Phytochemical and Hepato Toxicity Study of *Coriandrum sativum*

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Abstract

Coriandrum sativum is one of the most important species in the World. This study is aimed to estimate chemical constituent and chemical constituents. As previously reported that *C. sativum* is hepatoprotective drug as enzymetic and biochemical assay performed. Aqueous extract of *C. sativum* fruit was subjected to carried out in rats for studying the effect of the plant infusion on paracetamol induced free radicals and hepatotoxicity. The biochemical results showed that administration of paracetamol recorded a significant increase in plasma alanine imino transferase (ALT) aspartate amino transferase (AST) alkaline phosphate (ALP), bilirubin, urea and creatinine with significant decrease in plasma total protein, albumin and some antioxidant biomarkers compared to normal rats. ANOVA analysis indicated that rats which supplemented with aqueous extract and then administrated with paracetamol showed significant improvement in all biochemical parameters which become near to control.

Key words : *Coriandrum sativum*, fruit, liver enzyme, paracetamol, hepato-protection.

Introduction

Plants have been one of the important sources of medicines even since the beginning of human civilization. A review represents that 83% of the world's population relies on medicinal plants for their primary healthcare. The medicinal plants have some chemical substances or group of compounds that produce a definite physiological action in the human body. These chemical substances are called secondary metabolites[1].

Coriandrum sativum (Dhania) is an annual herb belong to the family Apiaceae. It has eleven components of essential oils, six types of acids, minerals and vitamins, each having a number of beneficial properties. It also contains antioxidants, which can delay or prevent the spoilage of food seasoned with this spice. A study found that both the leaves and seed are antioxidants, but the leaves were found to have a stronger effect.[2] *C. sativum* has been documented as a traditional treatment for diabetes. A study on mice found that *C. sativum* extract had both insulin-releasing and insulin-like activity. However they are good source of dietary fiber; recommended in cholesterol controlling and weight reduction programs[3-5].

The medicinal actions of plants are unique to particular plant species or groups and are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct. They are usually subdivided according to their substituent's into flavanols (kaempferol, quercetin), anthocyanins, flavones, flavonones and chalcones. These flavonoids display a remarkable array of biochemical and pharmacological actions viz., anti-inflammatory, antioxidant, anti-allergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities[6,7]. These compounds appear to play vital roles in defence against pathogens and predators and contribute to physiological functions such as seed maturation and dormancy. They are synthesized from phenyl propanoid and acetate derived precursors. Flavonoids are important for human beings due to their antioxidative and radical seavenging effects as well as their potential estrogenic and anticancer activities[8,9]. Quercetin belongs to this group of plant pigments called flavonoids that are largely responsible for the colours of many fruits, flowers and vegetables[10]. Quercetin works as anti-inflammatory, antioxidant, anticancer agents. Flavonoids possess a variety of biological activities that are thought to contribute towards protecting humans against degenerative diseases. Quercetin, kaempferol and isorhamnetin have been shown to have an anti-inflammatory effect on activated macrophages. In addition, quercetin and kaempferol show chemopreventive properties in brain tumours and synergistically suppress cell proliferation in human gut cancer lines. Quercetin a main aglycone in human nutrition is a potent free radical scavenger and antioxidant[9,11]. Antioxidant effects of quercetin on the mucosa of the nasal turbinate were demonstrated. Higher intakes of kaempferol resulted in a lower risk of coronary heart disease. In addition, isorhamnetin revealed distinct vasodilatory effects in animal models, suggesting vascular protective effects in human cardiovascular diseases.

Materials and Methods

Plant materials

The plant materials of *C. sativum* were collected from the plants grown in and around Jaunpur district U.P., India. The plant materials were dried in shade separately and powered coarsely.

Reagents

Reagents and chemicals Methanol and acetic acid were of HPLC grade and were purchased from Merck Company. Deionized water was prepared by a Milli-Q Water Purification system.

Extraction and separation

The dried powder samples (flower 300g) were extracted with methanol: water (80:20) by soaking for 40 Hrs at room temperature (25ºC). The methanolic extract was decanted, filtered under vacuum, concentrated in a rotary evaporator (36ºC). The resulting crude extract from the flower of CF (20.0g) was fractionated successively with ethyl acetate (EtOAc), n-butanol (BuOH), and the yield of soluble fractions of EtOAc (1.8g), BuOH (0.95g) and water (20 g). The ethyl acetate (methanol) soluble fraction from flower of CF (2.0g) was loaded into a flash chromatography column (silica gel for mesh 260-400 revelries grace column silisep) and eluted with different solvent system for gradient programme (like, EtOAc/n-hexane and MeOH/chloroform solvent systems). For this separation were separated by 5 sub fractions (EA5-EA10 and methanol: chlorofrom 7-10). Frequently each fraction was checked with TLC. For different mobile phase to getting single spot using by iodine vapour (solvent system for t1 and t2 chloroform: methanol: acetic acid; water 95:5:5:2 and t3).

