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PROTECTIVE EFFECT OF *APIUM GRAVEOLENS*, *CINNAMOMUM VERUM* IN CCL_4 INDUCED MODEL OF ACUTE LIVER INJURY

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ABSTRACT

Objective: The present study was designed to determine the hepatoprotective effect of *Apium Graveolens*(celery) ,*Cinnamomum verum*(Cinnamon) in acute lever injury induced by hepatotoxic agent CCL_4. **Materials & methods:** the normal serum values of ALP, AST, ALT, TSB &TSP were determined in 32 healthy domestic rabbits before CCL_4 induction &at two occasions 24,120hrs after ALI induction by CCL_4 and treatment with aqueous extract of herbs for five successive days. **Results:** showed significant decrease in ALP, AST&TSB level of both aqueous extracts measured at 120hr compared with control group &their levels measured at 24hr. **conclusion:** Both extract of celery & cinnamon possess mild hepatoprotective effect which was more powerful in the first extract. The histopathological examination showed a clear improvement in the sections of the liver tissue that support the effect of these aqueous extract on the liver.

KEYWORDS: *Apium Graveolens*(celery), *Cinnamomum verum*(Cinnamon), ALI,Hepato protection.

INTRODUCTION

Acute liver injury (ALI) is a clinical condition that results from sever & extensive damage of hepatocelluar tissue with reduced cell mass & blood flow caused by various reasons. It is associated with increase in serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST),serum alkaline phosphatase (ALP) & total serum bilirubin (TSB).[1]

Carbon tetrachloride (CCL_4) is a hepatotoxic agent used to induce (ALI) when administered orally to the rabbit due to formation of free radicals mediated lipid peroxidation.[2] A number of medicinal herbs had been proved to have hepatoprotective effect[3]in CCL_4 induced model of acute liver injury ,therefore, it is interesting to explorer the possiple hepatoprotective effect of *A.graveolens* & *C. verum* in the present study. *Apium Graveolens* (celery) is apopular aromatic herb & spice the active parts of the herb are fruits, roots & seeds. They contain essential oil ,glucoside of furo-coumarin & flavanoid in their structure.[4]also presence of apigenin.[5] linamarose & vitamin C&A.[6] it is used as appetizer, diuretic accelerate excretion of urinary calcium.[7] *Cinnamomum verum* (cinnamon) is a well known herb & spice. The chemical constituent of leaf & bark include essential volatile oil include (cinnamaldehyde, cinnamyl alcohol, eugenol, cinnamyl acetate, cinnamic acid).[8] the cinnamon is inhibiting bronchospasm induced by histamine,[9] also reducing fasting glucose, triglyceride ,LDL in type-2 diabetes mellitus.[10][11]Both herbs are cultivated in different countries of the world & it is worth to assess the potential hepatoprotective effect of these herbs.

MATERIALS AND METHODS

Chemicals

All chemicals used in the present study were of analytical grade. CCL_4 was procured form Merck /India ltd., India.

The kits for estimation of serum ALP, AST, ALT were purchased from Biomerieux-france while that for estimation of TSB & TSP were supplied from Randox-England.

Plant extraction

The test medicinal herbs were purchased from well known herbal shop (AL-Medina) in Baghdad city were identified and authenticated by Iraqi National Institute for herbs. The dried particles were cleaned carefully & powdered by electrical grinder then passed through sieve no. 40 to remove debris. The aqueous extract prepared by diluting one volume of well grinded powder to 10 volumes of water at 80C^o in the stoppered flask after shaking completely .thus, the solution was allowed to stand for 10 minutes to be cold the filtered to be used for practical purpose. The aqueous extract should be used within 12 hours.[12]

Animals

Thirty two healthy domestic rabbits weighing 850-950 gm were used in the present study, they were supplied by animal house of college of pharmacy. Animals were housed under good conditions at 28C^o in separated cages and were fed standard oxoid pellets & were given

water adlibitum The rabbits were randomly allocated to four groups (each contains eight rabbits) they were given a single daily dose of the followings at 9:00 a.m. for five successive days.

Group-1 (control) received distilled water (3ml) orally.

Group-2 (drug control) received distilled water (3ml) orally.

Group-3 received aqueous extract of celery (400mg/kg) orally

Group-4 received aqueous extract of cinnamon (1.5 mg/kg) orally.

The doses of celery & cinnamon had been chosen using many doses in pilot study. At 10:00 a.m. of the first day group 2,3and 4 were given CCL_4 as 1:1(v/v) mixture of CCL_4 olive oil at a dose of (1.25 ml/kg) orally given for induction of ALI. Blood samples were collected from marginal ear vein of the rabbits of all groups for biochemical analysis of serum AST,ALT,ALP and TSB&TSP at two occasions 24,120hr using spectrophotometer method.[13] for comparison between the values of these results. Later on, all the rabbits were sacrificed under light anesthesia of ethers to take liver specimen. the histopathological examination was performed to check the microscopic changes of the liver tissue using polarized microscope after fixating the section in 10% formalin for 48 hours and staining with hematoxylin & eosin.[14]

STATISTICAL ANALYSIS

All the obtained results were expressed as mean ± SEM. the difference among means had been analyzed by student's test using SPSS version12, p values <0.05 were considered to be statistically significant .

