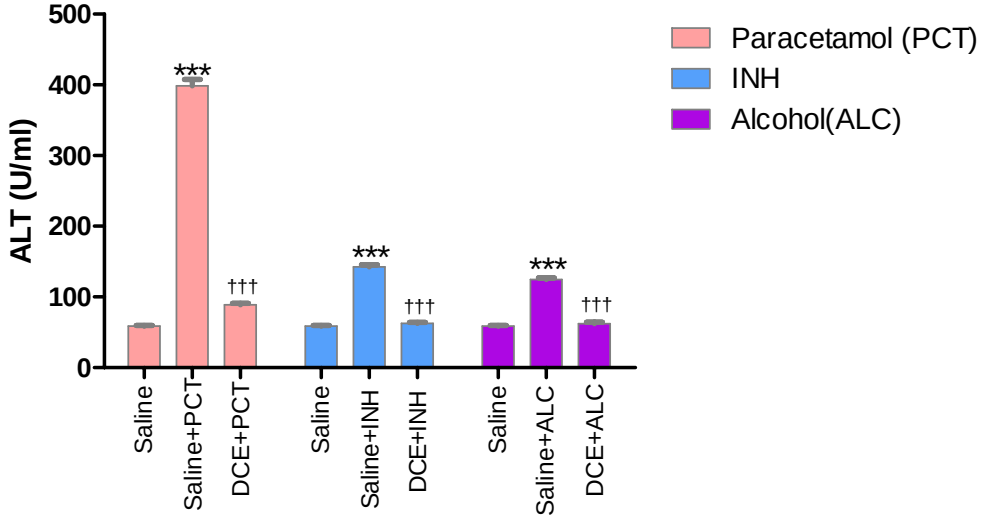


Fig 2. Effect of DCE on paracetamol, INH and alcohol induced changes in serum ALT.



***p<0.001 as compared to control.

†††p< 0.001 as compared to DCE untreated group.

Fig 3. Effect of DCE on paracetamol, INH and alcohol induced changes in serum Total Bilirubin.

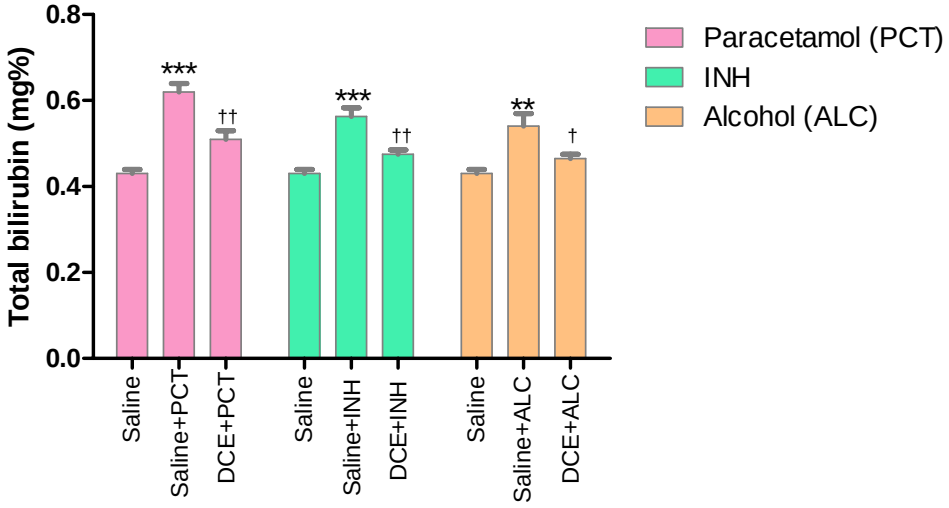
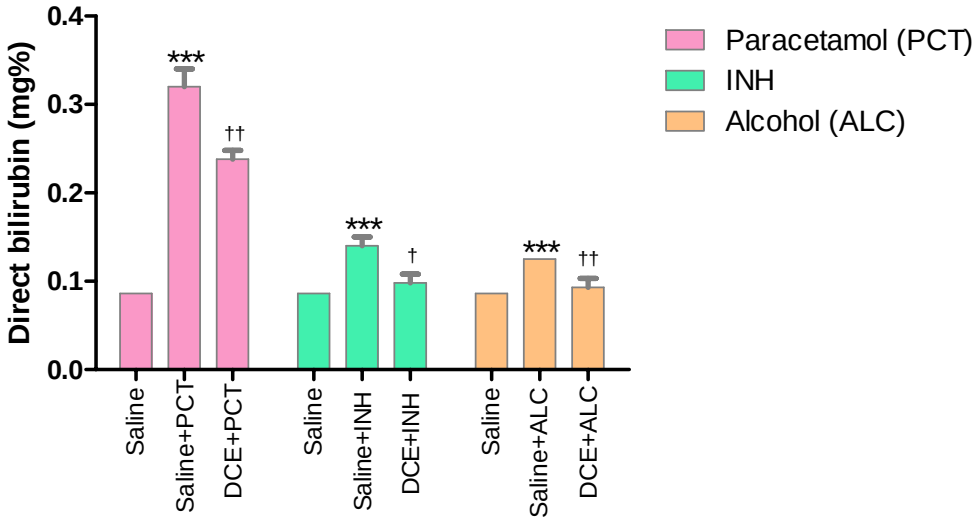


