

CHEMOPREVENTIVE EFFECTS OF EGYPTIAN *Balanites aegyptiaca* ON HEPATIC AND RENAL TOXICITY IN MALE RATS

(Received: 11.8. 2008)

By

K. Z. Ghanem, M. M. Ramadan, A. F . Mansour* and H. Z. Ghanem**

Food Science and Nutrition Department, Chemistry of Flavor and Aroma Department, Therapeutical Chemistry Department**, National Research Center, Dokki, Giza, Egypt.*

ABSTRACT

This work examined the protective effect of three different levels of the aqueous extract of the medicinal plant Hegleg (*Balanites aegyptiaca*) on liver damage induced by CCl₄ as well as kidney function and antioxidant biomarkers in albino rats. *Balanites aegyptiaca* volatile extract was isolated and analyzed by using GC and GC/MS and the total phenolic content was done by Folin–Ciocalteu reaction. Different groups of animals were pre-treated with plant extract (0.25%, 0.5% and 1% in drinking water) for two weeks and injected with CCl₄ (1.195ml/kg body weight) on the last day of the second week with contentious extract supplementation for another 15 days. The effect of the three levels of plant extract on the activity of transaminases (AST&ALT), γ -glutamyltransferase (γ -GT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), glutathione peroxidase (GP-X), superoxid dismutase (SOD), glutathione reductase (GR) and the levels of plasma total antioxidant capacity (TAC), total proteins, albumin, total bilirubin, creatinine, urea, uric acid as well as lipid profile were measured in the control group, CCl₄ intoxicated group and plant extracts co-treatment to the CCl₄ - intoxicated groups.

Values recorded for, lipid profile, uric acid as well as liver enzymes and kidney function tests were significantly higher while significant decreases were recorded for antioxidant biomarkers, hemoglobin and total protein levels in CCl₄ intoxicated rats compared to those recorded for the control rats. The plant extract produced significant ($P < 0.05$) hepatic and renal protective effects by decreasing the activity of liver enzymes (AST, ALT, ALP, γ -GT and LDH) as well as the level of urea, creatinine and uric acid. Extract of plant co-treatment to the CCl₄ - intoxicated rats increased activities of GP-X, SOD, GR, levels of TAC, total protein and hemoglobin compared to CCl₄ intoxicated rats. In the plant extract co-treated to CCl₄ groups, all of these oxidant responses were prevented significantly ($p < 0.05$). The values reported at level of extract 1% were near to the normal control group. Plant extract co-treatment to the CCl₄ - intoxicated rats, at the level 0.25% applied, produced no change in activity of liver enzymes and antioxidant biomarkers compared to CCl₄ - intoxicated rats. It was suggested that *B. aegyptiaca* extract at the level 1% could protect liver and kidney cells and maintains antioxidant biomarkers perhaps by eliminating the deleterious effects of toxic metabolites from the drug (CCl₄).

Key words: antioxidants, *Balanites aegyptiaca*, GC-MS, kidneys and liver functions, volatiles.

1. INTRODUCTION

Liver is the most important and main part of the animal body. It is highly affected primarily by toxic agents. Liver is the vital organ of metabolism and excretion. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2,500,000 new cases each year (Gupta and Misra 2006). In living systems, free-radicals are generated as part of the body's normal metabolic process, and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, by bacterial leucocytes, through xanthine oxidase activity, atmospheric pollutants, and from transitional metal catalysts, xenobiotics and drugs. Carbon tetrachloride (CCl₄) yields the reactive metabolite trichloromethyl radical (CCl₃). This free radical

can produce lipid hydroperoxides. These hydroperoxids can decompose to alkoxy (RO \cdot) and peroxy (ROO \cdot) free radicals that can oxidize other cell components (Albano *et al.*, 1982). Oxygen free-radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes. (Boynes, 1991 and Sabu and Kuttan, 2002). Similarly, in carcinogenesis, reactive oxygen species are responsible for initiating the multistage carcinogenesis process starting with DNA damage and accumulation of genetic events in one or few cell lines which leads to progressively dysplastic cellular appearance, deregulated cell growth, and finally carcinoma (Tsao *et al.*, 2004). Hence, therapy using free-radical scavengers (antioxidants) has potential to

prevent, delay or ameliorate many of these disorders (Delanty and Dichter, 2000). The plant antioxidant activity is mainly due to the phenolic components, such as flavonoids, phenolic acids and phenolic diterpenes (Javanmardi *et al.*, 2003). The hepatoprotective action combined with antioxidant activity has a synergistic effect to prevent the process of initiation and progress of hepatocellular damage (Gupta *et al.*, 2006). Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotenoids, glycosides, organic acids, lipids, alkaloids and xanthines. Plant extracts of many crude drugs are also used for the treatment of liver disorders. Extracts of 25 different plants have been reported to cure liver disorders (Sharma *et al.*, 2002). *Balanites aegyptiaca* is a savannah tree characterised by long straight green spines arranged spirally along the branches. Each spine has a two leaflet compound leaf below it. All the branches (whether bearing flower or not) are armed with spines which are usually simple and practically straight. Various parts of the tree is traditionally used for pharmaceutical purposes (Ojo *et al.*, 2006). *Balanites aegyptiaca*, a date like fruits called hegleg date is known in folk medicine for its hypoglycemic effect (Zaahkoul *et al.*, 2003).

