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# Hepatoprotective Effect of Carrot (*Daucus carota L.*) on Paracetamol Intoxicated Rats

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**Abstract** - Plant products play a crucial role in the hepatoprotection through its antioxidants property. Therefore, search for modern medicine of plant origin with this property has become a central focus on hepatoprotection today. This study investigated to search a new hepatoprotective agent from natural sources, the methanol extract of nutritional plant, *Daucus carota* leaves was tested against liver damage of albino rats. Levels of serum marker enzymes i.e. SGOT, SGPT (aminotransferases), ALP (alkaline phosphatase) and TB (total bilirubin) in serum respectively. The histopathological changes of liver sections were also compared with the respective controls. 30% paracetamol induced significant ( $P < 0.05$ ) increase in liver enzymes alongwith the hepatic necrosis and other visible disarrangements in hepatic tissues. Simultaneously, oral treatment with kaempferol (KF) isolated from *D. carota* plant reversed to all the serum and liver parameters, dose-dependently, in 30% paracetamol treated rats. The biochemical results were also compared with the standard drug i.e. silymarin. These findings indicate the hepatoprotective potential of *D. carota* fruit against liver damage might be due to the presence of flavonoid like KF constituents.

**Keywords**- Liver damage; kaempferol; *Daucus carota*; hepatoprotection; flavonoid.

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## I. INTRODUCTION

Carrot is a vegetable known scientifically as *Daucus carota L.* which belongs to family Umbelliferae (Apiaceae). Regarding its nutritive value, the carrot has abundant amount of beta carotenes (provitamin A) which give the vegetable its characteristic color. Moreover, carrots are rich in dietary fibers, antioxidants and minerals, especially potassium. The nutrient contents of each 100 g raw carrot were estimated to be as follow: water (89 g), carbohydrates (8.71 g), proteins (0.98 g), lipids (0.24 g), dietary fibers (2.24 g), vitamin A (12 mg), vitamin C (7.1 mg), calcium (33 mg), magnesium (18 mg) and potassium (240 mg), as recorded by Ensminger [1].

Concerning health benefits, the carrots are an excellent source of several antioxidant compounds such as phenolic compounds that play an important role in antioxidant properties of carrots and the other hydroxycinnamic derivatives such as dicaffeoylquinic acids in the extracts may exert some strong antioxidant activities along with chlorogenic acid [2]. The antioxidant compounds of carrot protect against cardiovascular diseases and cancer. Beta carotene protects especially night vision plus it acts as a powerful

antioxidant and provides protection against macular degeneration and development of snile cataracts [1].

Experimental and clinical studies on carrots (powder or extract) and its active constituents (mainly carotenoids) revealed that they have hyperglycemic effect [3, 4]; anticancer activity due to the presence of alpha carotene and falcarinol [5, 6] protective effect against coronary heart disease [7], hypocholesterolemic and hypolipidemic activities [8, 9].

On the other hand, excessive consumption of carotene rich foods as carrots may lead to a condition called "carotenoderma" in which the palms and other skin develop a yellow color because the body slowly convert carotene to vitamin A. Fortunately, this condition disappears after reduction of carrot consumption [1].

Hepatic damages are occurring due to infections, drugs induce toxicity such as Paracetamol (PCM), environmental and social factors such as alcoholism, and so forth [10]. Hepatocytes participates in the detoxification processes of the body and thus become vulnerable to damage through free radical generation [11]. Reactive oxygen free radicals have been known to produce tissue injury through covalent binding and lipid peroxidation and have been shown to augment fibrosis

as seen from increased collagen synthesis [12]. The currently observed rapid increase in consumption of herbal remedies worldwide has been stimulated by several factors, including the notion that all herbal products are safe and effective [13]. The parameters for assessment of degree of hepatoprotectivity are serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin (TB) along with the histopathological examination of hepatic tissues.

The aim of this research was to study the effect of feeding different concentrations of carrot on hepatotoxic rats.

## II. MATERIALS AND METHODS

### A. Animals

Young either male or female albino rats (Wistar strain), weighing 120-150 g were purchased from a local supplier (Sagar Biologicals) which were housed in standard cages and maintained at ambient temperature with 12:12 h light and dark cycles. Animals were allowed free access to food (standard laboratory rodent's chow) and water during the study. All procedures were conducted as per guidelines of a committee CPCSEA and the protocol use in this study was approved (No. Animal Eths. Comms./10/87/40, 22/05/2010) by Institutional Animal Ethics committee.

