experimental works

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STUDY OF THE EFFECT OF ALKALOIDS ISOLATED FROM *Typha domingensis Pers.* FRUIT ON HEPATOTOXICITY INDUCED BY CCl_4 IN RATS

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The present study was carried out to investigate the hepatoprotective effect of alkaloids isolated from Typha domingensis pers. fruit against hepatotoxicity induced by carbon tetrachloride in rats. Alkaloids from Typha domingensis per .fruit were isolated, preliminary phytochemical tests were done and the appropriate alkaloid dose for rats was determined. Rats were divided into three groups: group I (control group) received the normal saline, group II received CCl_4 only, and group III received both an alkaloid isolated, and CCl_4. The biochemical markers: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, and lipid profile (cholesterol, triglycerides, HDL, and LDL) were determined. A histopathological study of the liver was conducted. The results showed that CCl_4 significantly elevated the levels of AST, ALT, ALP, serum triglycerides, cholesterol, and LDL concentration as compared with the control group. While lower HDL and total protein were observed in the CCl_4-treated group compared with the control group. The result also showed that alkaloid isolated reduced the rise in liver index ALT, AST, ALP and serum triglycerides, cholesterol, and LDL levels caused by CCl_4. Alkaloid isolated increased HDL and total protein levels compared with the group treated with CCl_4 only. At the same time, the histological study of liver tissue proved that alkaloid isolation lessened the harm liver tissue caused by CCl4. The experiment demonstrates that alkaloids isolated have a good hepatoprotective effect.

k e y w o r d s: Typha domingensis pers .fruit, Alkaloids, Rats, Hepatotoxicity, CCl4

Introduction

The primary organ regulating homeostasis in the body is the liver. It is associated with all the biochemical processes related to growth, prevention of the disease, supply of the nutrient, production of the energy, and reproduction [1]. Exposure to harmful substances, certain drugs, and environmental pollutants such as CCl_4, Paracetamol, etc are the main causes of liver dysfunction [2]. Recent studies on free radicals revealed that oxidative stress and free radicals play a pathophysiological role in liver damage, both acute and chronic hepatic injury [3,4]. In these pathological states, where CCl_4 promotes the generation of reactive oxygen species (ROS) along significant lowering of antioxidant defense, numerous cellular functions are impaired and altered through the processes of lipid peroxidation, and oxidative damage occurs[5,6].

As a consequence, the advantages of antioxidants or free radical scavengers have been widely recognized as important for the prevention and treatment of both acute and chronic liver injuries[7,8]. The antioxidants in use are either generated synthetically or from naturally plants [5]. Hepatopathy is increasingly being treated with herbal medicine in many countries [2].

Typha domingensis pers. (T.domingensis) is a perennial herbaceous plant belonging to the Typhaceae family, these plants have long, slender green stalks that are capped with brown, fluffy, sausage-shaped flower heads that are 15–40 dm tall. It frequently grows in shallow water wetland and develops dense rhizomes close to ponds, canals, and river banks [9].

Alkaloids, phenols, flavonoids, tannins, and saponins are all present in the fruit of *Typha domingensis pers* [10]. *Typha domingensis* has antihyperlipidemic properties and is used externally for burns and wound healing [11], in addition to lowering the incidence of obesity, atherosclerosis, and hypertension [12]. *T. domingensis* has been used traditionally for bleeding disorders like hematuria, hematemesis, dysmenorrheal bleeding, neuroprotection, anxiety, depression, as a diuretic, astringent and in the food industry [13,14]. Determining the role of alkaloids isolated from *Typha domingensis pers*. fruit as a hepatoprotective agent is the aim of the present study.

Experimental

Plant Material

The flour of *Typha domingensis pers.* fruit was purchased from a local market in Thi-Qar city.

Isolation of alkaloid from Typha domingensis pers. fruit

A quantity of 50 gm of flour was extracted with n-hexane by the soxhlet extraction method to remove the oil [15]. The defatted flour was dried. The following procedure [16] was used to isolate the alkaloid:

Fifty grams of the defatted flour were combinated with 250 ml of (10%) ethanolic acetic acid and the mixture was stirred on a magnetic stirrer for 24 h. Then the precipitate was removed after the mixture was filtered. A vacuum rotator evaporator was used to concentrate the filtrate to a quarter of the previous volume at 70 C^o and the pH was adjusted to 9 by using ammonium hydroxide to precipitate the alkaloids. The mixture was put in a separation funnel, 20 ml of chloroform was added to the mixture and the mixture was mixed well. A collection of the organic layer was made. This step was repeated three times then the organic layers were dried using a rotator evaporator to produce (1.72) gm. The dry alkaloid was kept in a refrigerator in a capped container .