Biochemical study:

Experimental Animals: Adult male albino rats with initial weights ranging from 100-150 g were used as experimental animals for biochemical study. All experimental animals were provided from the Breeding Unit of the National Research Centre (T.D. College, Jaunpur). The animals housed individually in stainless steel wire mesh cages. They were maintained for one week, as an acclimatization period. Commercial standard pellets and tap water were supplied *ad libitum*.

Experimental Design: Twenty four adult rats were used for studying the effects of the plant infusion on panadol (paracetamol) induced free radicals and hepato-toxicity. The rats were equally divided into four groups (6 rats in each group):

Group 1: Normal control, rats were giving drinking tap water.

Group 2: Rats were supplemented with freshly prepared aqueous extract of *Coriandrum sativum* (2.5g/100ml water) for thirty days, to examine safety of plant extract concentration as a drink.

Group 3: Rats were intoxicated after 4 weeks by oral administration with paracetamol (2g/kg. B.W.).

Group 4: Protected rats: where rats were maintained on drinking freshly prepared Sudanese *C. sativum* infusion (2.5g/100ml boiled water) for (28 days) instead of tap water and then rats were intoxicated by oral administration with paracetamol (2g/kg B.W.).

The experiment duration was continued for 30 days. The rats were killed after two days from oral single dose of paracetamol administration.

Blood Sampling: Blood samples were withdrawn on heparinized tubes. Plasma was used for determination of liver and kidneys function and some antioxidant biomarkers. The (RBCs) was washed several times with cold saline solution. The packed RBCs were stored at 20ºC for determination of Glutathione peroxidase.

RESULT

Phytochemicals

Extract of green C. sativum contains major and minor constituents. Major chemical constituents are- volatile oils upto 1.7%. The distilled oil contains 65-70% of (+) – linalool (identifies coridandrol). Minor chemical constituents are – Monoterpene hydrocarbons viz α–pinene, β-pinene limonene, γ-terpinene, p-lymene, broneol, cilronwllol, Xmphone, Geraniol and Geranylacetate, hydrocylic compounds viz-pyranine, pyridine, thiazole, furan, tetrahydrofuran derivatives. Isocoumacin viz. coriandrin, dihydrocoriandrin, corian drones A-E glazonoids, phthalides viz- neochidiline, phenolic acids and sterioids flavonoids.

Three new isocoumarins coriandrones C-E were isolated from whole plant extract and their structure established from spectrol and chemical evidences[12].

Biochemical Assays:

Antioxidant Capacity: Plasma total antioxidant capacity, plasma catalase and cellular glutathione peroxidase were determined by using assay kits.

Liver and Kidney Functions: Plasma total protein, albumin, alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphates (ALP), total bilirubin and direct bilirubin were carried out by using assay kits [18-23]. Kidney functions (plasma Creatinine and urea) were also carried out [24, 25].

Table 1: Effect of administration of paracetamol and the aqueous extract of Sudanese *Coriandrum sativum* fruits on liver enzymes in plasma

	Parameters		
Groups	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**
Control	61.57±4.28[a]	16.43±1.54[a]	169.09±7.54[a]
Extract	61.17±3.97[a]	15.50±2.28[a]	166.87±10.15[a]
Paracetamol	85.00±5.48[b]	39.57±2.64[b]	240.19±8.49[b]
Paracetamol + extract	71.86±3.63[c]	30.00±2.38[c]	221.36±13.09[c]

Data presented as mean ± SE
Values in the same column with the same superscripts are not significantly at (P < 0.05).

Table 2: Effect of administration of paracetamol and the aqueous extract of Sudanese *Coriandrum sativum* fruits on some liver bio-indicator in plasma

Parameters					
Groups	**T.prot.(g/dl)**	**Alb(g/dl)**	**T.bili(mg/dl)**	**D.bil(mg/dl)**	**Ind. Bil (mg/dl)**
Control	6.47±0.39[a]	3.15±0.21[a]	0.34±0.03[a]	0.14±0.01[a]	0.19±0.02[a]
Extract	6.95±0.33[a]	3.22±0.20[a]	0.32±0.03[a]	0.14±0.01[a]	0.18±0.01[a]
Paracetamol	5.97±0.18[b]	2.88±0.19[b]	0.50±0.02[b]	0.24±0.02[b]	0.26±0.02[b]
Paracetamol + extract	6.63±0.25[a]	2.98±0.35[a]	0.41±0.03[c]	0.19±0.02[c]	0.22±0.03[c]

Data presented as mean ± SE
Values in the same column with the same superscripts are not significantly at (P < 0.05).

The aqueous extract showed strong DPPH free-radical scavenging activity (88.5% at 400ug/ml), compared with the standard reference (TBHQ) (99.73% at the same concentration).

