



Hepatoprotective and some haematological parameters effect of *Allium ampeloprasum* against carbon tetrachloride induced liver toxicity in albino rats

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Abstract:

The aim of the present study is to prepare ethanol extract of leek and to study the effect of its on body weight, liver weight, serum liver function enzymes, serum lipids profile and in carbon tetrachloride (CCL₄) intoxicated rats. Used thirty six male rats were divided into 6 equal groups. One group of rats was fed on basal diet and kept as a negative control while three groups were administrated by CCL₄ at a dose of 0.5 ml /kg body weight for induction of acute liver toxicity. The first group of the intoxicated rats was left as a positive control while the others were fed on experimental diets with leek extract at 200,400 mg/kg and other two groups were gives only 200,400 mg/kg of leek extract. At the end of experimental period (4 weeks), the rats were sacrificed and blood samples were collected for biochemical analyses. Livers of the sacrificed rats were removed and prepared for histopathological examination. Results showed that treatment effect of leek extract especially at 400 mg/kg in CCL₄ -intoxicated rats improved their body weight. Also decreased the levels of liver enzymes, total cholesterol, triglycerides and improved lipid profile. While the doses 200,400 mg/kg of leek extract groups appear normal values of all parameters in this study. Finally histopathological examination revealed alleviation of hepatic lesions caused by CCL₄ by increasing the concentration of leek extract used. In conclusion, it was suggested that leek extract could protect the liver cells from CCL₄-induced liver damages perhaps, by its antioxidative effect on hepatocytes, hence eliminating the deleterious effects of toxic from CCL₄.

Key words: *Allium*, Body weight, Liver weight, Liver enzymes, lipoprotein, Histopathology, CCL₄ and Rats.

تأثير الكراث في حماية الكبد وبعض معايير الدم ضد التسمم المستحث برابع كلوريد الكربون في الجرذ الامهق

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الخلاصة:

هدفت الدراسة الحالية إلى معرفة تأثير المستخلص الكحولي للكراث في معدل وزن الجسم والكبد ومستوى إنزيمات الكبد في والبروتينات الدهنية في الجرذان المعاملة بمادة رابع كلوريد الكربون لاستحداث التسمم فيها. استعمل 36 جرذ ذكر قسمت إلى

ست مجاميع متساوية ، المجموعة الأولى أعطيت الغذاء الاعتيادي وعدت مجموعة السيطرة السالبة بينما المجاميع الثلاثة الأخرى جرعت بمادة رابع كلوريد الكربون بجرعة 0.5 ملغم/كغم لاستحداث التسمم الكبدي الحاد، المجموعة الأولى منها هي السيطرة الموجبة أما المجموعتين الباقيتين أعطيت الغذاء الاعتيادي مع مستخلص الكراث الكحولي بالجرع 200 و 400 ملغم/كغم ، في حين المجموعتين الأخيرتين أعطيت مستخلص الكراث فقط بالجرع المذكورة فقط . في نهاية مدة التجربة (أربع أسابيع) خدرت الحيوانات وجمعت عينات الدم لإجراء التحاليل الكيموحيوية بينما عينات الكبد حفظت لإجراء الدراسة النسجية المرضية عليها . أظهرت النتائج إن المعاملة بالمستخلص الكحولي للكراث بالجرعتين 200 و 400 ملغم/كغم مع وجود رابع كلوريد الكربون حدوث تحسن ملحوظ في وزن الجسم وكذلك نقصان في مستويات الكوليستيرول الكلي والكليسيريدات الثلاثية وانخفاض واضح في مستوى البروتينات الدهنية بالإضافة إلى ذلك المجموعتين المعاملة بمستخلص الكراث فقط تكون القيم طبيعية لكل القيم المدروسة . أخيرا بينت الدراسة النسجية انخفاض الآفات الكبدية المستحثة بواسطة رابع كلوريد الكربون ويزداد الانخفاض بزيادة تركيز المستخلص . نستنتج من ذلك إن لمستخلص الكراث تأثير واقى للخلايا الكبدية ضد التسمم المستحث برابع كلوريد الكربون ربما يعود سبب ذلك إلى التأثير المضاد للأكسدة في الخلايا الكبدية الذي أدى إلى إزالة تأثير رابع كلوريد الكربون السام . توصي الدراسة باستعمال الكراث من قبل المرضى الذين يعانون من أمراض الكبد الناتج عن تأثيره الفعال في حماية الخلايا الكبدية والخافض للارتفاع تركيز البروتينات الدهنية في المصل.