Phytochemical Analysis

Chemical tests were performed on the alkaloid fraction to confirm that the extract contains alkaloid only by using standard procedures to identify the constituents, by characteristic color changes as described by [15].

Acute toxicity studies:

The acute toxicity study for alkaloid extracts were performed using albino rats. The animals were fasted overnight prior to the experiment and maintained under standard conditions. The extracts were administrated orally in increasing dose(1000,3000,5000 mg/kg) and animals were monitored for 72hours[17].

Experimental Animals:

For the experiment, male rats weighing 150–250 g were employed. The animals were kept at room temperature (25 C) and allowed access to food (standard laboratory chow) and tap water. Rats were acclimatized to laboratory conditions for 1 week before the start of experiments. All the animals were

cared for humanely and well maintained under standard ethical principles as per the university regulations (UMS/IP7.5/M3/ 4-2012). A total of 18 adult male rats were divided into three groups (6 rats/group) and treated as follows[18] :

GroupI (control group): Received normal saline (1ml/Kg) orally.

Group II: Injected intra peritoneal (I.P) with CCl_4 (1 ml/kg) every 72h for 10 days.

Group III : Received alkaloid extract (1000mg/kg) orally for 10 days + CCl_4 (1.0 ml/kg, I.P) administered at every 72h.

A suspension of the extract was prepared in normal saline and administered to the animals by gastric gavage needle.

Blood was collected by cardiac puncture using sterile syringes. Serum was obtained by centrifugation at 1500 rpm for 15 min and frozen at -20 ºC until further analysis. The liver was promptly removed from each rat and preserved in a 10% neutral buffered formalin solution for histopathological studies.

Estimation of Biochemical

Parameter serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by the method [19], serum alkaline phosphatase (ALP) was assessed using the method [20], serum total protein was determined by the method of [21] and [22], serum cholesterol was determined using the method of [23], serum triglycerides were assessed by the method of [24] and serum HDL was determined by the method [23].

Pathological studies:

Liver tissue specimens were taken from all groups immediately after the sacrifice of rats and placed in a 10% formalin solution. Paraffin sections of 5µ thickness were used, stained with Hematoxyline and Eosin (H & E), and viewed under a microscope [25].

Statistical Analysis

Univariate analysis of variance was used to analyze the study's results . The data were expressed as mean ±standard deviation (mean±SD). The statistical social science program SPSS was used to examine the difference between means (groups) using the least significant difference test (LSD). $p< 0.05$ was deemed significant.

Results and Discussion

Phytochemical screening revealed the presence of alkaloids only as shown in table (1)

Table 1: The phytochemical composition of the alkaloid isolated from *Typha domingensis pers*. fruit

Phytochemicals	*Typha domingensis pers*
Alkaloids	+
Phenoles	-
Flavonoids	-
Tannins	-
Saponins	-
Amino acids	-

Results of the acute toxicity test of the alkaloid extract *of Typha domingensis pers* .fruite show that the extract has no toxic effect on the animals. A dose of 1000 mg/kg was chosen as the concentration safe of the extract to be administered to the rats.

Results from Table2, show increases in the liver enzymes – aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) at the administration of CCl_4 compared with the control group, the total protein concentrations of the CCl_4 treated rats were significantly reduced ($P < 0.001$) as compared with the control group. As the majority of the proteins present in plasma are produced by the hepatocytes and released into circulation, this shows a loss in the liver's ability to manufacture proteins, which may be caused by potential hepatocyte damage brought on by CCl_4 [26]. results show a significant effect ($p> 0.05$) in ALP, AST, and ALT levels between the control and CCl_4 treated groups. The high serum concentration of ALP is typically evident during liver illness or bone disease, where ALP is found in the microvilli of the bile ducts and on the surface of the hepatocytes [27]. AST and ALT enzymes are typically present in the cytosol of hepatocytes and have a role in altering amino acids into alpha-keto acids. The enzymes leak into the blood and their

levels rise increase in cases of hepatotoxicity, where the cell loses the protective membrane[28]. The results of this study are consistent with the study [5]

The carbon tetrachloride (CCl_4) induced hepatotoxicity in rodents resembles viral hepatotoxicity in humans which renders it a suitable model for hepatoprotective drugs development [29].