The present work was therefore planned to study the volatile components, the antioxidant activity and the effects of aqueous extract of *Balanites aegyptiaca* on hepatic and renal toxicity in male rats.

2. MATERIALS AND METHODS

2.1. Preparation of Hegleg extracts 1, 0.5 and 0.25 gram of each ground dried fruits of *Balanites aegyptiaca* were infused with 100 ml freshly boiled water for 5 min. followed by filtration.

2.2. Isolation of headspace volatiles The volatiles of *Balanites aegyptiaca* were isolated using a dynamic headspace system. Hundred grams dried fruits were subjected to extraction for four hours using diethyl ether as a solvent. The volatile compounds obtained after extraction were dried over anhydrous sodium sulfate, evaporated and concentrated under gentle stream of nitrogen (Heath and Reineccius, 1986).

2.3. Separation and identification of volatiles The obtained volatiles were analyzed using GC-MS apparatus. Separation was performed on Thermo gas chromatograph (Walnut Creek, California, USA) equipped with Finnigan mat SSQ 7000 mass spectrometer and a 30m x 0.25mm DB-5 capillary column. The column

temperature was programmed from 40°C (isothermal for min.), to 300°C at a rate of 5°C/min with 10 min. isothermal hold. The injector temperature was 220°C and the transition line temperature was 300°C. The carrier gas was helium and the column pressure head was 10-15psi. The mass spectrometer had a delay of 3 min. to avoid the solvent peak and then scanned from m/z 50 to m/z 600. Ionization energy was set at 70eV. Identification of compounds was based on the comparison with the MS computer library (NIST and Wiley software package, ThermoFinnigan) and published spectra. A linear retention index was calculated for each compound using the retention times of a homologous series of C6 – C26 n-alkanes (Adams, 1995). Where no reference spectra were available, tentative identifications were made by comparison with spectra of related compounds.

2.4. Determination of total phenolic content Total phenolic compounds in the 1% aqueous extract were determined with Folin-Ciocalteu reagent using gallic acid as the standard (Kahkonen *et al.*, 1999).

2.5. Experimental animals Male albino rats with initial weights ranging from 150 to 160g were used as experimental animals. They were provided from the Breeding Unit of the National Research Centre, Giza, Egypt. The animals were housed individually in stainless steel wire mesh cages. They were maintained for one week, as an acclimatization period. Throughout the experimental period, the rats were fed on commercial standard pellets prepared by Cairo Company of Oil & Soap, Egypt, for experimental animals. The pellets contain 23% protein, 6.5% fat, 4% fibers, 8% ash, 2.5% added minerals and 56% carbohydrates. Rats were provided with food and water *ad libitum*.

2.6. Animal design Forty male Swiss albino rats, aged 5-6 weeks, were used for studying the effects of *Balanites aegyptiaca* infusion on CCl₄ hepatotoxicity. The animals were divided randomly into five equal groups as follows:

Control group: Rats were provided standard diet and tap water *ad libitum*.

CCl₄ Intoxicated group: Animals were intoxicated by intraperitoneally (i.p) injection with CCl₄ (1.195 ml / kg body weight) twice a week for two weeks.

Protected group: divided into three groups (Protected group I, II and III) of *Balanites aegyptiaca* extract: protected group I; rats which were maintained on standard diet and *Balanites aegyptiaca*, in drinking water (0.25g/dl instead of water), protected group II; rats which were maintained on standard diet and *Balanites*

aegyptiaca, in drinking water (0.5g/dl instead of water) and protected group III; rats which were maintained on standard diet and *Balanites aegyptiaca*, in drinking water (1g/dl instead of water), for two weeks. On the last day of treatment a hepatotoxic CCl₄ (1.195 ml /kg) was given intraperitoneally (i.p) twice a week with continuous supplementation with Hegleg fruits extracts for another two weeks.

2.7. Blood sampling Blood samples were collected from each rat by orbital puncture and withdrawn on heparinized tubes, plasma were collected after centrifugation at 3000 r.p.m. for ten min at 4°C and divided into aliquots to avoid freezing and thawing. Aliquots were then stored at -20°C.