RESULTS

Administration of CCL_4 to the rabbits in group-2 revealed a marked increase in serum AST, ALT, ALP and TSB levels with decrease in TSP level compared with control group. Both extracts of celery & cinnamon (group3&4) produced significant reduction in ALT,AST&TSB levels measured at 120hr. compared with control group & to their levels measured at 24hr. The effect of celery extract was more potent than cinnamon extract in lowering significantly serum levels of ALT,AST,ALP and TSB with values equal to (38±2.39), (93.5±1.95), (54±5.60), (11.70±0.27) versus (98.33±0.88), (96.83±3.07), (95.18±3.93) and (12.03± 0.42) respectively for cinnamon measured at 120 hr. compared to control group with (97.83±3.97), (146.52±6.15), (189.98±5.21), and (20.13±1.56) respectively & to their levels measured at 24 hr. Cinnamon showed significant increase in TSP levels with non significant increase to

celery level at 120 hr. compared to its level at 24hr.The histopathological examination of the liver in both extracts showed a clear improvement in the hepatocytes congestion, fatty changes, infiltration of lymphocytes necrosis. These improvements support the hepatoprotective effect of these extracts against CCL_4 induced ALI (fig.1, 2, 3, 4).

Figure 1 Normal rabbit liver section shows hepatocytes architecture with normal lobular appearance (Hand E stain X40)

Figure 2 Rabbit liver section 120 hr. after administration of CCL_4 as a single oral dose showing massive necrosis, fatty changes, lymphocyte infiltration and congestion (Hand E stain X40)

Figure 3: Rabbit liver section treated after CCL_4 & Apium Graveoleus administration showing minimal necrosis, mild fatty changes, mild congestion. (Hand E stain X40)

Figure 4: Rabbit liver section treated after CCL_4 & Cinnamomum verum showing minimal necrosis, mild fatty changes mild congestion. (Hand E stain X40)

Table-1 Effect of tested drugs & extracts on CCL_4 induced ALI

Group	Dose	SALT U/L	SAST U/L	SALP U/L	TSB Umol/L	TSP g/dl
Normal Control	----------	23.72±0.94	25.38±1.28	49.03±3.23	11.27±0.64	5.28±0.1
CCL_4 alon	1.25ml/kg	133.32±2.72 after 24 hr.	208.45±3.73 after 24 hr.	206.35±3.30 after 24 hr.	30.47±1.21 after 24 hr.	4.3±0.27 after 24 hr.
		97.83±3.97 after 120 hr.	146.52±6.15 after 120 hr.	189.98±5.21 after 120 hr.	20.13±1.56 after 120 hr.	4.58±0.16 after 120 hr.
Apium Graveolens +CCL_4		91.17±1.96* after 24 hr.	119.33±2.63* after 24 hr.	106.33±5.71* after 24 hr.	14.17±0.51* After 24 hr.	442±0.92 After 24 hr.
		38.00±2.39*	93.5±1.95*	54.0±5.60*	11.70±0.77*	4.72±0.25

	400mg/kg	**after 120 hr.**	**after 120 hr.**	**after 120 hr.**	**after 120hr.**	**after 120 hr.**
Cinnamonum Verum+CCL_4	**15mg/kg**	**124.50±4.60 after 24 hr.**	**110.50±3.51* after 24 hr.**	**120.00±2.24* after 24 hr.**	**13.85±0.41* after 24 hr.**	**14.58±0.14 after 24hr.**
		98.33±0.88 after 120 hr.	**96.83±3.07* after 120 hr.**	**95.18±3.93* after 24 hr.**	**12.03±0.42* after 120hr.**	**4.95±0.04 after 120hr.**

*significant lowering effect at P<0.05

DISCUSSION

The hepatotoxic compound CCL_4 is well known to be used for induction of ALI in experimental animal model. It is biotransformed in the cytochrome P 450 system to its metabolite trichloromethyl free radical (CCL_3) which in the presence of O_2 forms trichloromethylperoxyl free radical (CCL_3O_2 ·) that attacks lipids of endoplasmic reticulum ,eliciting lipid peroxidation with the leakage of hepatocellular enzymes like ALT,ALP,AST and causing an increase in TSB level & decrease in TSP level.[15] The same results were obtained by others.[16] who used rat as a model for ALI induction .The results of treated control (group-2) are compatible with results of others.[17] in using CCL_4 in their studies. The protective effect of celery extract (group-3) is related to its antioxidant & anti-inflammatory effect owing to the presence of apigenin & flavonoid compound in its contents that might have counteracted the pro-oxidant effect of formed 2-acetyl aminofluorine (2-AAF)by scavenging superoxide radicals that lead to suppression of lipid peroxidation.[18] in addition to the presence of vitamin C in constituents of extract may be helpful in suppression the lipid peroxidation of liver cells .[19] This effect was similar to the results of others who proved that vitamin C had hepatoprotecteive effect.[20] The anti-inflammatory effect of cinnamon (group-4) was obviously seen by.]21] in stopping inflammation of mouth & pharynx & in having fungicidal activity. Its anti oxidant effect can reduce both blood sugar & lipids.[22] The results of the present study confirm the role of oxidative stress in initiation of liver damage & support the convenience that antioxidants are important in prevention or attenuation of ALI. The histologeical architecture of the liver sections of (group 3and 4) showed a more or less lobular pattern with a mild degree of fatty changes & lymphocyte infiltration & mild congestion with minimal necrosis.(Fig.1,2,3,4)

CONCLUSION

Both extracts have mild hepatoprotective effect restoring the normal hepatic function, enhancing the biodefense of the liver against oxidative damage produced by CCL_4 administration.

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