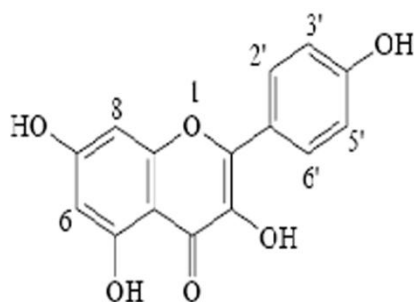


Figure 1: Chemical structure of Kaempferol

### B. Drugs and chemicals

Kaempferol (Cat. No. K0133) were purchased from Sigma-Aldrich Pvt. Ltd., USA. SGOT, SGPT kit, reduced glutathione, thiobarbituric acid and other chemicals were purchased from local suppliers.

### C. Preparation of plant extracts

Fruit of *Daucus carota* were purchased from the local supplier and was identified (No. Bot./Herb./A-1870) by Dr. Pradeep Tiwari, Deptt. of Botany, Dr. H. S. Gour Central University, Saugor (M.P.), India. The dried powdered fruits (2kg) of *D. carota* was extracted with methanol. The concentrated extract was dissolved in water and successively extracted with hexane, ethyl

acetate and butanol. The butanol extract was evaporated under vacuum to yield (30 g) flavonoidal fraction, which gave the positive Shinodha test. The extract was subjected to purification over silica gel column using chloroform: acetone: formic acid (75:16.5:8) as eluent; which yields (25.5 mg) compound 1-6 respectively. These compounds were identified as flavonol glycosides and acid hydrolysis of compound 1, 2 and 6 yield basic flavonoid, Kaempferol (KF) [14], compound 3 yield Isorhamnetin and compound 4 and 5 yield Quercetin [15]. The fraction 1, 2 and 6 were mixed and concentrate the mixture followed by recrystallisation with methanol. The yellow needle shaped crystals of kaempferol (Figure 1) were obtained.

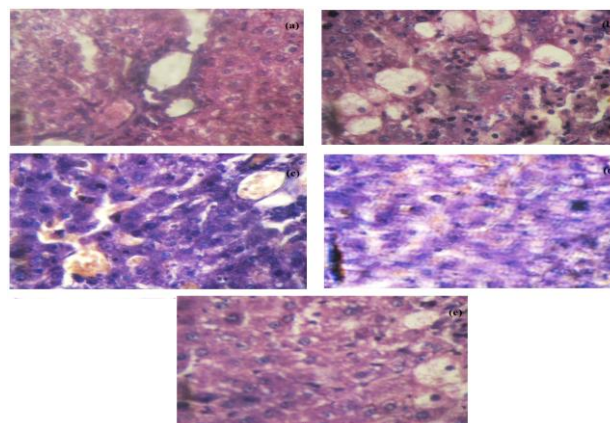


Figure 2: Histological photomicrographs of Vehicle control (a), Paracetamol control (b), Kaempferol control (100 and 200 mg/kg) (c and d) and Silymarin treated (e) rat livers.

TABLE 2. Qualitative chemical examination of various extracts obtained by successive solvent fractionation of the *Daucus carota* L. fruits

| S No | Phytoconstituents      | Pet. ether fraction | Hexane | Ethyl acetate fraction | Butanol fraction |
|------|------------------------|---------------------|--------|------------------------|------------------|
| 1.   | Alkaloids              | -                   | +      | -                      | -                |
| 2.   | Carbohydrates          | +                   | +      | +                      | +                |
| 3.   | Phytosterols           | -                   | -      | -                      | -                |
| 4.   | Flavonoids             | -                   | -      | +                      | +                |
| 5.   | Tannins                | -                   | -      | -                      | -                |
| 6.   | Proteins & Amino acids | +                   | +      | +                      | +                |
| 7.   | Saponins               | +                   | +      | -                      | -                |

#### D. Characterization of isolated flavonoid

##### 1. Solubility study

For the determination of solubility profile of KF, it was taken in accurately weight amount i.e. 2 mg in 10 ml of different solvent with decrease in polarity.

##### 2. Phytochemical investigations

Different phytochemical tests were applied on different extract for confirmation of alkaloids, carbohydrates, tannins, resins, volatile oils and also for flavonoids.

##### 3. Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) thermogram was recorded with a Mettler Star SW 9.00 DSC instrument. The sample was sealed in the aluminum crimp cell and heated from 20 to 400°C at 10°C/min. The onset and endset peak temperatures of KF were recorded.

