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HEPATOPROTECTIVE ACTIVITY OF *CARICA PAPAYA* AGAINST CARBON TETRACHLORIDE INDUCED HEPATIC DAMAGE ON RATS

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Abstract

Liver illnesses are an increasing global concern; nevertheless, there are currently no effective preventive or treatment options available, which contributes to an exceptionally dismal prognostic and high mortality rate associated with this type of disorder. Hepatoprotective is a key fitness problem in the field of modern medicine; as a result, the search for a novel effective treatment that does not have any side effects is continuing. Hepatoprotective potential of *Carica papaya* was evaluated against Carbon Tetrachloride induced hepatic damage on rats. Thirty six healthy albino rats were divided into six groups, each group with six rats with a weight range of 30–40 gm were selected for the experiment for a period of 14 days by using 36 rats. Biochemical Assay and Histopathological Studies of Rat Liver was performed. The Ethanolic extract of *carica papaya* in combination reduces the level of serum total protein specifically the elevated bilirubin levels. Single administration of CCl_4 adversely protein metabolism of body there by inhibiting protein synthesis. The Ethanolic extract of *carica papaya* plant have the potency to increase protein synthesis and reverse the effect of CCL_4.

Key words: *Carica papaya, Hepatoprotective, Histopathological, bioactive compounds.*

Introduction

Papaya plant which is commonly known as *Carica papaya* belongs to family Caricaceae. Some of the species of this family is used for treatment of various diseases. Leaves of this plant are known to contain certain bioactive compounds like papain, simopapain, cystatin, α-tocopherol, ascorbic acid, flavonoids, cyanogen, glycosides and glucosinolates. These bioactive compounds increase the amount of antioxidants in the blood and reduce the level of lipid peroxidation (Nisa *et al.*, 2019).The seeds of carica papaya rich source of fiber, sarcotesta linoleic acid, myristic acid, stearic acid, gadoleic acid ,oleic acid, palmitoleic acid and linolenic acid. The seed oil is rich phytoactive compounds like favonoids, alkaloids and saponins (Shaban *et al.*,2021).The leaves and fruit of these plant possess medicinal properties like hepatoprotective, anti-fertility, hypoglycaemic, anti-inflammatory, abortifacient as well as anti tumor and wound healing activites due to presence of carotenoids namely β- carotene, lycopene, anthraquinones glycoside etc. Traditionally leaves are used in various formulations and it is checked for

numerous parameters like ash values, swelling index, moisture content, extractive value etc (Yogiraj *et al.*, 2014).

The word liver is derived from Greek word "hepar". Liver is sometimes also called as "chemical factory" of the body, because it plays a key role in metabolism, secretion and storage of macro and micro molecules. It is also involved in detoxification of body. life style and higher use of toxins, pesticides, pharmaceuticals leads to liver intoxication which shows symptoms like cellular necrosis, increase in tissue lipid peroxidation, increase in level of serum glutamate oxaloacetate transaminase (SGOT/AST) , serum glutamate pyruvate transaminase (SGPT/ALT) triglycerides, cholesterol, bilirubin and alkaline phosphatase in blood as well as depletion in the tissue glutathione (GSH) levels. Drugs and other harmful chemical produces heptaotoxins that eventually damage liver and shows the symptoms that are similar to most of the naturally occurring liver disease (Arige *et al.*,2017). Recalcitrant compounds called "Xenobiotics" are persistent organic pollutants that persist for a long time in environment these include carbon tetrachloride (CCl_4), hydrocarbons, pesticides and drugs such as paracetamol etc. These compounds are major cause of hepatocyte injuries associating with acute or chronic liver diseases which is the major cause of death worldwide (Dash *et al*., 2007). The major cause of morbidity and mortality around the word is Liver related diseases. Drug induced therapies is the common reason for hepatic dysfunction. Research has shown that oxidative stress by chemicals like carbon tetrachloride (CCl_4) is major contributor in different hepatic disorders like liver lesions and liver fibrosis etc (AL-Mashhadani *et al*., 2019).