Also, Table 2 shows treatment of the animals with a concentration1000 mg/kg of the alkaloid extract significantly reduced ($P < 0.05$) the CCl_4-induced elevations in AST, ALT, and ALP levels, showing an increase significantly ($P < 0.05$) in total protein concentration in rats treated with alkaloid as compared to the CCl_4 treated rats only. Available data from earlier studies states that stimulation of the protein regeneration process and production of liver cell synthesis accelerates[30]. Increased levels of the total protein indicate hepatoprotective activity of alkaloid isolated, proves a protective effect against CCl_4 induced toxicity and therefore amelioration of the liver damage.

Table 2 : Effect of alkaloid isolated from *Typha domingensis pers*. fruite on some biochemical parameters in male rats.

parameters	Groups				P value	
	N	Group I N.S	Group II CCl_4 1ml/kg	Group III CCl_4(1ml/kg) + 1g/kg of alkaloid isolated	Group II vs Group I	Group III vs Group II
AST (U/L)	6	16.71 ± 1.07	21.40 ± 5.39 *	14.90 ± 5.04 *	0.05	0. 014
ALT (U/L)	6	21.35 ± 4.49	29.08 ± 0.64 * *	19.05 ± 3.98 ***	0.003	0.000
ALP (U/L)	6	24.66 ± 4.05	2.25*** 32.33±	22.00 ± 2.45 *	0.000	0. 014
Total Protein (g/dl)	6	6.49 ± 0.21	3.46 ± 0.17 ***	4.49 ± 0.12***	0.000	0.000

N =number of animals, Means ±SD, N.S = normal saline, * p< 0.05, ** p< 0.01, *** p< 0.001

The values of serum cholesterol, triglycerides, low-density lipoproteins (LDL), and high-density lipoproteins (HDL) are presented in Table 3. Table 3, shows increases in the levels of cholesterol, triglycerides, and LDL in serum while HDL decreased at the administration of CCl_4 Compared with the control group.

Significant differences were observed ($p < 0.05$) in cholesterol levels and ($p < 0.001$)) in the TG, HDL, and LDL in the serum between Group I and Group II. In the liver, CCl4 results in the formation of free radicals such as trichloromethyl and trichloromethyl peroxyl radicals that bind to macromolecules such as DNA, lipids, and proteins [31]. These free radicals extract hydrogen atoms from the lipid membrane of hepatocytes to form lipid hydroperoxides which ultimately leads to liver necrosis [31]. The increased level of lipid hydroperoxides and free radicals causes a reduction in the level of antioxidant enzymes, along with oxidative DNA damage, genetic mutation, and chromosomal alteration. The inbuilt antioxidant defense system of hepatocytes consists of catalase, superoxide dismutase, glutathione system, ascorbic acid, and tocopherol that protect against free radical-mediated damage [32]. Also, Table 3 shows treatment of the animals with a concentration1000 mg/kg of the alkaloid isolated reduced the CCl_4-induced elevations in cholesterol, TG, and LDL, while HDL increased. Significant differences were observed ($p < 0.05$) in cholesterol levels and ($p < 0.01$) in the TG and ($p < 0.001$) in the HDL and LDL between Group II and Group III, this indicates

the role of alkaloids isolated from *Typha domingensis pers.* Fruit in reducing the damage caused by CCl_4.

This study agreed with a study by Adnan Akram and Qaiser Jabeen that found that treating animals with different concentrations of *Typha domingensis* reduced the levels of cholesterol, triglyceride, and LDL and have antiatherosclerotic effects [12]. Alkaloids are known to have significant inhibitory effects on hepatocytes lipid accumulation therefore, it has a role in the treatment of the diseases related to lipid accumulation, such as non-alcoholic fatty liver disease and hyperlipidemia [33].