Introduction:

The liver is the most important organ in the body. It plays a pivotal role in regulating various physiological processes. It is also involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles (1,2). It helps in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. In addition, it aids metabolism of carbohydrate, protein and fat, detoxification, secretion of bile and storage of vitamins (3). The role played by this organ in the removal of substances from the portal circulation makes it susceptible to first and persistent attack by offending foreign compounds, culminating in liver dysfunction (4).

Liver diseases remain one of the major threats to public health and are a worldwide problem (5). They are mainly caused by chemicals like acetaminophen (in large doses), excess consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages (6,7,8).

Medicinal plants play a key role in

human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant material (9). The allium group is one of the world's most widely cultivated vegetable groups, with their culinary and medicinal uses. Equally varied are their health benefits, for they contain a range of phytochemicals with an array of biological effects. Evidence shows they play an important role in protecting against major lifestyle chronic diseases as well as health problems associated with ageing. Their antimicrobial activity, long recognized in folk remedies, has also now been scientifically validated (10,11).

The *Allium* genus includes approximately 500 species, the most widely used of which are onions, garlic, shallots, chives, scallions and leeks (12). Leeks (*Allium porrum* or *A. ampeloprasum* var. *porrum*), sometimes called "the gourmet's onion" have flat leaves instead of tubular and relatively little bulb development and is native to Western Asia and the Mediterranean countries. The thick leaf bases and slightly developed bulb look like a giant green onion, and are eaten as a cooked vegetable. Leeks contain saponins and the major flavonoid in leeks is kaempferol, with only a small amount of quercetin, carotenoids and chlorophyll mainly in the green tops (13,14).

Materials and methods:**Materials:**

Chemicals: Carbon tetrachloride (CCL₄) were obtained from the chemistry department/kufa university. the rats received a dose of (0.5) ml/kg of CCL₄ was suspended in olive oil (1:1v/v) by interagastric intubation method as well as leek extract was given at a dose 200 mg/kg , 400 mg/kg by interagastric intubation method also.

Leek leaves : were obtained from the local market in al Najaf city, Iraq.

Methods:

Preparation of leek extract :The leek leaves were dried at (45°C) and crushed to powder by using a blender, take a bout 100g of powdered were added to 500ml of 80% ethanol and put the mixture in soxhelt system during 24h . After that, resulting extracts were filtered using filter paper and concentrated to dryness in rotary evaporator in the room temperature and the recipient was used by several dilution (15).

Experimental Design : Thirty six male albino rats strain (*Rattus rattus*) weighting (230-250g) obtained from the animal house in the science faculty / Kufa university, Najaf. The rats kept under observation for one week before starting the experiment for acclimatization. fed on standard diet and water ad libitum . Then animals were divided into six groups of six rats in each. The first group was fed on the basal diet and served as a negative control (-Ve). The three groups were given carbon tetrachloride (CCL₄) for induction of acute liver damage. CCL₄ was diluted in an equal volume of olive oil as a vehicle and interagastric intubation method in a dose of 0.5 ml/kg body weight (16) . The first hepatotoxic group was fed basal diet and kept as a positive control (+ Ve) while the other hepatotoxic groups were fed on basal diets that substitute 200, 400 mg/kg of leek extract .The rest two groups were fed on basal diets that substitute 200 and 400 mg/kg of leek extract only .