2.8. Biochemical assays Transaminases (ALT and AST), alkaline phosphatase (ALP), γ -glutamyltransferase (γ -GT), and lactate dehydrogenase (LDH) activities were determined by the methods described by Bergmeyer *et al.* (1976), Rosalki *et al.* (1993), Szasz (1976), and Anon. (1972) respectively. Total bilirubin, total protein and albumin levels were determined in plasma samples according to the colorimetric method described by Jendrassik and Grof (1938), Peters (1968) and Dumas and Biggs (1972), respectively. Triglycerides (TG), total cholesterol and HDL cholesterol levels in plasma were carried out according to the methods of Wahlefeld (1974), Allain *et al.* (1974) and Finley *et al.* (1978) respectively. Plasma samples were analyzed for urea (Tabacco *et al.*, 1979), creatinine (Bartel *et al.*, 1972) and uric acid (Fossati *et al.*, 1980). The activities of glutathione reductase (GR), Glutathione Peroxidase (GPx), superoxide dismutase (SOD), and plasma total antioxidant capacity (TAC) were measured using methods of Goldberg and Spooner (1983), Paglia and Valentine, (1967), Nishikimi *et al.* (1972), Koracevic *et al.* (2001) respectively. Malondialdehyde (MDA) was determined spectrophotometrically according to Ohkawa *et al.*, (1979). Hemoglobin was determined by using cyanomethemoglobin method (ICSHESH, 1965).

2.9. Statistical analysis Results were subjected to

ANOVA analysis according to SGCG(1987).

3. RESULTS

3.1. Chemical constituents of the headspace volatiles of the *Balanites. aegyptiaca* Gas

Table (1): Analysis of *Balanites aegyptiaca* volatiles by GC-MS

No.	RT	Components*	KI**	Concentration (relative area %)
1	7.940	2-Furancarboxaldehyde	830	0.67
2	12.00	Verbenene	967	0.23
3	13.35	Cineole, dihydro-1,8-	991	0.09
4	14.57	Cymene, o-	1022	0.09
5	14.73	Menthene, 1-para-	1023	0.12
6	16.18	Mentha-3,8-diene(para)	1072	0.21
7	16.79	Campholenal, alpha-	1125	0.16
8	17.13	Pinocarvone	1162	0.1
9	17.81	Verbenone	1204	0.86
10	18.02	6-Camphenyl acetate	1237	1.18
11	18.81	Cinnamyl alcohol (Z)-	1259	9.34
12	18.95	Cinnamyl alcohol (E)-	1300	8.62
13	19.22	Verbenyl acetate	1282	12.95
14	20.19	Sabinyl acetate	1291	16.27
15	20.84	Carvacrol	1298	14.49
16	21.04	Undec-9-en-1-al	1308	9.82
17	21.21	Verbanol acetate	1318	13.46
18	21.49	Carvyl acetate (trans-)	1337	6.53
19	21.87	Dihydro Carveol Acetate	1356	0.45
20	22.16	Carvyl acetate (cis-)	1362	1.4
21	22.66	Carvacrol acetate	1371	1.13
22	23.31	Menth-1-en-9-ol acetate	1420	0.17
23	23.56	Ionone (6-methyl-gamma-E-)	1479	0.65
24	24.33	Indipone	1949	0.14
25	25.76	Bisabolene	1533	0.87

*Compounds listed according to their retention time (RT) on DB5 Column

**Compounds identified by GC-MS (MS) and by kovats index (KI) on DB5column.

Chromatography – Mass Spectrometry (GC / MS) analysis of the *B. aegyptiaca* volatiles is presented in Table (1). Twenty – seven compounds were positively identified. The analysis showed that, Sabinyl acetate (16.27%), Carvacrol (14.49 %), Verbanol acetate (13.46%) Undec-9-en-1-al (9.86%) , Cinnamyl alcohol (Z) (9.34%) Cinnamyl alcohol (E)- (8.62%) and Carvyl acetate (trans-) (6.54%) were the main compounds of the extracted volatile compounds.

3.2. Liver function CCl₄ treatment resulted in a

Table (2): Effect of *Balanites aegyptiaca* extract on liver function parameters.

Parameters / Groups	liver enzyme activities (U/l)					(mg/dl)		
	γ -GT	LDH	ALT	AST	ALP	T.P	ALB	T. Bili
Control	19.72±1.1a	436±26a	41.2±2.8a	96± 8.2a	246±11a	9.1±0.5a	5.4±0.38a	0.512±0.02a
CCl ₄	30.22±1.6b	977±61 b	93±7.1 b	188±11 b	266±10 b	4.9 ±0.30b	2.9±0.13 b	1.13±0.05b
Protected I	29.18±1.3b	928±72b	90.1±6.2b	181±12.2b	267±11 b	5.1±0.21b	3.1±0.15 b	1.06±0.016 b
Protected II	25.31±1.2c	621±51c	70.1±4.2c	151±11.5c	255±14 c	6.9±0.21c	4.9±0.11 c	0.78±0.016 c
Protected III	21.11±1.0a	451±21 a	48±.3.1a	101±7.2a	250±10 ac	8.9 ±0.55a	5.0±0.32 ac	0.522±0.03 a

T.P: Total protein; ALB; Albumin; T.Bili: Total bilirubin; a,b and c: same scrips in the same coloumn indicate no significant differences

significant increase in activities of ALT, AST, ALP, γ -GT, LDH and total bilirubin level. A significant decrease in plasma total protein and albumin was reported after CCl₄ intoxication. However, supplementation with Hegleg extract normalized these effects, that became comparable to the normal control at the level of (1%). No significant change occurred at (0.25%) level compared to CCl₄ intoxicated group (Table 2).

