Curative effect of basil on liver injury in experimental rats

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Abstract: Forty two albino male rats were classified into six groups. Group I (n=7) served as control (-ve), and animals in groups II–VI CCl4 were induced liver injury. Group II served as control(+ve) and treated groups from III to VI rats received daily oral doses of ursofalk drug , basil ethanolic extract, basil aqua extract, and basil powder. The results revealed that control (+ve) rat group showed a significant decrease in final body weight, body weight gain, food intake & food efficiency ratio (FER); serum total protein, globulin, glutathione transferase (GST) & catalase and liver triglyceride, total lipid, superoxide dismutase (SOD), glutathione peroxidase (GPX) & GST but a significant increase in serum alanine and aspartate aminotransferase , alkaline phosphates, gamma glutamyle peptidase (ALT, AST**,** ALP & γGT), total bilirubin & nitrite (NO); albumin/ globulin ratio and liver glycogen, cholesterol & malondialdehyde (MDA) compared with control (-ve) group. All treated groups showed a significant decrease in body weight gain; serum globulin, GST& catalase and liver glycogen but a significant increase in serum ALP, total bilirubin & NO and albumin/ globulin ratio compared with control (- ve) group. Drug group showed a significant increase in serum AST & total bilirubin and liver cholesterol and MDA but a significant decrease in liver triglyceride, total lipid, SOD, GPX, GST compared with control (- ve) group. Basil ethanol extract and basil aqua extract rat showed a significant increase in serum ALT albumin/ globulin ratio and liver cholesterol & MDA and a significant decrease in serum total protein, liver triglyceride while basil powder showed a significant increase in serum ALT, AST, γGT and albumin/ globulin ratio and a significant decrease in serum total protein, albumin, liver total lipid and liver SOD compared with control (-ve).
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Key word: Basil- Liver injury- Rats.

1-Introduction

Liver is a major metabolic organ affected by various chemicals and toxins. Carbon tetrachloride (CCl4) is a well-known hepatotoxin and induces oxidative stress by the formation of free radicals. Extensive evidence demonstrated that CCl3 and Cl are formed as a result of the metabolic activation of CCl4 which in turn initiate lipid peroxidation process (Abd El-Ghany 2006).

Plants have played a significant role in maintaining human health and improving the quality of human life. Some herbal extracts are known to prevent the oxidative damages in different organs by altering the levels of cytochrome P-450 through their antioxidant properties (Kandasamy et al., 2010).

Ocimum basilicum is an aromatic herb known as African basil and wild basil and is used as an anthelmintic, deodorant, stimulant, anti-inflammatory, cardiotonic, blood purifier, used in skin diseases and as an antipyretic particularly in malarial fevers. Leaves and flowering tops are used for the essential oil which contains eugenol and found to have antibacterial, antiyeast and insecticidal action while extracts showed a hepatoprotective effect by promote liver health by reducing damage due to radiation exposure or environmental pollution or toxicants (Oboh et al., 2009).

The present investigation was undertaken to determine curative effects of concurrent use of basil leaves either extract or powder on CCl4 induced liver injury.

2. Materials and methods

2.1. Chemicals

Carbon tetrachloride was obtained from SIGMA Company for harmaceutical Industries and given 0.5 ml/rat CCl4 by back subcutaneous injection according to Moritz and Pankow (1989). Ursofalk drug is obtained from Minapharm Company. It is a white capsule and contains 250 mg ursodeoxycholic acid used in improvement of liver functions. Rats administered ursofalk drug dissolved in distilled water in dose 10 mg/kg of rat intragastric.

2.2. Basil plant and extracts

Dry plant leaves of basil (*Ocimum basilicum*) were purchased from Agricultural Research Center, Giza, Egypt. Basil leaves were dried with hot air (40–60 ˚C) and grinded to powder which was added to the diet as 5 % of the constituent of fiber. Basil aqua extract was prepared by boiling dried plant with 100 ml distilled water for 15 min. The extract was then filtered with a

clean cotton cloth and the volume of the extract was adjusted to 100 ml by evaporation. Basil ethanolic extract was prepared from basil powdered which was refluxed eight times with an 80% ethanol solution for two times, 1 h each time. The solvent was removed by evaporation under reduced pressure using Rotary Evaporator. Yield of the aqua and ethanol extract were given to rats as 250 mg/kg b.w intragastric.