At the end of experiment period (4 weeks), rats were anaesthetized by ether and blood samples were collected from the portal vein divided in two parts the one put into dry centrifuge tubes and were centrifuged for 20 minutes at 3000 rpm and other parts put in EDTA tube for haematological analyses (Hb, PCV). After separate the sera which were kept at -10°C till biochemical analysis. Livers of the sacrificed rats were removed for histopathological examination.

Biochemical Analyses: The collected serum samples were used for estimating aspartate amino transferases (AST), alanine amino transferases (ALT) (17), and alkaline phosphatase enzymes (ALP) (18). Serum total cholesterol (TC) (19) , triglycerides (TG) (20) and high density lipoprotein (HDL) (21) were determined calorimetrically. Low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL) were calculated mathematically according to Friedwald's equations (22).

$$LDL = TC - [HDL + (TG/5)]$$

$$VLDL = Triglycerides / 5.$$

Histopathological Studies of the Liver: Livers of the scarified rats were dissected, removed, washed with normal saline and put in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. The tissue specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness, stained with Hematoxylen and Eosin (H and E) and then studied under an electronic microscope (23).

Statistical Analysis: Results were expressed as means $\pm$ S.E. Statistical analysis was carried out using computerized SPSS program (version 17) with one way ANOVA (24).

Results and Discussion:

The liver, the key organ involved in numerous metabolic functions and detoxification of hazardous substances, is

a frequent target of a number of toxicants (25). There is no doubt that reactive oxygen species play an important role in pathological changes in the liver, particularly in the cases of alcoholic and toxic liver diseases (26). It is now generally accepted that the hepatotoxicity of CCl_4 is the result of reductive dehalogenation, which is catalyzed by P-450 enzyme system and which forms highly reactive trichloromethyl free radical. This readily interacts with molecular oxygen to form the trichloromethyl peroxy radical. Both trichloromethyl and its peroxy radical are capable of binding to proteins or lipids, or of abstracting a hydrogen atom from an unsaturated lipid, initiating lipid peroxidation and liver damage and by doing so playing a significant role in pathogenesis of diseases (27,28).

In the table (1) showed the body weight significant decrease ($p < 0.05$) as a result of CCl_4 administration was considered to be the result of direct toxicity of CCl_4 and indirect toxicity related to the liver damage. Changes in the body weight after CCl_4 dosing have been used as a valuable index of CCl_4 -related organ damage (29,30).

CCl_4 is one of the chemicals that affect on the liver causing hepatic injury leading to acute inflammation followed by its chronic form, which may be complicated by cirrhosis and hepatocellular carcinoma, all these affects causes significant decrease ($p < 0.05$) in the liver weight (31).

Vegetables are an important source of mineral and phenolics which play an important role in nutritive value. *Allium* vegetables and related organosulfur compounds inhibit of mutagenesis, modulation of enzyme activities, inhibit of DNA adduct formation, scavenge of free-radical, and effect on cell proliferation and tumor growth (32). Leeks are a good source of dietary fiber, folic acid and calcium. Leeks are easy to digest and

have laxative, antiseptic, diuretic, and anti-arthritis properties (33). Leeks support healthy digestion by promoting the growth of useful bacteria in the gut due to contain prebiotics carbohydrates that serve as fuel for good bacteria in the digestive tract. These probiotic bacteria fortify the immune systems and keep digestive processes running smoothly. Leeks fiber energizes the human body to perform many types of biological functions like digestion, metabolism and growth therefore leeks digest causes significant increase ($p < 0.05$) in the body weight and other organs as liver (34).

Table (2) show in positive control group significant decreased ($p < 0.05$) hemoglobin and packed cell volume compared to negative control group. The treated groups with ethanol extract of leek 200 mg/kg and 400 mg/kg and CCl_4 showed non significant decrease ($p < 0.05$) in hemoglobin and packed cell volume compared to negative control group. The treated groups with ethanol extract of leek 200 mg/kg and 400 mg/kg only showed significant increase ($p < 0.05$) in hemoglobin and packed cell volume compared to negative control group, this results were similarity with (35).