Carbon tetra chloride is one of the most potent Xenobiotic compound that induces damage to kidney and liver. Therefore it is used for screening against hepatoprotective or liver treatment drugs(Mahmoodzadeh *et al*.,2017). Experimental test on animal like drug induced liver injury , carbon tetra chloride is commonly used for this purpose because it is the most common hepatotoxin agent. Traditional system of medicine like Ayurveda, Siddha, Unani etc have leave behind modern system of medicines in terms of reliable liver protective drugs. Now a day's various researches have been made on medicinal plants for hepatoprotective activity. Numerous plants have been used for preparing medicines for curing hepatotoxicity (Singhal and Gupta, 2012). The major source of CCl_4 is chlorine producing factories, chloromethane production units these industries emit CCL_4 directly into air as well as in soil and water. Carbon tetra chloride produce free radicasl which induces lipid peroxidation (LP), protein oxidation, and DNA damage by cytochrome (CYP) 450 mediated cytotoxicity this compound metabolize into trichloromethyl radical; CCl_3 and trichloromethyl peroxyl radical; CCl_3OO etc. which leads to various diseases including Liver diseases (Shaban *et al.*,2021).The present investigation is based on potential hepatoprotective activity of *C. papaya* against carbon tetrachloride (CCl_4) induced toxicity in comparisons with silymarin a well known antihepatotoxic agent.

Materials and Methods

Plant material

Carica papaya belongs to family Caricaceae, leaves of plant are carefully collected from agriculture field at Raipur district of Chhattisgarh in the absence of fungal infection. Harvested leaves were identified and classified with the help of herbarium specimens at MATS University, Raipur, Chhattisgarh.

Extraction

Collected leaves of plant were washed in tap water two to three times to remove surface dirt. Leaves were then air dried at

room temperature for about 2 weeks. Dried leaves were grinded into fine powder using a mixer grinder. This fine powder was used for extraction in a Soxhlet apparatus.Leaf extract of *Carica papaya* was prepared by adding 25 g of leaf powder in 80% ethanol in Soxhlet extractor for 48 h. The obtained extract was then filtered by using many layers of muslin cloth and concentrated in a rotator evaporator for 12 h at 50^0 C. It was then preserved in airtight bottles that were used for further studies.

Experimental Animals

For experiment 6–8 age weeks, 36 healthy albino rats with a weight range of 30–40 gm were purchased from animal house. These rat were grouped and kept in polypropylene cages under standard environmental conditions with light-dark cycle of 12/12 h. Before experimental procedure rat were given normal diet and water under laboratory conditions for 1 week. The above mentioned procedures were reviewed and approved by the University Animals Ethical Committee.

Experimental Induction of CCl_4 Hepatotoxicity

The experiment was performed for a period of 14 days by using 36 rats that were divided into 6 groups, each group has 6 rats. Group I was named as control group in this group rats were treated with only normal saline at the dosage rate of 1 ml/kg/day body weight daily. In group II rats were treated with CCl_4 intra peritoneally at the dosage rate of 1ml/kg/day (1:1 of CCl_4 in olive oil) body weight over the whole period of experiment. In group III rats were treated with CCl_4 1ml/kg/day (1:1 of CCl_4 in olive oil) and silymarin at the dosage of 100 mg/kg/day orally. Group IV were administered with ethanolic extract of *C.papaya* leaves at 100 mg/kg/day body weight and a dose of CCl_4 1ml/kg/day (1:1 of CCl_4 in olive oil) Group V rats were treated with ethanolic extract of *C. papaya* leaves at 200 mg/kg/day body weight and dose of CCl_4 1ml/kg/day (1:1 of CCl_4 in olive oil) daily and in group VI rats were treated with ethanolic extract of leaves of *C. papaya* at 400 mg/kg/day body weight and dose of CCl_4 1ml/kg/day (1:1 of CCl_4 in olive oil).All groups were incubated for 14 days and exposed to the above procedure daily.