##### 4. Thin Layer Chromatography

Thin layer chromatography of KF was performed using the Silica gel 60 F254 was used as an adsorbent and ethyl acetate:formic acid:water 6.5:2.0:1.5 (v/v/v) as mobile phase. After development, the plate was placed in iodine chamber. The  $R_f$  value of KF was noted and compared with the standard.

##### 5. Melting Point determination

Melting point of KF was determined by melting point apparatus (Scientific International, New Delhi, India).

#### 6. Infrared Spectroscopic studies

Fourier transform infrared (FTIR) spectra of KF was obtained on the Perkin Elmer 400 FTIR spectrometer using the wave number 500-4000  $\text{cm}^{-1}$ . Homogenous KBr pellets were prepared by mixing and grinding 500 mg potassium bromide and 5 mg of sample in a mortar and then compressing this fine powder to get hard disc which was inserted into the infrared sample holder carefully.

#### 7. Nuclear Magnetic Resonance Spectroscopy

The  $^1\text{H-NMR}$  of KF was recorded in Bruker 400 Ultrashield<sup>TM</sup> using DMSO as a solvent.

#### E. Treatment schedule

Rats were divided into four groups (n=6). Group I (Vehicle control) and Group II (toxic control) received olive oil as a vehicle for 7 days. Group III received KF suspension in CMC (0.5%) orally. Group IV animals were treated with standard drug i.e. silymarin (SL) orally for 7 days. All the groups except vehicle control group received an equal mixture of PCM (750 mg/kg; orally) and olive oil for 7 days after 30 minutes on the last day of respective treatment.

#### F. Evaluation of hepatoprotective activity

Blood samples were withdrawn on the 8<sup>th</sup> day from the retro-orbital plexus of rats and kept in separate centrifuge tubes. The blood was allowed to coagulate at room temperature for about 1 h and then centrifuged at 3000 rpm for 10 minutes. The serum was separated and kept at -20°C for estimation of SGOT, SGPT, ALP and TB.

TABLE 3. Hepatoprotective activity of isolate containing Kaempferol

| S. No. | Treatment Design                            | Serum Glutamateoxaloacetate transaminase (IU/L) (SGOT±SEM) | Serum Glutamatepyruvate transaminase (IU/L) (SGPT±SEM) | Serum Alkaline Phosphatase (IU/L) (ALP±SEM) | Serum Total Bilirubin (mg/100ml) (TB±SEM) |
|--------|---|--|--|---|---|
| 1.     | Normal Control                              | 115.26±2.18  | 97.24±2.73   | 16.72±1.15                                  | 0.79±0.1                                  |
| 2.     | Paracetamol Control                         | 419.18±4.39*   | 325.32±4.36*   | 42.17±2.26*                                 | 2.36±0.37*                                |
| 3.     | Isolate (Kaempferol) Control (100 mg/Kg BW) | 279.41±4.37**  | 217.42±3.74**  | 33.14±1.02**                                | 1.73±0.11**                               |
| 4.     | Isolate (Kaempferol) Control (200 mg/Kg BW) | 149.63±3.53**  | 129.14±3.24**  | 27.36±1.10**                                | 1.06±0.13**                               |
| 5.     | Standard (Silymarin) Control                | 133.26±3.64*   | 112.67±3.07*   | 22.15±1.37*                                 | 0.94±0.16*                                |

TABLE 4. Percentage protection of different treatment groups

| S. No. | Treatment Design                            | Serum Glutamateoxaloacetate transaminase (SGOT) % Protection | Serum Glutamatepyruvate transaminase (SGPT) % Protection | Serum Alkaline Phosphatase (ALP) % Protection | Serum Total Bilirubin (TB) % Protection |
|--------|---|--|--|---|---|
| 1.     | Normal Control                              | -  | -  | -   | -                                       |
| 2.     | Paracetamol Control                         | -  | -  | -   | -                                       |
| 3.     | Isolate (Kaempferol) Control (100 mg/Kg BW) | 45.98  | 47.31  | 35.48   | 40.13                                   |
| 4.     | Isolate (Kaempferol) Control (200 mg/Kg BW) | 88.69  | 86.01  | 58.19   | 82.8                                    |
| 5.     | Standard (Silymarin) Control                | 94.07  | 93.23  | 78.66   | 90.44                                   |

### G. Histopathological study

Histological study of liver sections was done with hematoxylin and eosin staining. The stained sections were observed under the microscope at 40X [16].