Leeks are a good source of allyl sulfides and also rich in the flavonoid especially kaempferol. Leeks contain excellent amounts of vitamin C, as well as folate, and some useful amounts of B vitamins, vitamin E, copper, potassium and iron. These vitamins and minerals work together to help stabilize blood. Calcium in leeks is also used for the proper clotting of blood in the human body in addition of iron was increased the haemopoiesis process in the bone marrow due to significant increased levels ($p < 0.05$) of the hemoglobin and packed cell volume in the blood (36). *Allium* species also have immune enhancing actions that include promotion of lymphocyte synthesis, cytokine release, phagocytes and natural killer-cell activity

(37).

Assessment of liver function in the table (3) can be made by estimating the activities of serum AST, ALT and ALP which are enzymes originally present higher concentration in cytoplasm. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage (38). The elevated level of these entire marker enzymes observed in the positive control group corresponded to the extensive liver damage induced by toxin. These results are in agreement with previous finding that the activity levels of serum AST, ALT and ALP were significantly elevated ($p < 0.05$) in rats after CCl₄ administration (39). The reduced concentrations of AST, ALT and ALP as a result of leek extract administration, the tendency of these marker enzymes to return towards a near normalcy in ethanol extract of leek administration groups point towards an early improvement in the secretory mechanism of the hepatic cell and is a clear manifestation of anti-hepatotoxic effect of leek extract. This effect was similar to that reported in another study that ethanol extract of leek was observed to lower effectively the increased levels of AST, ALT and ALP

in CCl₄ intoxication rats in comparison with negative control group (40).

In addition, the results in the table (4) and (5) showed in positive control significant increased levels ($p < 0.05$) of TC, TG and serum lipoproteins. This perhaps due to the presence of damage in the liver. Also the benefit effect of leek extract on serum lipids and improvement in the levels of TC, TG and serum lipoproteins is probably indicative of hepato-protective effect of leek extract in CCl₄ administration rats. In another study (41) suggested that one reason for the preventive effect of leek against the atherosclerotic process may be the changes in the portions of lipoprotein cholesterol fractions. Moreover, previous studies have shown that ingestion of leek appears to inhibit hepatic fatty acid synthesis by lowering key enzymes activities in supplying substrates, thus reducing lipid accumulation in the liver and TG level in plasma. With respect to the cholesterol lowering property of leek, it has been suggested that some constituents of leek may act as inhibitors for some enzymes such as hydroxyl methyl glutaryl CoA reductase, which participates in cholesterol synthesis (42).

Table (1) Effect of leek extract on body weight and liver weight in CCL4 – intoxicated rats.

Groups	Controls- ve	Controls+ ve	200 mg/kg leek	400 mg/kg leek	200 mg/kg leek+ CCL4	400 mg/kg leek+ CCL4
Variables						
Body weight(g)	273±9.15	218±8.21 A	272±3.03 *	277±7.10 *	7.18±247 b *	11.14±254 b *
Liver weight(g)	35.11±1.02	27±0.22 A	34±3.10 *	37±2.61 *	29±2.15 b *	31±7.65 b *

Values are mean±S.E. Values in the same row sharing the different letters are significantly different with negative control.

*significantly different with positive control.

Six rats in each groups

Table (2) Effect of leek extract on blood hemoglobin and packed cell volume in CCL4 – intoxicated rats.

Groups Variables	Controls- ve	Controls+ ve	200 mg/kg leek	400 mg/kg leek	200 mg/kg leek+ CCL4	400 mg/kg leek+ CCL4
Hb(gm/dl)	12.08±2.18	7.99±1.39 A	12.11±1.13 *	12.75±1.98 *	±1.2410.14 b *	2.01±10.55 b *
PCV(%)	38.61±3.62	29±0.55 A	37.81±3.17 *	38.31±4.11 *	33.79±1.47 b *	35.71±2.12 b *

Values are mean±S.E. Values in the same row sharing the different letters are significantly different with negative control.

*significantly different with positive control.

Six rats in each groups

Table (3) Effect of leek extract on liver function enzymes in CCL4 – intoxicated rats.