2.3. Animals and experimental design

The experiment was performed on growing male Sprague dewally rats (n = 42) weighing about 151± g with approval from Helwan Farm of Laboratory Animals were provided with standard diet (Nelson 2000). After 15 days of acclimatization period; they were randomly assigned into six groups. Group I (n=7) served as negative control and animals in groups II–VI were given CCl4 to induce liver injury. Treated groups from III to VI rats received daily oral doses of uroflok drug, basil ethanolic extract, basil aqua extract, and basil powder .The daily food intake and weekly body weight of the individual rat was measured during the entire period of experiment (8 weeks). Food efficiency ratio was calculated according to Chapman et al., (1950). Rats were sacrificed under light chloroform anesthesia at the end of experiment. Blood samples and the liver were collected for biochemical estimation.

2.4. Biochemical estimation

2.4.1. Plasma ALT, AST, γGT and ALP

Serum aminotransferase (ALT, AST), gamma glutamyle peptidase (γGT) and alkaline phosphates enzymes activity (ALP) were estimated according to Reitman and Frankel (1957), Henry, (1974) and Kind and King (1954), respectively.

2.4.2. Serum total protein, albumin and globulin

Serum total protein, albumin and globulin (G) were estimated following the method of Weichselbaum (1946), Bartholomev and Delany (1966) and Coles (1974), respectively.

2.4.3. Serum glutathione transferase (GST), catalase and nitrite (NO)

Serum GST, catalase and NO were estimated according to Habig et al., (1974), Luck (1965) and Green et al., (1981), respectively.

2.4.4. Liver total lipid, cholesterol, triglyceride and glycogen.

Liver total lipid, cholesterol, triglyceride and glycogen were determined spectrophot- ometrically following the methods of Folch et al., (1957), Abell et al., (1952), Young and Pestaner ,(1975) and Rerup and Lundquist, (1967), respectively.

2.4.5. Liver SOD, GPX, GST and MDA

liver superoxide dismutase (SOD), glutathione peroxidase (GPX), Glutathione-S-transferase (GST) and malondialdehyde (MDA) were determined spectrophotometrically following the methods of Minami and Yoshikawa (1979), Flohe and Gunzler (1984), Habig et al .,(1974) and Placer et al .,(1966), respectively.

2.5. Statistical analysis

The data were analyzed statistically using analysis of variance to compare the means of different treatment groups with that of negative control and positive control groups (Snedecor and Cochran, 1994).

3. Results

3.1. Weekly body weight of rat groups

There was a steady decrease in final body weight, body weight gain food intake and FER of control (+ve) rat group ($P<0.05$, 0.01&0.001). However, all treated groups showed a significant decrease in body weight gain ($P<0.05$) compared with control (- ve) group and a significant increase in final body weight, body weight gain, food intake and FER compared with control (+ve) group as given in table (1).

3.2. Serum ALT, AST, γGT and ALP

Table (2) showed a significant increase in serum ALT, AST, γGT and ALP in control (+ve) rat group ($P<0.001$) and a significant increase in serum AST in drug, basil aqua extract and basil powder ($P<0.05$) but a significant increase in ALT in treated groups with basil either ethanolic or aqua and powder ($P<0.05$) compared with control (-ve) group. All treated groups showed a significant increase in ALP ($P<0.05$) compared with control (-ve) and a significant decrease in serum ALT, AST, γGT and ALP compared with control (+ve) rat group.

3.3. Serum total protein, albumin and globulin

The control (+ve) rat group showed a significant increase in serum total bilirubin and albumin/ globulin ratio ($P<0.001$&0.01) and a significant decrease in serum total protein and globulin ($P<0.001$) compared with control (-ve) group. Drug group showed a significant increase in serum total bilirubin ($P<0.01$) and a significant decrease in serum globulin ($P<0.05$) compared with control (-ve) but showed a significant decrease in serum total bilirubin and a significant increase in serum total protein, globulin and albumin/ globulin ratio compared with control (+ve). Drug group showed a significant increase in serum total bilirubin ($P < 0.01$) and a significant decrease in serum globulin ($P<0.05$) compared with control (-ve) but showed a significant decrease in serum bilirubin and albumin/ globulin ratio and a significant increase in serum total protein and globulin compared with control (+ve).