### H. Statistical analysis

Statistical analysis was carried out using Sigmaxstat 3.5; statistical software. Data were analysed using one-way ANOVA followed by Tukey test. In all the results, the criterion for statistical significance was  $p < 0.05$ .

## III. RESULTS

### A. Solubility study

Solubility profile of the KF was observed in different solvents and findings were enlisted in Table 1.

### B. Phytochemical investigations

The results obtained from phytochemical investigations were reported in Table 2.

### C. Thin Layer Chromatographic study

The  $R_f$  value of KF was found to be 0.78 and also compared with standard.

### D. Infrared Spectroscopic Analysis

The IR spectrum of KF showed characteristic peaks at 3411 (O-H stretching), 1663.1 (aryl ketonic stretching), 1608.8, 1523.5, 1496 (aromatic C-C stretching), 1383.1 (in plane O-H bending of phenols), 1318.9 (in plane bending of C-H bond in aromatic hydrocarbon), 1265 (C-O stretches of aryl ether), 1203 (C-O stretches of phenol), 1167 (C-CO-C stretch and bending in Ketone) and 940.6, 821.4, 677, 602.3  $\text{cm}^{-1}$  (out of plane C-H bending of aromatic hydrocarbon).

### E. Nuclear Magnetic Resonance Spectroscopy

$^1\text{H}$  NMR (DMSO, 400 MHz) of KF showed major chemical shift ( $\delta$ ) at: 12.843 (s, 2H, 5-OH), 10.869 (s, 1H, 7-OH), 9.350 (s, 1H, 3-OH), 7.35 (s, 1H, H-8), 6.85 (s, 1H, H-5'), 6.37 (s, 1H, H-4'), 4.58 (d, 1H, H-1'), 4.04 (t, 1H, H-2'), 3.68 (d, 1H, H-6'a), 3.41 (dd, 1H, H-6'b) 3.20 (m, 1H, H-3'), 3.16 (m, 1H, H-5'), 3.14 (m, 1H, H-4') ppm.

### F. Evaluation for hepatoprotective activity

The results obtained for serum levels of SGOT, SGPT, ALP and TB along with its percentage protection on liver tissue are depicted in Table 3 and 4 respectively. The SGOT ( $F_{(4,29)} = 121.03$ ,  $P < 0.001$ ), SGPT ( $F_{(4,29)} = 754.09$ ,  $P < 0.001$ ), ALP ( $F_{(4,29)} = 46.01$ ,  $P < 0.001$ ) and TB ( $F_{(4,29)} = 10.71$ ,  $P < 0.001$ ) levels were significantly increased in PCM control groups as compared to vehicle control. This effect was reversed by co-treatment with KF which showed significantly ( $P < 0.05$ ) decreased levels of SGOT, SGPT, ALP and TB as compared to PCM treated control group.

### G. Histopathological study

Histopathological profile of liver of PCM treated rats showed intense centrilobular necrosis and vascularisation. Fatty degeneration was observed in areas other than the antrilobular zones. Mononuclear infiltration in the areas of fatty change was observed. Liver of rats treated with KF showed significant signs of amelioration of PCM induced liver injury as evident from the presence of normal hepatic cords, absence of necrosis and vacuoles and less degree of infiltration by inflammatory cells which was comparable to the SL. Histological changes with different control groups are presented in Figure 2.

#### IV. DISCUSSION

Although, *Daucus carota* has antioxidant activity due to its free radical scavenging effect i.e. why it also protect hepatocytes from oxidation due to PCM intoxication hence reduce hepatic injuries.

In the present study, KF was isolated from *D. carota* and characterized by solubility study, DSC, TLC, melting point, IR and NMR spectroscopic analysis. These physicochemical and spectroscopic investigations clearly showed evidence for the presence of KF in isolate.

*In vivo* hepatoprotective study showed that the KF was active therapeutic phytoconstituent for ameliorating the effect of hepatotoxic agent i.e. paracetamol and lowering the serum level of SGOT, SGPT, ALP and TB.

Oxidative free radicals increases during hepatotoxicity are reported to play an important role in the pathogenesis of this condition. There are many evidences which have shown KF to have antioxidant effects [17]. This makes us interested to evaluate the antioxidant effect of KF for the possible therapeutically supportive role in hepatoprotection.