Groups Variables	Controls- ve	Controls+ ve	200 mg/kg leek	400 mg/kg leek	200 mg/kg leek+ CCL4	400 mg/kg leek+ CCL4
AST(μ/ml)	41.16±3.51	97.31±8.61 A	45.37±2.10 *	43.71±0.77 *	±6.0173.07 b *	1.81±61.20 b *
ALT(μ/ml)	13.24±7.08	28.05±2.12 A	15.10±2.60 *	16.18±1.14 *	25.23±0.50 b *	21.34±1.12 b *
ALP(μ/ml)	4.22±31.17	2.41±55.24 A	2.44±33.60 *	3.15±32.62 *	3.10±47.02 b *	6.27±43.11 b *

Values are mean±S.E. Values in the same row sharing the different letters are significantly different with negative control.

*significantly different with positive control.

Six rats in each groups

Table (4) Effect of leek extract on serum total cholesterol and triglycerides in CCL4 – intoxicated rats.

Groups Variables	Controls- ve	Controls+ ve	200 mg/kg leek	400 mg/kg leek	200 mg/kg leek+ CCL4	400 mg/kg leek+ CCL4
TC(U/I)	92.98±2.49	110.96±2.31 A	92.16±1.84 *	93.11±2.75 *	±1.79102.56 b *	3.23±98 b *
TG(U/I)	53.32±2.56	62.16±1.99 A	53.39±2.95 *	54.42±1.43 *	59.40±2.30 b *	57.22±3.66 b *

Values are mean±S.E. Values in the same row sharing the different letters are significantly different with negative control.

*significantly different with positive control.

Six rats in each groups

Table (5) Effect of leek extract on lipoprotein fractions in CCL4 – intoxicated rats.

Groups Variables	Controls- ve	Controls+ ve	200 mg/kg leek	400 mg/kg leek	200 mg/kg leek+ CCL4	400 mg/kg leek+ CCL4
HDL(U/I)	63.94±1.01	76.02±2.20 A	67.07±1.13 *	65.24±0.24 *	±1.3174.19 b *	0.39±72.08 B *
LDL(U/I)	17.13±0.54	21.84±0.72 A	16.60±0.56 *	17.40±0.96 *	19.21±3.27 b *	18.05±4.24 B *
VLDL(U/I)	10.65±0.41	15.52±0.30 A	11.63±1.27 *	10.70±0.19 *	13.22±6.02 b *	12.10±3.42 B *

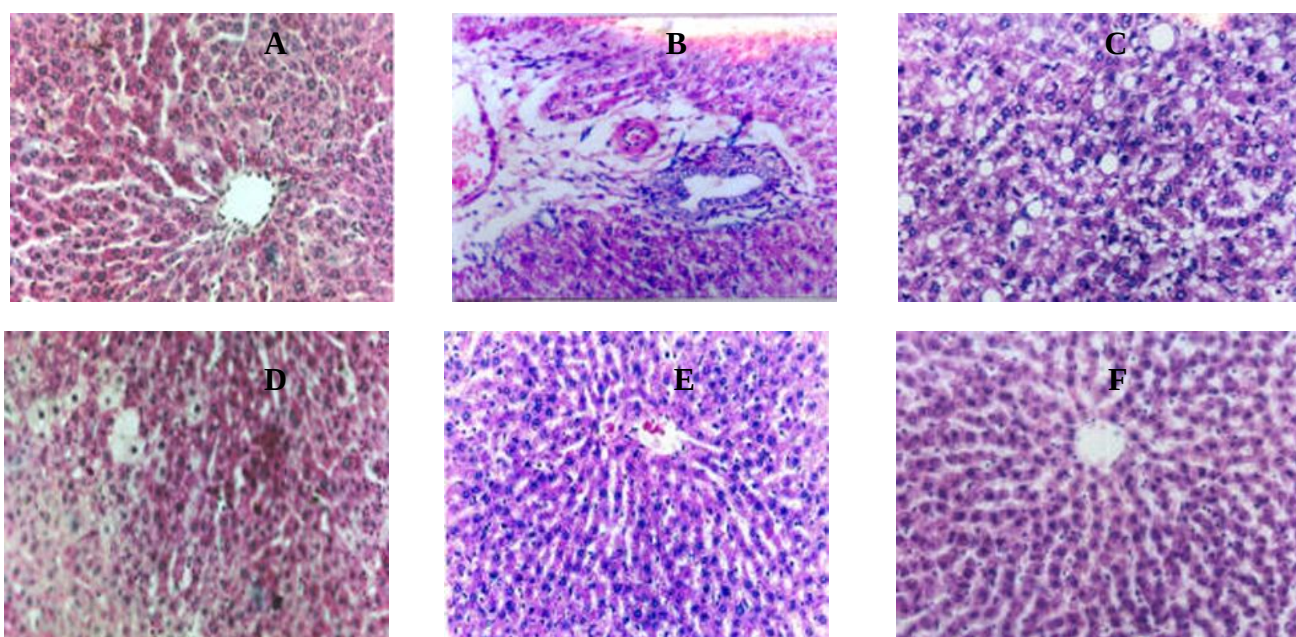
Values are mean±S.E. Values in the same row sharing the different letters are significantly different with negative control.