Basil ethanol extract, basil aqua extract and basil powder rat groups showed a significant increase in serum total bilirubin and albumin/ globulin ratio ($P < 0.001, 0.01 \& 0.05$) and a significant decrease in serum total protein and globulin ($P < 0.05 \&$) compared with control (-ve). Basil ethanol extract group showed a significant decrease in serum total bilirubin and albumin/ globulin ratio and a significant increase in serum total protein and globulin while basil aqua extract group showed a significant decrease in serum total bilirubin, albumin and albumin/ globulin ratio but basil powder rat group showed a significant decrease in serum albumin and albumin/ globulin ratio compared with control (+ve) as shown in table (3).

3.4. Serum GST, catalase and NO

Table (4) showed a significant decrease in serum GST and catalase and a significant increase in serum NO ($P < 0.05, 0.01 \& 0.001$) in control (+ve) and all treated groups compared with control (-ve) but a significant increase in serum GST and catalase and a significant decrease in serum NO in all treated groups compared with control (+ve).

Table (1): Mean values±SD of body weight gain, food intake and food efficiency ratio (FER) of experimental rat groups

Groups / Variables	Initial. weight(g)	Final weight(g)	Weight gain (g)	Food intake (g/w)	FER
Control	151.33±	238.48±	87.15±	18.49±	0.078±
(-ve)	3.47 [a]	11.41 [a]	5.67 [a]	1.24 [a]	0.002 [a]
Control	150.14±	199.05±	48.91±	15.45±	0.052±
(+ve)	3.24 [a]	12.28 [b**]	5.01 [c***]	1.45 [b*]	0.001 [c**]
drug	152.37±	229.73±	77.36±	18.10±	0.071±
	4.21 [a]	15.71 [a]	7.11 [b*]	1.81 [a]	0.003 [a]
Basil ethanolic	152.44±	228.38±	75.94±	18.04±	0.070±
extract	4.29 [a]	13.14 [a]	6.19 [b*]	1.77 [a]	0.003 [a]
Basil Aqua	153.22±	227.35±	74.13±	18.02±	0.068±
extract	3.21 [a]	15.20 [a]	7.18 [b*]	1.36 [a]	0.002 [ab]
Basil powder	151.41±	224.70±	73.29±	17.80±	0.068±
	5.25 [a]	16.33 [a]	7.03 [b*]	2.01 [a]	0.001 [ab]

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

Table (2): The Mean values ± SD of serum ALT, AST, γGT and ALP of experimental rat groups

Groups / Variables	ALT (µ /ml)	AST (µ /ml)	γGT (µ /ml)	ALP (µ /ml)
Control	29.91±	55.17±	8.15±	40.27±
(-ve)	3.21 [c]	5.12 [c]	1.22 [c]	5.61 [c]
Control	78.11±	102.76±	15.37±	88.98±
(+ve)	6.66 [a***]	11.44 [a***]	1.14 [a***]	8.33 [a***]
drug	35.77±	63.81±	9.88±	50.47±
	4.11 [bc]	5.99 [b*]	1.41 [c]	6.17 [b*]
Basil ethanolic	38.24±	60.65±	9.11±	56.14±
extract	3.99 [b*]	6.11 [bc]	1.30 [c]	6.03 [b*]
Basil aqua extract	41.33±	66.24±	9.67±	61.60±
	4.80 [b*]	7.12 [b*]	1.21 [c]	7.10 [b*]
Basil powder	43.78±	71.30±	11.17±	65.21±
	4.71 [b*]	7.67 [b*]	1.33 [b*]	7.30 [b*]

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

Table (3): The Mean values ± SD of serum bilirubin, total protein, albumin, globulin and albumin/globulin ratio (A/G) of experimental rat groups