Blood Collection and Biochemical Assay

At the end of 4 weeks experimental period the rats were sacrificed by anesthetizing with diethyl ether (Shaban *et al.*,2021 and Adeneye *et al.,*2009) after that blood samples of rats were collected into plain sample bottles (Adeneye *et al.,*2009). The collected blood samples were allowed to clot for about 45 min at room temperature (Dash *et al*., 2007) and then the blood serum of samples were obtained by centrifuging blood samples at 2500 rpm at 300^0 C for 15 min (Wahid *et al.,*2016) as per the standard procedures the total protein conc. (Shaban *et al.*,2021) and serum marker enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), bilirubin in blood serum were estimated (Gond and Khadabadi,2008).

Histopathological Studies of Rat Liver

After blood collection , the liver of rats of different group were excised immediately and was washed in ice cold normal saline (Dash *et al*., 2007).The tissue sections of liver was obtained by fixing it in 10% formalin solution , followed by dehydration with 100% ethanol solution, cleared in xylene and embedding in paraffin wax . The liver sectioned was cut into 5 µm thickness and was stained with hematoxylin-eosin solution and then section were then examined under microscope for visualizing the changes during CCl4 challenge in Ethanolic extract of *C. papaya* in treated as well as in control groups (Prakash et al.,2008)The sections were examined for changes in

hepatic cells like necrosis, steatosis and fatty cells (Adeneye et al.,2009,Dash *et al*., 2007).

Result

Effect of Ethanolic extract of *C. papaya* on CCl_4 induced liver damage in rats with reference to biochemical changes in serum enzyme, bilirubin and total protein are shown in Table 1.

Table 1: Effect of Ethanolic extract of *C. papaya* on CCl_4 induced liver damage in rats

Groups	AST	ALT	ALP	Bilirubin	Total protein
Group I	22.38 ± 0.6	18.34 ± 0.5	28.32 ±0.61	1.20 ±0.04	7.8±0.4
Group II	66.46 ± 0.4	54.02 ± 1.3	54.62 ± 0.90	5.20 ±0.43	5.6 ± 0.5
Group III	25.21 ± 0.7	17.29 ± 0.6	27.49 ± 0.72	1.59 ±0.03	7.2 ± 0.6
Group IV	36.52 ± 0.6	22.14 ± 0.6	40.56 ± 0.52	2.97 ±0.03	6.3 ± 0.2
Group V	23.53 ± 0.2	16.11 ± 0.3	25.62 ± 0.65	1.23 ±0.04	6.5 ± 0.3
Group VI	23.61 ± 0.7	18.28 ± 0.8	27.32 ±0.75	1.21 ±0.06	6.2 ± 0.4

There was a significant increase in serum AST, ALT and ALP. Treatment with silymarin (100mg/kg) and EECP (100, 200, 400 mg/kg) prevented the elevation of serum marker enzymes AST, ALT, ALP. The decreases in the level of AST, ALT and ALP were found to be greater in standard drug silymarin followed by EECP. A significant elevation of total bilirubins and decreased level of total protein in the serum of CCl_4 intoxicated rats (Group II) when compared to normal control (Group I). The extracts of EECT and EECA reduced the levels of total bilirubins and reversed the altered total protein (Shanmugasundaram *et al*., 2015). Histopathological studies of rat liver tissue from Group I animals show normal hepatic cells with central vein (V) and sinusoidal dilation. In CCl_4 treated group (Group II), severe hepatotoxicity was observed by severe necrosis (N) with disappearance of nuclei. Mild degree of necorsis (N) with normal cells (C) was observed in Group III and mild degree of necrosis (N) with areas of inflammation adjacent to necrosised area was observed in Group V animals, treated with EECP (200 mg/kg/day) respectively, while normal hepatocytes with regenerating hepatocytes and mild inflammation in the portal area (M) was observed with Group IV and normal hepatocytes with mild inflammation of portal tried (M) was observed with group VI animals at the dose of 400mg/kg/day of EECP respectively. The liver taken from animals treated with standard drug Silymarin (100 mg/kg) showed the normal hepatic cells with portal vein (V) and portal artery.