* significantly different with positive control.

Six rats in each groups

Histopathological examination of livers of the negative control rats fed on basal diet revealed normal histological picture of hepatic lobule which consists of central vein surrounded by normal hepatocytes as shown in Fig. (1-A). Examination of liver of the CCL4-intoxicated positive control rats showed severe fatty degeneration of hepatocytes and infiltration of leucocytes in hepatic sinusoid (Fig.1-B). Livers of CCL4-

intoxicated rats fed on diet+200mg/kg leek extract showed little vacuolar degeneration of hepatocytes as shown in Fig. (1-C) while livers of CCL4-intoxicated rats fed on diet+400mg/kg leek extract showed almost normal histology of the hepatic lobule (Fig.1-D). Finally Livers of groups gives only leeks extract showed as negative control rats (Fig.1-E,F).The higher concentration of leek extract the improvement in liver histopathology.



Figure(1) Histopathological changes detected in the liver of (A) control-ve (B) control + ve (C) CCL4+200 mg/kg leek (D) CCL4+400 mg/kg leek (E) 200 mg/kg leek and (F) 400 mg/kg leek.(40X).

Conclusion: From these results, it was suggested that ethanol extract of leek could protect the liver cells from CCl₄-induced liver damages perhaps, by its antioxidative effect on hepatocytes, hence eliminating the deleterious effects of toxic metabolites from CCl₄. So the present study recommended that the use of the leeks may be useful for patients suffering from liver diseases due to its hepatoprotective and hypolipidemic activities .

References :