3.3. Antioxidant biomarkers Administration of CCl₄ to rats produced a significant increase in MDA level of red blood cells (RBCs). On the other hand, pretreatment of rats with Hegleg extract (at levels 0.5% and 1%) caused a marked reduction in MDA. In rats treated with CCl₄, there was a significant decrease in GR, SOD, GPx activities and plasma TAC. Non-significant change in their activities were noted at extract level (0.25%). On the other hand, supplementation of rats intoxicated with CCl₄ with extract at levels 0.5% and 1% normalized these levels (Table 3).

elevations, and returned back to normal control at extract level (1%).

3.5. Biochemical evaluation of the three levels of *B. aegyptiaca* extract The results showed that level 1 % of extract supplementation had the highest protective effect against CCl₄-induced hepatotoxicity followed by level of 0.5%. The level of 0.25 % has no protective effect. It is obvious from results that level 1 % exhibits the highest scores for biological effects against CCl₄-induced hepatotoxicity in comparison with the other two levels.

4. DISCUSSION

Much attention has been focused on the protective biochemical function of naturally occurring antioxidants in biological system and on the mechanisms of their actions (Freil and Higdon, 2003 and Manach *et al.*, 2005). Liver injury has often been induced in animals by the administration of carbon tetrachloride (CCl₄); these hepatotoxins characteristically produce cytotoxic injury by destroying hepatocytes by

Table (3): Effect of *Balanites aegyptiaca* extract on some antioxidant biomarkers.

Parameters Groups	Antioxidant biomarkers (U/l)				(mg/dl)
	GR	SOD	GPx	TAC	MDA in RBCs
Control	1099±74 a	414±23a	1500±91 a	3015± 160a	3.88±0.12 a
CCl ₄	839 ±49b	165±11b	974±38 b	1980± 92b	5.12±0.23 b
Protected 1	864±50b	172±14 b	991±41 b	2003±100 b	5.1±0.21 b
Protected 11	975±64c	343±18 c	1266±±88 c	2661±117 c	4.5±0.31 c
Protected 111	1006±58a	362±21c	1493±98 a	2960±115a	3.76±0.13 a

GR: Glutathione reductase; SOD: Superoxid Dismutase; GPx: Glutathion peroxidase (U/l); TAC Total antioxidant capacity and MDA; Malondialdehyde. a, b and c: same scrips in the same coloumn indicate no significant differences ($P \leq 0.05$).

3.4. Lipid profile and kidneys function Urea and creatinine concentrations were studied to assess the renal function. Plasma urea and creatinine levels as well as uric acid were increased significantly after CCl₄-intoxication compared to control group ($p < 0.01$), while *B. aegyptiaca* extract treatment decreased these elevations at extract levels 0.5% and 1%. CCl₄-induced in rats, at the dose applied, produced increase of plasma total cholesterol, triglyceride, LDL and HDL levels (Table 4) when compared with those of the control group ($p < 0.01$), while *B. aegyptiaca* extract treatment decreased these

lipid peroxidation through microsomal cytochrom P-450 (Mac-Sween *et al.*, 1994). The activities of plasma ALT, AST ALP, γ GT and LDH; sensitive indicator of liver function; were studied, since they could be used as an indirect biochemical index of hepatocellular damage (Naziroglu, *et al.*, 1999). In the present study, it was found that CCl₄ caused a significant increase in plasma γ -glutamyl transferase activity. These results are in accordance with El-Demerdash *et al.* (2002). They found that a significant increase in liver GSH level was accompanied by a significant increase in hepatic γ -GT activity. Drinking of *B.*

Table (4): Effect of *Balanites aegyptiaca* extract on lipid profile, kidneys function and blood hemoglobin.

Parameters Groups	Lipid profile (mg/dl)				Kidney function (mg/dl)		mg/dl	g/dl
	Tri-G	TC	LDL	HDL	Urea	Creatinine	Uric acid	Hemoglopine
Control	167±6.5a	188±6.3a	103±5.5a	74±3.9a	42.2±2.1a	0.41±0.020a	2.9±0.11a	12.12±0.55a
CCl ₄	211±10.2b	251±14b	149±10.2b	87±6.4b	93.1±6.1b	3.13±0.08b	5.2±0.18b	10.11±0.31b
Protected 1	197±13.6 b	243±16b	141±11.5b	91±6.8b	90.5±4.7b	2.91±0.08b	4.9±0.21b	10.61±0.52b
Protected 11	186±9.2c	209±14c	122±9.6c	81±6.3c	82.4±5.1c	0.97±0.018c	3.3±0.09c	10.96±0.44c
Protected 111	170±7.3a	196±11a	110±5.3a	75±8.2a	61.5±4.0c	0.91±0.017c	3.1±0.10ac	11.97±0.40 a