Groups / Variables	Bilirubin (mg/dl)	T.protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin /Globulin
Control (-ve)	0.33± 0.01^{e}	7.10± 1.53^{a}	3.31± 0.66^{a}	3.79± 0.55^{a}	0.87± 0.11^{c}
Control (+ve) drug	1.45± 0.33^{a***}	5.33± 1.10^{c***}	3.25± 0.78^{a}	2.08± 0.53^{c***}	1.56± 0.19^{a**}
	0.88± 0.17^{cd**}	6.30± 1.32^{ab}	3.11± 0.45^{ab}	3.19± 0.24^{b*}	0.97± 0.10^{c}
Basil ethanolic extract	0.77± 0.15^{d**}	5.99± 1.13^{b*}	3.01± 0.44^{ab}	2.98± 0.28^{b*}	1.04± 0.13^{b*}
Basil aqua extract	0.99± 0.11^{b**}	5.55± 0.88^{bc*}	2.91± 0.19^{b*}	2.64± 0.35^{bc**}	1.10± 0.18^{b*}
Basil powder	1.03± 0.13^{ab***}	5.45± 0.66^{bc**}	2.85± 0.39^{b*}	2.60± 0.41^{bc**}	1.09± 0.22^{b*}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

Table (4): The Mean values ± SD of serum GST, catalase and NO of experimental rat groups

Groups / Variables	**GST (µ /ml)**	**Catalase (µ /ml)**	**NO (µ mOl/l)**
Control (-ve)	261.81± 33.16^{a}	301.41± 45.32^{a}	1.03± 0.16^{a}
Control (+ve) drug	108.22± 10.27^{d***}	171.20± 20.14^{c***}	9.67± 1.89^{a***}
	189.14± 18.33^{c**}	217.33± 31.13^{b*}	3.77± 0.55^{b*}
Basil ethanolic extract	220.35± 28.26^{b*}	235.14± 25.31^{b*}	3.51± 0.67^{b*}
Basil aqua extract	218.82± 27.70^{b*}	230.17± 25.31^{b*}	3.51± 0.67^{b*}
Basil powder	215.41± 30.11^{b*}	233.81± 22.21^{b*}	3.16± 0.73^{b*}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

3.5. Liver SOD, GPX, GST and MDA

The control (+ve) rat and drug groups showed a significant decrease in liver SOD, GPX, GST and a significant increase in liver MDA (P< 0.05, 0.01&0.001) compared with control (-ve) group. Basil ethanol extract and basil aqua extract rat groups showed a significant increase in Liver MDA (P<0.05) while basil powder rat group showed a significant decrease in Liver SOD (P<0.05) compared with control (-ve) group. All treated groups showed significant increase in liver SOD, GPX, GST and a significant decrease in liver MDA compared with control (+ve) group as shown in table(5)

Table (5):The Mean values ± SD of some liver glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPX) of experimental rat groups.

Groups Variables	SOD (µ /mg)	GPX (µ /mg)	GST (mg/g)	MDA (mmol/g)
Control	55.91±	43.91±	4.33±	8.21±
(-ve)	6.17 [a]	6.01 [a]	1.11 [a]	1.87 [c]
Control	25.89±	21.81±	1.27±	20.17±
(+ve)	3.20 [d***]	2.15 [d***]	0.12 [d***]	2.76 [a***]
drug	37.22±	32.11±	2.77±	11.21±
	4.77 [c**]	3.02 [c*]	0.33 [c*]	2.11 [b*]
Basil ethanolic extract	53.41±	39.29±	3.87±	10.14±
	5.67 [ab]	4.41 [ab]	0.54 [ab]	1.75 [b*]
Basil aqua extract	50.21±	38.14±	3.67±	10.51±
	6.12 [ab]	3.82 [ab]	0.67 [ab]	1.57 [b*]
Basil powder	49.31±	36.35±	3.59±	9.99±
	4.19 [b*]	4.50 [ab]	0.56 [ab]	1.44 [bc]

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

3.6. Liver total lipid, cholesterol, triglyceride and glycogen

Table (6) showed a significant increase in liver glycogen and cholesterol (P<0.001) and a significant decrease in liver triglyceride and total lipid (P<0.01) in control (+ve). The drug group, basil methanol extract and basil aqua extract rat groups showed a significant increase in liver cholesterol (P<0.05) and a significant decrease in liver triglyceride and total lipid (P<0.05) but basil powder group showed a significant decrease in liver triglyceride (P<0.05) compared with control (-ve).All treated groups showed a significant decrease in liver glycogen and cholesterol and significant increase in liver total lipid compared with control (+ve) rat group.