On the basis of investigations and their results, we are able to reach the conclusion that the *D. carota* has therapeutic potential against hepatotoxic agent like PCM due to presence of flavonoid which is named as kaempferol.

#### V. ACKNOWLEDGMENT

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#### REFERENCES

- [1] A.H. Ensminger and M.K.J. Ensminger, "Food for health: A Nutrition Encyclopedia", Clovis, California, Peguss press, USA, 1986.
- [2] D. Zhang and Y. Hamazu, "Phenolic compounds and their antioxidant properties in different tissues of carrots", *J Food Agric Environ*, vol. 2(issue 1), 2004, pp. 95-100.
- [3] K. Suzuki, Y. Ito and S. Nakamura, "Relationship between serum carotene and hyperglycemia: A population-based cross-sectional study", *J Epid*, vol. 12(issue 5), 2002, pp. 357-366.
- [4] K. Ylonen, G. Alfthan and L. Groop "Dietary intake and plasma concentrations of carotenoids and tocopherols in relation to glucose metabolism in subjects at a high risk of type 2 diabetes", *Am J Clin Nutri*, vol. 73(issue 6), 2003, pp. 424-414.
- [5] D.S. Michaud, D. Feskanich and W.B. Rimm, "Intake of specific carotenoid and risk of lung cancer in 2 prospective US cohorts", *Am J Clin Nutri*, vol. 72(issue 4), 2002, pp. 990-997.
- [6] M. Kobaek-Larsen, L.P. Christensen, W. Vach, J. Ritskes-hoitinga and K. Brandt, "Inhibitory effects of feeding carrots of falcarinol on development of azoxymethane-induced preneoplastic lesions in the rat colon", *J Agric Food Chem*, vol. 53( issue 5). 2005, pp. 1823-1827.
- [7] J.M. Gaziano, J.E. Mason and L.G. Branch, "A prospective study of consumption of carotenoids in fruits and vegetables decreased cardiovascular mortality in the elderly", *Ann Epidmiol*, vol. 5, 1995, pp. 255-282.
- [8] C. Nicolle, N. Cardinault, O. Aprikan, et al., "Effect of intake on cholesterol metabolism and antioxidant status in cholesterol fed rat", *Europ J Nutr*, vol. 42(issue 5), 2003, pp. 254-261.
- [9] C. Nicolle, E. Gueux, C. Lab, et al., "Lyophilized carrot ingestion lowers lipemia and beneficially affects cholesterol metabolism in cholesterol fed C57BL/6j mice", *Europ J Nutr*, vol. 43(issue 4), 2004, pp. 237-245.
- [10] M. Ali, R. Ramachandran, M.R.M. Rafiullah, et al., "Prevention of carbon tetrachloride-induced hepatotoxicity by the ethanol extract of *Capparis moonii* fruits in rats", *Pharmaceutical Biology*, vol. 42, 2004, pp. 286-288.
- [11] E. Porchezian and S.H. Ansari, "Hepatoprotective activity of *Abutilon indicum* on experimental liver damage in rats", *Phytomedicine*, vol. 12, 2005, pp. 62-64.
- [12] J.C. Geesin, J.S. Gordon and R.A. Berg "Retinoids affect collagen synthesis through inhibition of ascorbate-induced lipid peroxidation in cultured human dermal fibroblasts", *Archives of Biochemistry and Biophysics*, vol. 278, 1990, pp. 350-355.
- [13] O. Said, K. Khalil, S. Fulder, et al., "Ethnobotanical survey of medicinal herbs of the middle eastern region", *Journal of Ethnopharmacology*, vol. 83, 2002, pp. 251-265.
- [14] L. Bin and H. Luo, "Isolation of Flavonol glycosides from *Daucus carota*", *Chemical Journal on Internet*, vol. 5, 1992, pp. 3.

- [15] D.D. Sawikar and E.D. Phandre, "Chemical constituents of *Maytenus emarginata* wild" Indian J Chem, vol. 31, 1992, pp. 639.
- [16] G.L. Humanson, "Animal Tissue Technique", San Francisco:WH Freeman and Company, 2<sup>nd</sup> ed, 1962, pp. 136-137.
- [17] J.N.T. Simplice, D.T. Jean, H. Léopold, et al., "Antimicrobial and antioxidant activity of kaempferol rhamnoside derivatives from *Bryophyllum pinnatum*", BMC Research Notes, vol. 5, 2012, pp. 158-60