Tri-G; triglyceride, TC; total cholesterol, LDL; low density lipoprotein, HDL; high density lipoprotein. a, b and c: same scrips in the same coloumn

aegyptiaca infusion before CCl_4 injection improved the activity of γ -GT, which becomes comparable to the control. The present data show that, CCl_4 induced significant elevation in LDH activity compared to the control. This finding was also reported by Naziroglu *et al.*, (1999) and El-Khatib and Mansour (2001). The metabolism of CCl_4 involves electron oxidase system to yield the trichloromethyl radical, $\text{CCl}_3\cdot$ (Albano *et al.*, 1982), or possibly, the trichloromethyl peroxy radical, $\text{CCl}_3\text{OO}\cdot$ (Britton and Bacon, 1994). Such free radicals can act in two ways: either in a direct way by covalent binding to membrane proteins and lipids (Feher *et al.*, 1992), in particular to those of the endoplasmic reticulum, with resulting alkylation reactions and possible enzyme inactivation; or in an indirect way, through interactions with membrane of lipid peroxidation (Comporti, 1985), which is an important pathogenic mechanism of liver necrosis. Ojo *et al.* (2006) reported that phytochemicals have medicinal uses, stem bark extracts of the *Balanites aegyptiaca*, produced significant ($P < 0.05$) hepatoprotective effects, *Balanites aegyptiaca* extracts significantly suppressed the increase of ALT and AST in rats treated with paracetamol. A higher inhibition of serum level elevation of ALP was observed with the *Balanites aegyptiaca* extracts. From these results, it was suggested that the extracts could protect the liver cells from paracetamol induced liver damages perhaps by eliminating the deleterious effects of toxic metabolites from the drug (Ojo *et al.*, 2006). Present results indicate that rats protected with *B. aegyptiaca* infusion before CCl_4 injection, improved the liver enzyme activities, being in accordance with the findings of Ojo *et al.*, (2006). It is prior that protection with *B. aegyptiaca* infusion- suppressed mainly the toxic elevation in plasma activity of LDH induced by CCl_4 . In the present study, CCl_4 caused significant elevation in all tested liver enzyme activities compared to control. Parallel results were observed by El-demerdash *et al.* (2002), Song and Yen, (2003) and Ojo *et al.* (2006).

Ojo *et al.* (2006) reported that saponin was present in a concentration greater than 100 mg/kg of plant part in the leaf, stem bark and root extracts of *B. aegyptiaca*. The concentrations of cardiac glycosides and phlobatannin recorded for the plants ranged between 10 and 50 mg/kg plant part. Anthraquinone occurred in a very low concentration (< 10 mg/kg) in the leaves of *B. aegyptiaca*. Plant-derived phenolic compounds are well known to exhibit antioxidant activity through a variety of mechanisms, including free radical- scavenging, lipid peroxidation and

chelating of metal ions (Shahidi,1997). It could be estimated that the phenolic compounds (2.34g/100g dry mater) present in the *Balanites aegyptiaca* aqueous extract played an important role in antioxidant activity, directly through the mechanism of reduction of oxidized intermediates in the chain reaction. The results showed that phenolic compounds inhibited the reactive oxygen species (ROS) generation malondialdehyd in RBC decreased from 5.12 mg/dl in CCl_4 -induced hepatotoxicity in rats to 3.76 mg/dl in *B. aegyptiaca* protected rats. The present data demonstrate that *Balanites aegyptiaca* aqueous extract has hepatoprotective effect through its free radical scavenging and antioxidant properties. The antioxidant properties of carvacrol (one of the main volatile compounds identified in the present results) were characterized in *B. aegyptiaca* by Aeschbach *et al.* (1994). Carvacrol, decreased peroxidation of phospholipid liposomes in the presence of iron (III) and ascorbate. This compound was good scavenger of peroxy radicals ($\text{CCl}_3\text{O}_2\cdot$). Uyanoglu *et al.*, (2008) investigated the possible effects of carvacrol obtained from origanum oil upon the regenerative feature of the liver subsequent to partial hepatectomy in rats. Aspartate transaminase (AST), alanine transaminase (ALT) activities and tumor necrosis factor-alpha (TNF- alpha) levels were determined in serum samples. They reported also the liver regeneration, mitotic index and proliferating cell nuclear antigen (PCNA) index increased significantly in carvacrol and hepatectomy (73 mg/kg) rats group over the hepatectomy rats group at the 72nd hour after partial hepatectomy. Also histological evaluations were also similar with the results of PCNA and mitotic indexes. In AST, ALT and TNF- alpha levels, there was no statistically significant difference. According to these results, it was concluded that carvacrol increases the liver regeneration rate (Uyanoglu *et al.* 2008). Chan *et al.* (2005) suggested that carvacrol may act as effective agents to modulate the functions of immuno-responsive cells *via* different intracellular signalling pathways. Koparal *et al.* (2003) demonstrated that carvacrol is very potent inhibitor of a human cancer through its antioxidant power. Ipek *et al.*, (2003) reported that carvacrol exhibited a significant antigenotoxic activity in mammalian cells, indicating its potential for use as an antigenotoxic agent. Ipek *et al.*, (2005) indicated significant antimutagenicity of carvacrol *in vitro*, suggesting its pharmacological importance for the prevention of cancer mainly hepatoma. Zeytinoglu *et al.* (2003) demonstrated that carvacrol inhibits