- 1-Shanani S (1999). Evaluation of hepatoprotective efficacy of APCL-A polyherbal formulation in vivo in rats. Indian Drugs. 36: 628-631.
- 2-Subramoniam A, Pushpangadan P(1999). Development of phytomedicine for liver diseases. Indian J. Pharmacol., 31: 166-175.
- 3-Ahsan MR, Islam KM, Bulbul IJ (2009). Hepatoprotective activity of Methanol Extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. Eur. J. Sci. Res. 37(2): 302-310.
- 4-Bodakhe SH, and Ram A (2007). Hepatoprotective Properties of Bauhinia variegata Bark Extract. Yakugaku Zasshi., 127: 1503-1507.
- 5-Asha V, Pushpangadan P (1998). Preliminary evaluation of the anti-hepatotoxic activity of Phyllanthus kozhikodanus, Phyllanthus maderspatensis and Solanum indicum, Fitoterapia 59: 255-259.
- 6-Dianzani MU, Muzia G, Biocca ME, Canuto RA (1991). Lipid peroxidation in fatty liver induced by caffeine in rats. Int. J. Tissue React. 13: 79-85.
- 7-Recknagel RO (1983). A new direction in the study of Carbon tetrachloride hepatotoxicity. Life Sci. 33: 401-408.
- 8-Wendel A, Feurensteins S, Konz KH (1987). Acute paracetamol intoxication of starved mice leads to lipid peroxidation in vivo. Biochem. Pharmacol., 28: 2051-2053.
- 9-WHO (1993). Regional Office for Western Pacific, research guidelines for evaluating the safety and efficacy of herbal medicines. Manila.
- 10-Riley, D.M., Bianchini, F. And Vainio, H.(2008): *Allium* Vegetables and Organosulfur Compounds: Do They Help Prevent Cancer.. Environ Health Perspect. Sep; 109(9):893-902.
- 11-Gupta SS (1994). Prospects and perspectives of natural plant products in medicine. Indian J. Pharmacol. 26: 1-12.
- 12-Malairajan, P., Geetha, G., Narasimhan, S. And Jessi, K. (2006): Analgesic Activity Of Some Indian Medicinal Plants. J. Ethnopharmacol., 19: 425-428.
- 13-Garth, L. N. And Rita, E. (2006): Lipid Replacement And Antioxidant Nutritional Therapy For Restoring Mitochondrial Function And Reducing Fatigue In Chronic Fatigue Syndrome and Other Fatiguing Illnesses. Journal of Chronic Fatigue Syndrome; 13(1): 57-68.
- 14-Onyeagba, R.A., Ugbogu, O.C., Okeke, C.U And Iroakasi, O.(2004):Studies on the Antimicrobial Effects Of Garlic (*Allium Sativum* Linn), Ginger (*Zingiber Officinale* Roscoe) And Lime (*Citrus Aurantifolia* Linn). Afr.J.Biotechnol. 3:552-554.
- 15-Irkin, R. And Korukluoglu, M. (2007):

Control Of *Aspergillus Niger* With Garlic, Onion And Leek Extracts. African Journal Of Biotechnology Vol.6 (4), 384-387.

16-Manoj, B. and K. Aqueed, (2003). Protective Effect of *Lawsonia alba* L. Against CCl₄-Induced Hepatic Damage in Albino Rats. Indian J. Exp. Biol., 4: 85-87.

17-Bergmeyer, H.U., P. Schreiber and A. Wahlefeld, (1978). Optimization of Methods for Aspartate and Alanine Amino Transferases. Clin. Chem., 24: 58-61.

18-King, J. (1965). The phosphohydrolases acid and alkaline phosphatases. In: Practical and Clinical Enzymology, Eds., King, J. Van Nostrand Co. Ltd., London, pp: 191-208.

19-Allain, C., L.S. Poon, C.S. Chan, W. Richmond and P.C. Fu, (1974). Enzymatic Determination of Total Serum Cholesterol. Clin. Chem., 20: 470-475.

20-Wahlefeld, A.W., (1974). Methods of Enzymatic Analysis, pp: 1831-1835, Academic Press.

21-Lopes-Virella, M.F., P. Stone, S. Ellis and J.A. Colwell, (1977). Cholesterol Determination in High-Density Lipoproteins Separated by Three Different Methods. Clin. Chem., 23(5): 882-884.

22-Friedewald, W.T., R.I. Levy and D.S. Friedrickson, (1972). Estimation of Plasma or Serum Low Density Lipoprotein Cholesterol Concentration without Use of Preparaline Ultracentrifuge. Clin. Chem., 18: 499.

23-Carleton, H., (1979). Histological Techniques, 4th Edition, London, Oxford University Press, New York, USA.

24-Snedecor, G.W. and W.G. Cochran, (1986). Statistical Methods. 4th Edition. Iowa State University Press, Ames, Iowa,

USA.

25-Meyer, S.A. and A.P. Kulkarni, (2001). Hepatotoxicity. In: Introduction to Biochemical Toxicology, 3rd ed. Eds., Hodgson E. and R.C. Smart. John Wiley and Sons, New York, pp: 487-490.

26-Poli, G. and M. Parola, (1997). Oxidative Damage and Fibrogenesis. Free Radic. Biol. Med., 22: 287-305. 28.