Table (6): The Mean values ± SD of liver glycogen, cholesterol, total lipids and triglyceride of experimental rat groups

Groups Variables	Glycogen (mg/100g)	Cholesterol (mg/g)	Total lipids (mg/g)	Triglyceride (mg/g)
Control	40.11±	3.67±	2.56±	6.11±
(-ve)	5.14[c]	0.33[c]	0.88[a]	1.06[a]
Control	55.16±	6.21±	1.13±	4.01±
(+ve)	6.32[a***]	1.13[a***]	0.46c[**]	0.50[bc**]
drug	43.40±	4.11±	1.78±	4.99±
	4.24[bc]	0.55[b*]	0.55[b*]	0.33[b*]
Basil ethanolic extract	45.51±	4.57±	1.66±	4.57±
	5.14[bc]	0.65[b*]	0.43[b*]	0.47[b*]
Basil aqua extract	44.12±	4.60±	1.59±	4.87±
	4.40[bc]	0.53[b*]	0.37[b*]	0.45[b*]
Basil powder	38.61±	3.55±	2.01±	4.55±
	3.22[bc]	0.54[bc]	0.76[a]	0.34[b*]

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant.

4-Discussion

It is clear that exposure to CCl4 can cause cellular damages through metabolic activation of those compounds to highly reactive substances such as reactive oxygen species. Carbon tetrachloride causes elevation in ALT and AST .The increase in the level of serum transaminase reflects the liver damage as these enzymes are released in the blood circulation after the administration of hepatotoxin as carbon tetrachloride (Szymonik-Lesiuk et al., 2003). The toxicity is initiated

by formation of a reactive metabolite trichlormethyl radical by microsomal fixed function oxidase which binds covalently to the macromolecules and induces peroxidative degradation of membrane lipids resulting in hepatotoxicity and subsequent increase in serum transaminase (Cabre et al .,2000 and Kandasamy et al., 2010).

The nutritional results were agreed with the fact that basil is a good source of beta carotene, calcium, vitamin C and also contains zinc, manganese and sodium. Volatile oil of basil has estragol, linalool, eugenol, methyl chavicol and small quantities of methyl cinnamate, cineole, and other terpenes, apigenin, luteolin, orientin and vicenin (Samudralwar and Garg 1996). O. sanctum leaves are used in bronchitis, gastric and hepatic disorders. Chewing a couple of leaves before a meal helps to stimulate the appetite and a tea taken after a meal promotes digestion by increasing the flow of gastric juices, while reducing gas and bloating. Leaves of O. sanctum are commonly used in mild indigestion, diminished appetite and malaise (Vats et al., 2004).

The biochemical results were agreed with many previous researches. The Liver enzymatic superoxide dismutase, catalase, glutathione-S-transferase, non-enzymatic antioxidants (reduced glutathione) and lipid peroxidation end product, malondialdehyde levels were significantly modulated by the O. sanctum oil treatment (Geetha and Vasudevan 2004). O. sanctum leaf powder for a month reduced fasting blood sugar, uronic acid ,total amino acids, total cholesterol, triglyceride, phospholipids and total lipids in diabetic rats indicating the hypoglycemic and hypolipidemic effect of O. sanctum (Rai et al., 1997). Fresh leaves lowered total cholesterol, triglyceride, phospholipid and LDL-cholesterol levels and increased HDL-cholesterol in rabbits (Sarkar et al., 1994). O. sanctum water and alcohol leaf extracts showed a significant ability to scavenge free radicals. Both extracts and their fractions inhibit in vitro lipid peroxidation at very low concentrations. In vivo, lipid peroxidation was also inhibited by aqueous extracts of O. sanctum in a dose-dependent manner in male albino rabbits (Suanarunsawat et al., 2010). O. sanctum for 15 days in mice decreased hepatic lipid peroxidation and glucose-6-phosphatase activity, while the activities of endogenous antioxidant enzymes, SOD and CAT were increased (Chintalwar and Chattopadhyay 2005). O. sanctum was found to lower cholesterol, lactate dehydrogenase and alkaline phosphatase levels without affecting blood glucose and urea levels in rats. Ethanolic O. sanctum leaf extract inhibits oxidative stress by modulating xenobiotic-metabolizing enzymes, reducing the extent of lipid and protein oxidation and up-regulating antioxidant defenses (Vats et al., 2004).

On the basis of above results it may be inferred that basil exhibited significant anti hepatotoxic activity

5-Referance

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