Table3: Effect of alkaloid isolated from *Typha domingensis pers*. fruite on lipid profile parameters in male rats.

Parameters	Groups				P value	
	N	Group I N.S	Group II CCl_4 1ml/kg	Group III CCl_4 (1ml/kg) + 1g/kg alkaloid isolated	Group II vs Group I	Group III vs Group II
Cholesterol (mg/dl)	6	50.60 ± 4.20	44.57 ± 6.54*	50.02 ± 0.57 *	0.028	0.045
Triglycerides (mg/dl)	6	80.58 ± 4.29	64.20 ± 3.42 ***	72.38 ± 4.38**	0.000	0.003
HDL (mg/dl)	6	5.30 ± 2.38	12.32 ± 0.74 ***	23.22 ± 0.81 ***	0.000	0.000
LDL (mg/dl)	6	22.35 ± 2.36	14.98 ± 1.94***	21.80 ± 2.89 ***	0.000	0.000

N =number of animals , Means ±SD, N.S = normal saline , * p< 0.05, ** p< 0.01, *** p< 0.001

Histological study of cross sections of the liver:

Fig. (1a) shows liver normal like looking at liver architecture, normal hepatocytes, and blood vessels filled by RBCs in the control group.

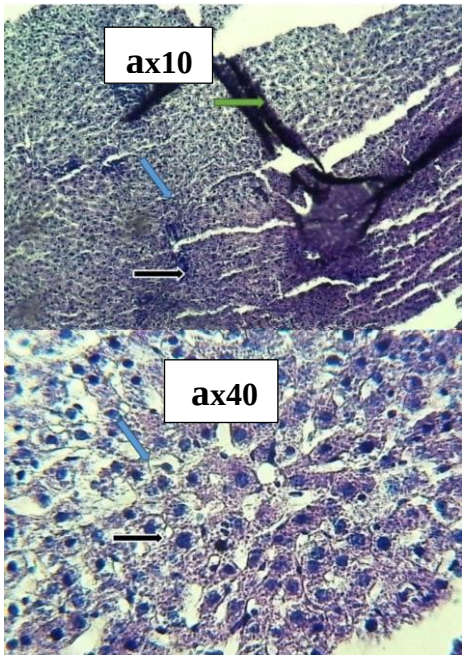
Fig. (1b) shows fat vacuoles, glycogen or lipofuscin pigment, Cuboidal epithelial cells with round, centrally located nuclei hepatocytes and hepatocyte necrosis, severe fatty changes, sinusoidal congestion, and lymphocytic in the group treated with CCl_4 (Group II) In such cases, the deterioration and damage of membrane lipids disrupt the balance between oxidants and antioxidants. Therefore, oxidative damage occurs[6].

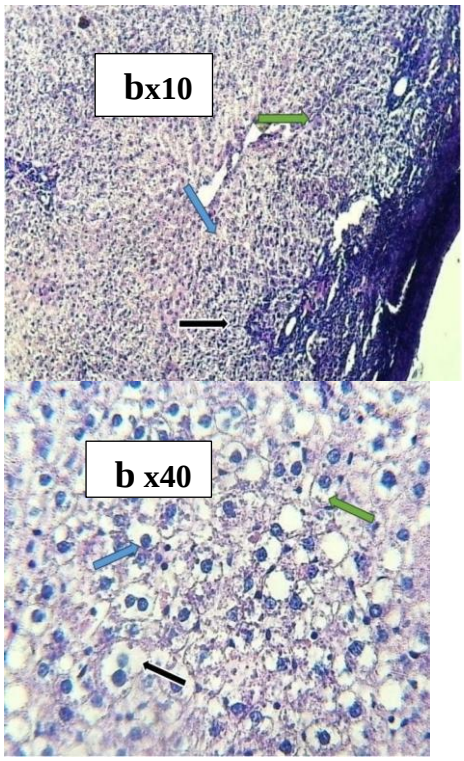
Exposure to CCl_4 has been reported to induce free radical generation in tissues such as the liver, heart, lung, testis, brain, and blood[26]. The first metabolite of CCl_4; trichloromethyl free radical, is believed to initiate the biochemical processes leading to oxidative stress, which is the direct cause of many pathological conditions such as diabetes mellitus, cancer, hypertension, kidney damage, liver damage, and death[34]. Liver damage caused by acute exposure to CCl_4 shows clinical symptoms such as jaundice, swollen and tender liver, and elevated levels of liver enzymes in the blood[35]. Oxidative stress, occurring cause pathophysiological changes associated with various liver disorders such as hepatitis, hepatocellular carcinoma, and liver cirrhosis [36].