growth of myoblast cells even after activation of mutated N-ras oncogene, suggesting the possibility that carvacrol may find application in cancer therapy. Santos *et al.* (2004) suggested that 1,8-cineole has potential value as a dietary flavoring agent in the prevention of gastrointestinal inflammation and ulceration. SOD, GX-P and GR, each of which plays a role in the antioxidant system. The free radical on the liver detoxification enzymes (SOD, Gp-x and GR) affected the enzyme activity, mainly peroxides. Enzymes such as SOD and GX-P play important roles in the protection against free radical damage and are considered the primary antioxidant enzymes since they are involved in direct elimination of active oxygen species (Serafini *et al.* 1996). Significant reduction in activities of GPx, SOD, and GR was noted in CCl₄ intoxicated rats groups. Supplementation of plant extract to rats maintain their normal levels. This may reflect the antioxidant activity of *B. aegyptiaca* infusion.

5. REFERENCES

- Adams R. P. (1995). Identification of essential oil components by GC-MS. Allured, Carol Stream.
- Aeschbach R, Loliger J, Scott B.C. Murcia A., Butler J., Halliwell B. and Aruoma O.I. (1994). Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food-Chem-Toxicol.* 32 (1): 31-6
- Albano E, Lott K.A., Slater T.F., Stier A., Symons M.C. and Tomasi A. (1982). Spin-trapping studies on the free-radical products formed by metabolic activation of carbon tetrachloride in rat liver microsomal fractions isolated hepatocytes and *in vivo* in the rat. *Biochem J.* 204:593-603.
- Allain C. C., Poon L. S., Chan C. S., Richmond W. and Fu P. C. (1974): Enzymatic determination of total serum cholesterol. *Clin Chem*, 20: 470-475.
- Anon. (1972). Determination of lactate dehydrogenase activity. *Z Klin Chem U Klin Biochem*, 8: 658-660.
- Bartel H., Bohmer M. and Heierli C. (1972): Serum creatinine determination without protein precipitation. *Clin Chem Acta*, 37:193-197.
- Bergmeyer H.U, Bowers G.N., Horder M. and Moss D.W. (1976). Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes. Part 2. IFCC method for aspartate aminotransferase. *Clin Chem Acta* . 70: 19-29.
- Boynes J.W. (1991). Role of oxidative stress in the development of complication in diabetes. *Diabetes* 40:405 – 411
- Britton R.S. and Bacon B.R. (1994). Role of free radicals in liver diseases and hepatic fibrosis. *Hepatogastroenterology* 41:343-8.
- Chan A.S.L, Pang-Hai H, Yip E.C.H, Tam Y. K. and Wong Y.H. (2005). Carvacrol and eugenol differentially stimulate intracellular Ca²⁺ mobilization and mitogen-activated protein kinases in Jurkat T-cells and monocytic THP-1 cells. *Planta-Medica*.71(7):634-639.
- Comporti M. (1985). Lipid peroxidation and cellular damage in toxic liver injury. *Lab Invest* 53:599-623.
- Delanty N. and Dichter M.A. (2000). Antioxidant therapy in neurologic diseases. *Arch. Neurol.* 57(9):1265-1270
- Dumas B. T. and Biggs H. G. (1972). In standard methods of clinical chemistry. Vol. 7 Academic Press, New York, p. 175-180.
- El-Demerdash E., El-Denshary E.S., El-Ridi M. Al-Gharabli N. and Osman A.M. (2002). Probucol and liver efficiency during chemically-induced hepatocarcinogenesis. *Anticancer Res.* 22:977-84.
- El-Khatib A.S. and Mansour M.A. (2001). Prior treatment with captopril attenuates carbon tetrachloride-induced liver injury in mice. *Res. Commun. Mol. Pathol. Pharmacol.* 110: 3-16.
- Feher J., Csomos G. and Vereckei A. (1992): Role of free radical reactions in liver diseases In: *Free Radical and the liver.* (Csomos. G. and Feher J., eds). Springer-Verlag: Berlin, Heibelberg.
- Finley P. R., Schiffman R. B. and Williamson R. J. (1978). Cholesterol in high-density lipoprotein: use of Mg²⁺/dextran sulfate in its enzymic measurement. *Clin Chem*, 24: 931-933.
- Fossati P., Prencipe L. and Berti G. (1980): Use of 3,5-dichloro-2 hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *Clin Chem*, 26: 227-231.
- Freil B. and Higdon J.V. (2003). Antioxidant activity of tea polyphenols *in vivo*: evidence from animal studies. *J. Nutr.* 133: 3275-84.
- Goldberg D.M. and Spooner R.J. (1983): *Methods of enzymatic analysis*,