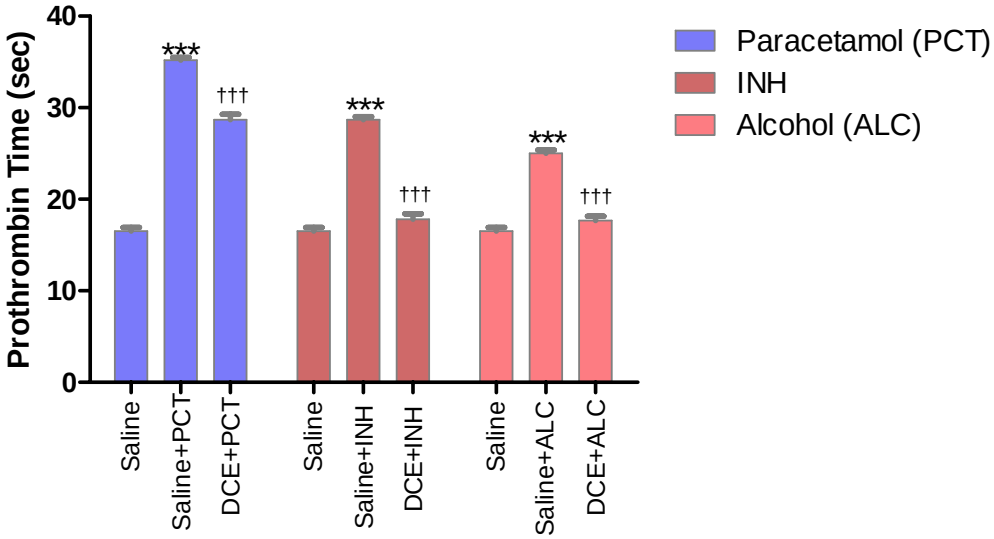
Fig 4. Effect of DCE on paracetamol, INH and alcohol induced changes in serum Bilirubin (Direct).



** $p<0.01$, *** $p<0.001$ as compared to control.

† $p<0.05$, †† $p < 0.01$, ††† $p< 0.001$ as compared to DCE untreated group.

Fig 5. Effect of DCE on paracetamol, INH and alcohol induced changes in plasma ptothrombin time.



$^{***}p<0.001$ as compared to control.

$^{†††}p< 0.001$ as compared to DCE untreated group.

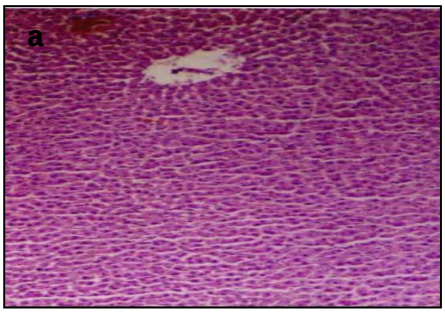
Alcohol induced hepatotoxicity:

Alcohol challenge in saline pretreated group increased significantly ($p<0.001$) serum ALT, AST and total ($p<0.05$) as well as direct ($p<0.001$) bilirubin level as compared to that of control group. Similarly alcohol also increased PT significantly ($p< 0.001$). Pre-treatment with DCE restored the elevated enzyme (ALT & AST) levels to normal (Fig 1&2) and significantly ($p<0.05$) lowered bilirubin (total as well as direct) (Fig 3&4) and PT (Fig 5). (Table I)