Biochemical Results:

Table 1 showed the effect of administration of paracetamol and the aqueous extract of Sudanese *C. sativum* fruits on some liver enzymes. The results indicated that rats which administrated only paracetamol (group 3) recorded a significant increase in plasma AST, ALT, ALP and GGT (85±5.48, 39.57±2.64 and 240.19±8.49) compared to normal control rats (group 1) (61.57±4.28, 16.43±1.54 and 169.09±7.54) respectively. ANOVA analysis indicated that rats supplemented the aqueous extract then administrated with paracetamol (group 4) showed a significant decrease in plasma AST, ALT, and ALP (71.86±3.63, 30±2.38 and 221.36±13.09), respectively compared to the group of paracetamol intoxicated rats (group 3). Table 2 showed the effect of administration of paracetamol and the aqueous extract of Sudanese *C. sativum* fruits on some liver bio-indicators, total proteins, albumins and bilirubin group. A significant decrease in plasma total proteins and albumin was noticed in paracetamol rats (5.97±0.18 and 2.88±0.19) compared with control group (6.74±0.39 and 3.15±0.21). A significant increase in plasma total bilirubin, direct bilirubin and indirect bilirubin were obtained in paracetamol rats (0.50±0.02,

0.24±0.02 and 0.26±0.02) compared with control group (0.34±0.03, 0.14±0.01 and 0.19±0.02), respectively. ANOVA analysis indicated that rats which supplemented the aqueous extract then the paracetamol showed a significant increase in total proteins and albumin with a significant decrease in bilirubin, which become near to normal control. Results of liver function tests, as shown in Tables 1 and 2, revealed that supplementation of the aqueous extract to rats (group 2) had no significant effect on all the tested parameters included in liver function tests (AST, ALT, ALP total proteins, albumin, total bilirubins, direct bilirubin, indirect bilirubin) compared to control rats. The results of kidneys function tests, rats which were supplemented with aqueous extract (group 2) recorded a significant decrease in plasma creatinine with non-significant change in plasma urea level (36.17±2.17, 0.61±0.04) compared to control (37.78±1.59 and 0.67±0.04). A significant increase in plasma urea and creatinine occured in group of paracetamol intoxicated rats (42.69±1.08 and 0.76±0.03) compared to control group, with a significant decrease occurred in rats protected with Coriandrum sativum (37.01±2.51 and 0.67±0.04) (group 4). The effect of aqueous extract of *C. sativum* fruits and the administration of paracetamol on some antioxidant biomarkers (plasma total antioxidant capacity (TAC) and plasma catalase activity (CAT). ANOVA analysis indicated that rats supplemented with aqueous extract (group 2) recorded a significant increase in TAC and GPx with non-significant change in CAT activity (1.79±0.19 mM/L, 412.86±50.00 U/ml and 0.25±0.07 U/ml) compared to control (1.15±0.13 mM/L, 0.16±0.03 U/ml and 386.17±28.02 U/ml), respectively. Administrated only paracetamol recorded a significant decrease in TAC (0.92±0.06), CAT (198.91±19.54) and Gpx (0.12±0.02), compared to normal control rats. Rats supplemented with aqueous extract and then administrated with paracetamol showed a significant increase in TAC (1.12±0.15), CAT (289.43±41.89) and GPx (0.16±0.03) levels compared with paracetamol toxicated rats. On the other hand, compared to normal control, the values of aqueous extract supplemented group return back near to control values in all previous parameters.

DISCUSSION

Hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites, when a part of paracetamol activated by hepatic cytochrome P-450 a highly reactive metabolite; N-acetyl-p-benzen-qunone imine is generated[14-16]. Generally, this metabolites disactivates antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase. N-acetyl-p-benzoquinone imine (NAPQI) is normally detoxified by conugation with reduced glutathione to form mercapturic acid which is excreted in urine[17]. The NAPQI then cause acylation or oxidation of cytosolic membrane protein and generation of reactive oxygen which leads to further oxidation of protein thiols and lipid peroxidation, DNA fragmentation and ultimately cell necrosis. Damage to the liver is not due to the drug itself but to a toxic metabolite NAPQI, which is produced by cytochrome P450 enzymes in the liver. In normal circumstances this metabolite is detoxified by conjugating with glutathione in phase 2 reaction. In overdose large amount of NAPQI is generated which overwhelm the detoxification process and lead to damage to liver cells[18-20]. Paracetamol hepatotoxicity was reflected in an increase ($P<0.05$) in serum ALT, AST, ALP activity and significant decrease ($P<0.05$) in total proteins and albumin level. Co-administration of the aqueous extract of Sudanese *C. sativum* fruits (30 mg/ml water) decreased the elevated serum enzyme activities and increased total protein and albumin[21-23]. Bilirubin is one of the most useful clinical markers to diagnose the severity of necrosis, is a measure of binding conjugation and excretory capacity of hepatocytes[24-25]. The level of serum bilirubin was significantly increased in paracetamol treated group and returns back near to normal evel in aqueous extract treated rats group. Antioxidants constituents of the plant material act as radical scavengers and help in converting the radicals to less reactive species[26-28]. The liver breaks down or modifies toxic substances and most medicinal products in a process called drug metabolism[29,30]. This sometimes results in toxication, when the metabolite is more toxic than its precursor. Excess consumption of certain toxic chemicals

such as antibiotics, chemotherapeutic, peroxidised oils, acetaminophen, aflatoxin, carbon tetrachloride, chlorinated hydrocarbon, alcohol lead to infection and autoimmune disorder[31-33].

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