- Bergmeyer, H. U. (ed.) vol.3, p.258- 265, Verlag Chemie, Deerfield beach, Fl.
- Gupta A.K. and Misra N.(2006). Hepatoprotective activity of aqueous ethanolic extract of *Chamomile capitula* in paracetamol intoxicated Albino rats. Am. J. Pharma. Toxicol. 1 (1): 17-20.
- Gupta A.K., Chitme H., Dass S.K. and Misra N. (2006). Hepatoprotective activity of *Rauwolfia serpentina* rhizome in paracetamol intoxicated rats. J. Pharmacol. Toxicol., 1: 82-88.
- Heath H. B. and Reineccius G. (1986). Flavouring materials of natural origin. In Flavour chemistry and Technology. Macmillan publisher USA, p. 198-251.
- ICSHEESH (1965) [International committee for standardization in hematology of the European society of hematology] Recommendations and requirements for hemoglobinometry in human blood. J Clin Path. 18: 335-341.
- Ipek E., Tuylu B.A., Zeytinoglu H. , Yagasaki K. Shirahata S. (2003) Effects of carvacrol on sister chromatid exchanges in human lymphocyte cultures. Cytotechnology. 43 (1/3): 145-148.
- Ipek E., Zeytinoglu H., Okay S., Tuylu, B. A., Kurkcuoglu M. and Baser K.H.C. (2005). Genotoxicity and antigenotoxicity of Origanum oil and carvacrol evaluated by Ames Salmonella/microsomal test. Food Chem. 93 (3): 551-556.
- Javanmardi J., Stushnoff C., Locker E. and Vivanco J.M. (2003). Antioxidant activity and total phenolic content of Iranian basil. Food Chem. 83: 547- 50.
- Jendrassik L . and Grof P. (1938). Vereinfacht photometrische methoden zur bestimmung des blutbilirubin. Biochim Z . 297:81-84.
- Kahkonen M. P., Hopia A. I., Vuorela H. J., Rauha J. P., Pihlaja K ., Kujala T. S. and Heinonen M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem, 47: 3954-3962.
- Koparal A.T., Zeytinoglu M., Yagasaki K. and Shirahata S. (2003). Effects of carvacrol on a human non-small cell lung cancer (NSCLC) cell line, A549. Cytotechnology. 43(1/3): 149-154.
- Koracevic D., Koracevic G., Andrejevic V., Koracevic V and Cosic V. (2001). Method for the measurement of antioxidant activity in human fluids. J Clin Pathol, 54: 356- 361.
- Mac Sween R.N.M., Anthony P.P. and Schever P.J. (1994). Pathology of liver, 3rd ed., Longman group limited, Churchill Living Stone Edinburgh, London..
- Manach C., Williamson G., Morand C., Scalbert A and Remesy C. (2005). Bioavailability and bioefficacy of polyphenoles in humans. I. Review of 97 bioavailability studies. Am. J. Clin. Nutr. 81: 230-42.
- Naziroglu M., Cay M., Ustundag B., Aksakal M. and Yekeler H.(1999). Protective effects of vitamin E on carbon tetrachloride-induced liver damage in rats. Cell Biochem Funct 17:253-9.
- Nishikimi M., Rao A.N. and Yagi K. (1972). The occurrence of superoxide anion in the reaction of reduced pheuzazine methosulfate and molecular oxygen. Biochem Biophys Res. Commun, 46: 849-854.
- Ohkawa H., Ohishi N., and Yogi K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95:351-358.
- Ojo O. O., Nadro M. S. and Tella I. O. (2006). Protection of rats by extracts of some common Nigerian trees against acetaminophen-induced Hepatotoxicity. African J. Biotech. 5 (9): 755-760.
- Paglia D.E. and Valentine W.N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70: 158-69.
- Peters T.Jr. (1968). Proposals for standardization of total protein assays. Clin Chem. 14: 1147-59.
- Rosalki S.B., Foo A.Y., Burlina A., Perllwitz W., Stieber P., Neumeier D., Klein G., Poppe W.A. and Bodenmuller H. (1993). Evaluation of ISO- ALP test kit for measurement of alkaline phosphatase activity in serum and plasma. Clin Chem, 39: 648- 652.
- Sabu M.C. and Kuttan R. (2002). Antidiabetic activity of medicinal plants and its relationship with their antioxidant property. J. Ethnopharmacol, 81:155 – 160.
- Santos F.A, Silva, R. M., Campos A.R., Araujo R. P., Lima-Junior, R.C.P. and Rao V.S.N. (2004). 1,8-cineole (eucalyptol), a monoterpene oxide attenuates the colonic damage in rats on acute TNBS-colitis. Food Chem. Toxic. 42(4): 579-584 .
- Serafini M., Ghiselli A. and Ferro A. (1996). *In vivo* antioxidant effect of green and black tea in man. Eur. J. Clin. Nutr. 50: 28-32.
- SGCG (1987). Statistical Graphic Corporation, Graphic. Statgraphics: Statistical Graphics System Version 2.6. SGCG software System Inc. U.S. SGCG Inc.