Discussion

In previous search literature it was revealed that certain chemicals like Carbon tetrachloride (CCl_4) and toxic chemicals when given in higher amount to experimental animals and humans causes hepatotoxicity leading to kidney and liver injury. Carbon tetrachloride (CCl_4) shows analgesic and antipyretic property which effect liver function, necrosis in hepatic lobule as well as death in some cases (Alam *et al*., 2017).Leaf extract protein(CI-1), isolated from *Carica papaya* were given to experimental animal and histological test conducted shown that the serum parameters like AST, ALT and ALP was reduced as compared CCl_4 treatment(1ml/kg) for a period of 14 days(Ahsan *et al.,*2009).After completion of experimental dosage there was a varied difference in results. The hydroalcoholic extract of plant showed significant hepatoprotective effect while silymarin

was used as standard drug because it reduced the level of hepatotoxicity. The paracetamol treated group showed liver blooming, necrosis and inflammation (Qadir *et al.,* 2014).

Dosage application of CCl_4 in experimental animal induces liver damage which somehow resembles viral hepatitis. Liver toxicity is seen with changes in endoplasmic reticulum which loses its intracellular metabolic enzymes and it is measured by serum marker enzymes like AST, ALT, ALP, ACP, LDH etc. These enzymes are normally present in higher concentration in cytoplasm but in case of hepatic injury these enzymes leaks into blood stream leading to hepatotoxicity. The *Carica papaya* leaf extract (EECP) when given at dosage of 300mg/kg can significantly restores the normal level of serum marker enzymes(Shanmugasundaram *et al*., 2015). Antioxidants like α-tocopherol (Vitamin E), and silymarin can protect liver against heptaotoxins like CCl_4 and other harmful chemicals. Various herbal formulations in Ayurvedic medicines have been used as hepato-protectant against CCl_4-induced hepatic injury. Carbon tetrachloride induces hepatotoxicity which inturn effect glucose, protein and lipid metabolism in body. As these macromolecules are synthesized in liver any dysfunction in liver affects synthesis, circulation and metabolism of these biomolecules (Adeneye *et al.,* 2009). In experimental test rats were treated with various solvent extract of CCl_4 and *carica papaya*. Various studied showed that CCl_4 dosage causes centrizonal hemorrhagic hepatic necrosis in test animals and humans. In the above experiment rat were administered with CCl_4 which showed liver enlargement leading to infiltration, vacuolization, and inflammation of liver. Thus, there was increase in liver weight and decrease in body weight of rat. As compared to negative control, the pre and post treatment of rat with 80% methanol and n-butanol extract showed no significant changes in body weight but relative increase in liver weight. This methanolic extract when given in higher dosage amount it was able to decrease serum AST, ALT, and ALP level (Meharie *et al*., 2020).

As compared to CCl_4 treated group, the ethanolic extract of *carica papaya* in combination with CCl_4 reduces the level of serum total protein specifically the elevated bilirubin levels.Single administration of CCl_4 adversely protein metabolism of body there by inhibiting protein synthesis. The Ethanolic extract of *carica papaya* plant have the potency to increase protein synthesis and reverse the effect of CCL_4.This shows the hepatoprotective activity of *carica papaya* plant against CCl_4 damage (Komalavalli *et al*.,2014).The present study revealed that in comparison with control group the rats injected with carbon tetra chloride shown severe liver damage like degeneration, inflammation and fat deposition in liver as well as increase in serum ALT, AST,ALP level and decrease in albumin levels(Shaban *et al.*,2021).

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