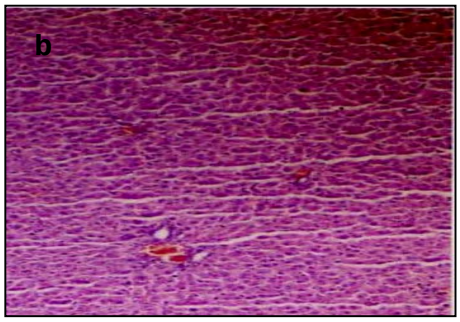
Histopathological studies:

Microscopic observations of Saline alone treated and DCE alone treated showed in fig 6 (a &b). Microscopic observations of H & E stained sections of liver revealed centrilobular necrosis in paracetamol group(fig 6c), while focal hepatocellular necrosis along with micro and macro fatty changes in INH (fig 6e). In alcohol (fig 6g) challenged animals moderate fatty changes were observed. Pre-treatment with DCE restored the liver histology to normal only in INH (fig 6f) and alcohol (fig 6h) challenged groups, but failed to normalize the pathology in paracetamol group (fig 6d) though there was marked improvement.

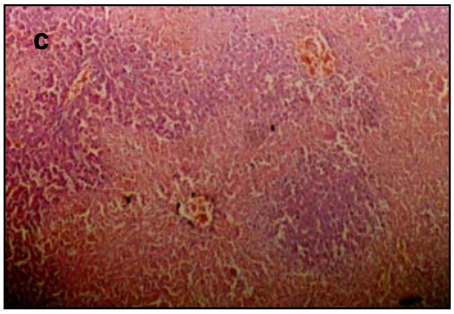
Fig 6: Microphotographs of H&E stained sections of liver



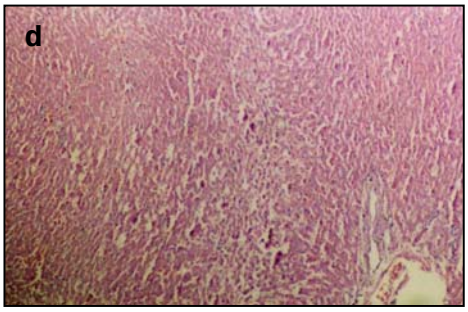
a. Control group (Saline alone 100X)



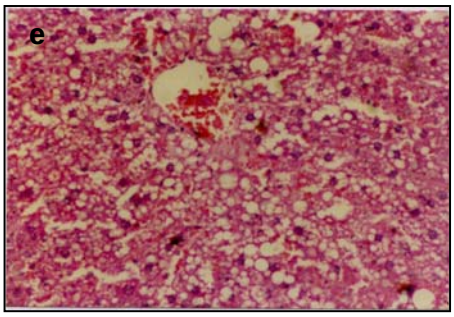
b. Control group (DCE alone 100X)



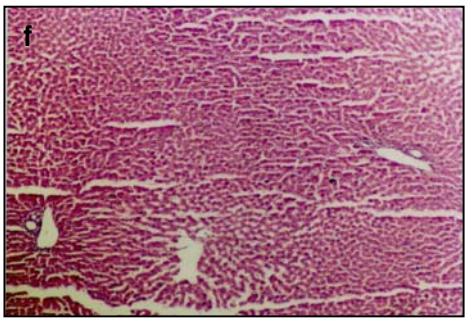
c. Saline **+** Paracetamol (100X)



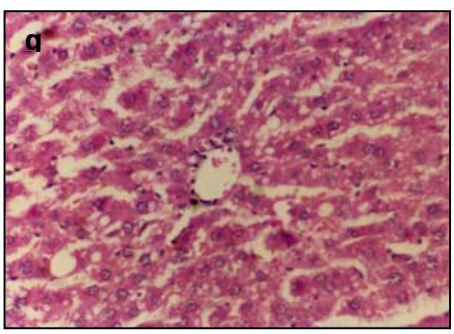
d. DCE **+** Paracetamol (100X)



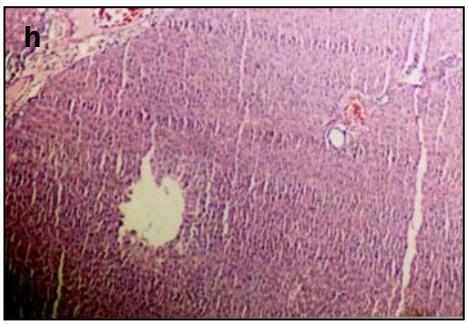
e. Saline + INH (400X)



f. DCE + INH (100X)



g. Saline **+** Alcohol (400X)



h. DCE **+** Alcohol (100X)

Table I: Liver function tests in treated and control animals.