- Shahidi F. (1997). Natural antioxidants. An overview. In F. Shahidi (Ed.), Natural antioxidants, chemistry, health effects and applications,; (pp.1–11). Champaign, IL: AOCS Press.
- Sharma S.K., Ali M. and Gupta J. (2002). Phytochemistry and Pharmacology, 2: 253-270.
- Song T.Y. and Yen G.C. (2003). Protective effects of fermented filtrate from *Antrodia camphorata* in submerged culture against CCl₄-induced hepatic toxicity in rats. J. Agric. Food Chem. 51:1571-7.
- Szasz G. (1976). Reaction-rate method for gamma-glutamyltransferase activity in serum. Clin Chem 22:2051-5.
- Tabacco A., Meiattini. F., Moda E. and Tarli P. (1979). Simplified enzymatic-colorimetric serum urea nitrogen determination. Clin Chem, 25: 336-337.
- Tsao A.S., Kim E.S. and Hong W.K. (2004). Chemoprevention of Cancer. CA Cancer J. Clin.54:150 – 180.
- Uyanoglu M., Canbek M., Aral E, and Baser K.H.C. (2008). Effects of carvacrol upon the liver of rats undergoing partial hepatectomy. Phytomedicine. 15(3): 226-229.
- Wahlefeld A.W. (1974). In methods of enzymatic analysis, Vol. 5, Bergmeyer, Eds. Academic Press, New York. 1831- 1835.
- Zaahkouk A.M., Rashid Z.A. and Mattar A.F.(2003). Anti – diabetic properties of water and ethanolic extracts of *Balanites aegyptiaca* fruits flesh in senile diabetic rats. The Egyptian J. Hospital Med. 10: 90 – 108.
- Zeytinoglu H., Incesu Z. and Baser. K.H.C. (2003). Inhibition of DNA synthesis by carvacrol in mouse myoblast cells bearing a human N-RAS oncogene Phytomedicine. 10(4):292-299.

التأثير الكيمياءى الواقى لنبات الهجليج المصرى ضد تسمم كبد وكلى ذكور الجرذان البيضاء

قدري زكى غانم- منال محمد رمضان*- عمرو فاروق منصور*- حسن زكى غانم**

قسم التغذية و علوم الاطعمة- *قسم كيمياء مكسبات الطعم و الرائحة-قسم الكيمياء العلاجية
**المركز القومى للبحوث الدقى – الجيزة - مصر

ملخص

يهدف هذا البحث الى التعرف على التأثير الكيمياءى الواقى لنبات الهجليج المصرى ضد تسمم كبد وكلى ذكور الجرذان البيضاء بمادة رابع كلوريد الكربون وتأثيره كمضاد للاكسدة .
تم فصل المركبات العطرية من المستخلص المائى والتعرف عليها باستخدام جهاز الفصل الكروماتوجرافى ذات مطياف الكتلة وكذا تقدير المحتوى الكمى من مركبات البولى فينولات. تم عمل ثلاثة تركيزات مختلفة (0.25% و 0.50% و 1%) للمستخلص المائى لنبات الهجليج حيث اعطيت الى الجرذان البيضاء لمدة اسبوعين وفى نهاية الاسبوعين تم حقن الجرذان البيضاء برابع كلوريد الكربون (1.19 ملل / كيلوجرام من وزن الجسم) واستمرت التجربة لمدة اسبوعين آخرين. وقد تم ايضا دراسة التأثير الكيمياءى الواقى للمستخلص على وظائف الكبد. (الانزيمات الناقلة لمجموعة الامين ولاكتات دى هيدروجينيزو الجاما جلوتاميل ترانسفيراز) و الكلى (اليوريا و الكرياتينين وحمض اليوريك) و نشاط بعض الانزيمات المضادة للاكسدة ومستوى الدهون بالدم (الكوليسترول الكلى والكوليسترول عالى الكثافة والمنخفض الكثافة والتراى جلسريد) تم مقارنة النتائج بالمجموعة الضابطة وايضا بالمجموعة المحقونة برابع كلوريد الكربون.
اظهرت النتائج ان رابع كلوريد الكربون ادى الى ارتفاع معنوي ملحوظا فى انزيمات الكبد وفى مستوى اليوريا و الكرياتينين وحمض اليوريك وكذلك مستوى الدهون بالدم بينما احدث انخفاضا ملحوظا فى مستوى البروتينات و الهيموجلوبين وايضا نشاط الانزيمات المضادة للاكسدة وذلك فى المجموعة المحقونة برابع كلوريد الكربون فقط مقارنة بالمجموعة الضابطة.

واوضحت النتائج ان تناول حيوانات التجارب المحقونة برابع كلوريد الكربون للمستخلص المائى لنبات الهجليج ادى الى حماية خلايا الكبد و الكلى من التلف و الحفاظ على مستوى بعض الانزيمات المضادة للاكسدة ومستوى الدهون بالدم الى قرب المستوى الطبيعى عند تركيز 1% بينما لم تظهر اى نتائج معنوية ايجابية واضحة عند تركيز 0.5 % و 0.25 % .
واظهرت النتائج ان لنبات الهجليج المصرى عند مستوى 1 % تأثير واقى ضد تسمم كبد وكلى الجرذان البيضاء بمادة رابع كلوريد الكربون و له تأثير واضح فى المحافظه على مستوى مضادات الاكسدة بالجسم.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (59) العدد الرابع (اكتوبر 2008): 273-280.