27-Strader D.B., Wright T., Thomas D.L. and Seeff L.B. (2004): "Diagnosis, management, and treatment of hepatitis "C.", Hepatology; 39: 1147-1171.

28-Recknagel, R.O., E.A. Glende, J.R. Dolak and J.A. Waller, (1989). Mechanisms of carbon tetrachloride toxicity. Pharmacol. Ther., 43: 139-154. 29.

29-Bruckner, J.V., W.F. MacKenzie, S. Muralidhara, R. Luthra, G.M. Kyle, D Acosta, (1986): Oral Toxicity of Carbon Tetrachloride: Acute, Subacute and Subchronic Studies in Rats. Fundam. Appl. Toxicol., 6: 16-34.

30-Pradeep, K., C.V. Mohan and K.G. Karthikeyan, (2005). Effect of Pretreatment of *Cassia fistula* Linn. Leaf Extract Against Subacute CCL₄ Induced Hepatotoxicity In Rats. Indian J. Exp. Biol., 43: 526-530.

31-Dorant, E., Van Den, P.A. And Goldbohm, R.A. (1996): A Prospective Cohort Study On The Relationship Between Onion And Leek Consumption, Garlic Supplement Use and The Consumption, Garlic Supplement Use And The Netherlands. Carcinogenesis Mar; 17(3): 477-84.

32-Argho, E.M., Ekpo, K.E. and Ibeh, G.O. (2010). Effect of aqueous extracts of *Allium cepa* on Carbon Tetrachloride liver damage in wister albino

rats.Eur.J.Sci.Res.,26(1):122-130.

33-Fortin, F. (2004): Protection Against Co-Carcinogenesis By Antioxidants. Editorial Director. The Visual Foods Encyclopedia. Macmillan, New York. 22:116.

34-Merchant, R.E.(2003): Dietary Chlorella Pyrenoidosa For Patients With Malignant Glioma:Effects On Immunocompetence, Quality of Life, And Survival. Phytother.Res. 2003; 4(6):220-31.

35-Nkosi, C.Z., A.R. Opoku and S.E. Terblanche, (2005).Effect of Pumpkin Seed (Cucurbita Pepo) Protein Isolate on the Activity Levels of Certain Plasma Enzymes in CCl4-Induced Liver Injury in Low Protein Fed Rats. Phytotherp. Res., 19(4): 341-345.

36-Mitra, S.K., M.V. Venkataranganna, R. Sundaram,S. Gopumadhavan, (1998). Effect of HD-03, A Herbal Formulation on the Antioxidant Defence System inRats. Phytotherapy Research, 12(2): 114-117.

37-Lin, C.C., M.H. Yen, T.S. Lo and J.L. Lin, (2009).Evaluation of the Hepatoprotective and Antioxidant Activity of *Boehmeria nivea* and *Allium cepa*. J.

Ethnopharmacol., 60(1): 9-17.

38-Aouadi, R., A. Aouadet, A. Elkadhi, C. Ben Rayana, H.Jaafoura, B. Tritar and K. Nagati, (2011). Effect of fresh leek (*Allium Porrum*) on lipid metabolism in male rats. Nutrition Research, 20: 273-280.

39-Gebhardt, R.,(1991). Inhibition of cholesterol biosynthesis by a water soluble leek extract in primary cultures of rat hepatocytes. Arzneimittelforschung, 41: 800-804.

40-Durak, L., M. Kavutcu, B. Aytac, A. Avci, E. and Devrim, H. (2004). Effect of leek extract consumption on blood lipid and oxidant/antioxidant parameters in humans with high blood cholesterol.J. Nutr. Biochem., 15: 373-377.

41-Gebhardt, R. and H. Beck, 1996. Differential inhibitory effects of leek-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte cultures. Lipids, 1269-1276.

42-Joanne Barnes Linda A. Anderson and J. David Phillipson (2007): Herbal Medicines, 3rd Edition, Pharmaceutical Press Publication.