Pre treatment	Treatment 8th day	Enzymes (U/ml)		Bilirubin (mg%)		Prothrombin Time (sec)
		AST	ALT	Total	Direct	
Saline	Saline (control)	141.0±1.53	59.0±0.86	0.430±0.01	0.086±0.00	**16.50±0.43**
	Paracetamol	597.0±4.16***	398.5±9.24***	0.620±0.02***	0.320±0.02***	**35.17±0.31*** **
	INH	212.7±2.95***	142.7±2.86***	0.563±0.02***	0.140±0.01***	**28.67±0.33*** **
	Alcohol	169.3±3.71***	124.7±2.29***	0.540±0.03**	0.125±0.00***	**25.00±0.37*** **
DCE	Paracetamol	174.7±2.11†††	89.0±1.98†††	0.51±0.02††	0.238±0.01††	**28.67±0.61††† **
	INH	147.3±2.86†††	62.67±1.33†††	0.475±0.01††	0.098±0.01†	**17.83±0.60††† **
	Alcohol	147.0±4.16††	62.33±2.22†††	0.465±0.01†	0.093±0.01††	**17.67±0.49††† **
	DCE	142.7 ± 1.20	59.33 ± 0.67	0.4367±0.01	0.0900±0.01	**17.50 ± 0.43**
	$F_{7,40}$	2639	952.6	14.55	78.69	**230.6**

*p<0.05, **p<0.01, ***p<0.001 as compared to control.

†p<0.05, ††p < 0.01, †††p< 0.001 as compared to DCE untreated group.

Discussion

In the present study aqueous extract of *Daucus carota L.* has been investigated for its protective action against hepatotoxicity induced by commonly used drugs like paracetamol, INH and widely abused substance like alcohol. The findings of the present study clearly indicate the hepatoprotective activity of DCE against above mentioned drugs induced hepatotoxicity. In the INH and alcohol models of hepatotoxicity DCE significantly lowered (almost to the extent of restoration to normal) the serum level of marker enzymes *viz.* AST, ALT and also bilirubin as well as PT. However in paracetamol challenged animals DCE though significantly lowered these parameters, failed to normalize them, indicating its lower efficacy, as compared to that in INH and alcohol induced hepatotoxicity.

There is paucity of information regarding similar studies, however DCE has been reported by Bishayee et al.[10] to lower plasma levels of AST, ALT and bilirubin in CCl_4 induced hepatotoxicity.

Histopathological findings of the liver in the present study were parallel to (other findings) marker enzyme level, indicating hepatoprotective activity of DCE.

It is not possible to pinpoint hepatoprotective mechanism of DCE from the findings of the present study. DCE has been reported by Straub[18] and Olson[19] to contain carotenes including β -carotene, α -carotene, γ -carotene, lycopene, cryptoxanthin, leutein, many partly degraded carotenoids such as abscisic acid, trisporic acid, β-*apo-carotenals,* crocetin and many common polar carotenoids, like violaxanthin. It is well known that oxygen free radicals are strongly associated with cellular injury. As reported by Burton some of the above compounds have the potential to scavange the free radicals including the peroxy radicals and thereby, might contribute for the hepatoprotective activity of *Daucus carota*.[20]

DCE has been reported by Bishayee and Chatterjee (1993) to inhibit lipid peroxidation and normalize glutathione related enzymes in CCl_4 evoked liver damage in mice.[21] Findings of the present study suggest that hepatoprotective activity of DCE is mainly linked to other antioxidant enzymes rather normalization of glutathione related enzymes, since DCE failed to provide complete protection against paracetamol induced hepatotoxicity.

Regular consumption of *Daucus carota L.* (carrot), a widely used vegetable, whether provides protection against hepatotoxic insults and the mechanism involved need to be explored.

Acknowledgement

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