Fig. (1c) shows fat vacuoles, glycogen or lipofuscin pigment, Cuboidal epithelial cells with round, centrally located nuclei distracted hepatocytes and inflammatory mononuclear, phagocytic cells (macrophages) and moderate fatty changes accompanied by mild necrosis In the group treated with CCl_4 and alkaloid extract(Group III).

The present study indicates that alkaloids isolated from *Typha domingensis pers*. the fruit has

hepatoprotective effects. This agrees with the study [10].





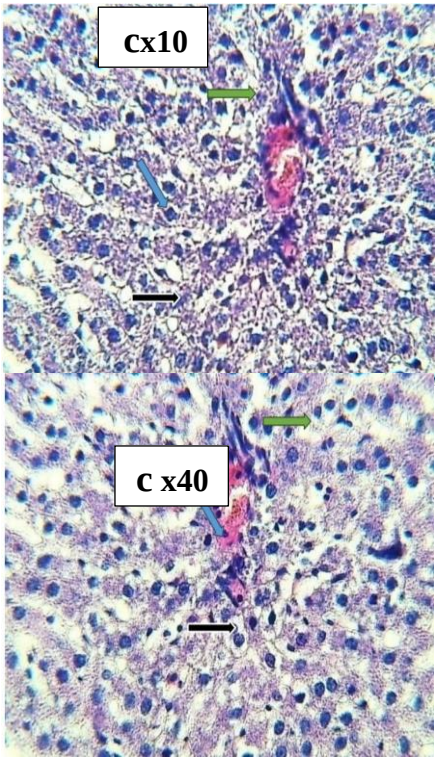


Figure 1: Photo of rat liver of Group I (a), Group II (b), and Group III (c).

Conclusion

The present study revealed that the alkaloids isolated from *Typha domingensis pers*. fruit can act hepatoprotective agent.

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Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ВИВЧЕННЯ ВПЛИВУ АЛКАЛОЇДІВ, ВИДІЛЕНИХ З Typha domingensis Pers. ФРУКТИ НА ГЕПАТОТОКСИЧНІСТЬ, ІНДУКОВАНУ Ccl4 У ЩУРІВ

Дане дослідження було проведено з метою вивчення гепатопротекторної дії алкалоїдів, виділених з Typha domingensis pers. фрукти проти гепатотоксичності, викликаної чотирихлористим вуглецем у щурів. Було виділено алкалоїди з плодів Typha

domingensis, проведено попередні фітохімічні тести та визначено відповідну дозу алкалоїдів для щурів. Щури були розділені на три групи: група I (контрольна група) отримувала фізіологічний розчин, група II отримувала лише CCl4, а група III отримувала як виділений алкалоїд, так і CCl4. Визначали біохімічні маркери: аспартатамінотрансферазу (АСТ), аланінамінотрансферазу (АЛТ), лужну фосфатазу (ЛФ), загальний білок і ліпідний профіль (ХС, тригліцериди, ЛПВЩ, ЛПНЩ). Проведено патогістологічне дослідження печінки. Результати показали, що CCl4 значно підвищив рівні АСТ, АЛТ, ЩФ, сироваткових тригліцеридів, холестерину та концентрації ЛПНЩ порівняно з контрольною групою. Тоді як у групі, що отримувала CCl4, спостерігалося зниження ЛПВЩ і загального білка порівняно з контрольною групою. Результат також показав, що ізольований алкалоїд зменшив підвищення печінкового індексу ALT, AST, ALP і рівнів сироваткових тригліцеридів, холестерину та ЛПНЩ, викликане CCl4. Ізольований алкалоїд збільшив рівень ЛПВЩ і загального білка порівняно з групою, яка отримувала лише CCl4. У той же час гістологічне дослідження тканини печінки довело, що виділення алкалоїдів зменшує пошкодження печінкової тканини CCl4. Експеримент показує, що виділені алкалоїди мають хороший гепатопротекторний ефект.

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