Effects of Garlic Oil in Correction of Hepatotoxicity Induced by CCl_4 in Rabbits

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Abstract

This study was designed to evaluate the physiological effects of garlic oil in hepatotoxicity induced by intubation of CCl_4in both sex rabbits. Thirty local domestic rabbits were used and divided randomly into three groups each of which contain ten animals, the 1st group was served as control, received no treatment, the 2nd group were received carbon tetra chloride (CCl_4) at a single dose of 1.5 ml/kg/B.W orally to induce hepatotoxicity, and the 3rd group received garlic oil at a dose of 1ml/kg/B.W intraperitonealy in one hour before administration of CCl_4 in a same dose and manner like that of the 2nd group, while the garlic oil treatment continues for three days. Liver functions were assessed by estimating serum alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total serum bilirubin & total serum protein with histopathological examination after 72 hours of induction. The results revealed that the use of garlic oil produced significant reduction in the level of these enzymes except level of total serum protein that elevated, and greatly reverted the tissue of the liver to normal state, so the use of garlic oil possess hepatoprotective activity by restoring the normal hepatic functions and potentiating the bio- defense system of the liver against hepatotoxicity produced by CCl_4 administration.

تأثير زيت الثوم في تصحيح تسمم الكبد المستحدث برابع كلوريد الكاربون في الأرانب

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الخلاصة

صممت هذه الدراسة لتقييم التأثيرات الفسلجية التي يحدثها زيت الثوم بعد استحداث تسمم الكبد التجريبي. استخدم 30 أرنباً محلياً من كلا الجنسين قسمت عشوائياً إلى ثلاث مجموعات ضم كل منها 10 أرانب، استخدمت المجموعة الأولى كمجموعة سيطرة، المجموعة الثانية أعطيت رابع كلوريد الكاربون CCl_4 بجرعة منفردة مقدارها 1.5 مل/ كغم من وزن الجسم عن طريق الفم لاستحداث تسمم الكبد الحاد، أما المجموعة الثالثة فقد عوملت بزيت الثوم بجرعة 1 مل/ كغم عن طريق الحقن في الخلب قبل إعطاء CCl_4 بساعة واحدة، أعطي CCl_4 بنفس الجرعة والكيفية كما في المجموعة الثانية في حين استمرت المعاملة بزيت الثوم لثلاثة أيام. تم تقييم وظائف الكبد من خلال قياس مستويات إنزيمات النين امينو ترانسفيريز، اسبارتيت امينو ترانسفيريز، الفوسفاتيز القلوي، البليروبين الكلي والبروتين الكلي في مصل الدم مع الفحص النسيجي للكبد بعد 72 ساعة من أعطاء CCl_4.

أظهرت النتائج ان استخدام زيت الثوم أدى إلى خفض مستويات هذه الأنزيمات ما عدا مستوى البروتين الكلي الذي شهد ارتفاعاً، وإعادة نسيج الكبد إلى وضع قريب من الوضع الطبيعي إلى حد كبير. لذا فأن زيت الثوم يمتلك

فاعلية واقية من خلال عمله في إعادة وظائف الكبد الاعتيادية وتقوية الجهاز الحيوي الدفاعي للكبد ضد الضرر الناتج عن إعطاء رابع كلوريد الكاربون.

Introduction

Garlic (*Allium sativum Linn*) is a member of plant kingdom, Asparagales order, Alliaceae family, and Allioideae subfamily, which is widely consumed herb in food stuffs and medicines (1). Garlic has been proposed to have a healing power in the treatment of hyper tension, heavy metal poisoning, athletes foot, in addition to its uses in treatment of various diseases due to its antimicrobial, antitumor, hypolipidemic, hypocholesterolemic, cholagogue, antiatherosclerotic, antispasmodic, antioxidant and immunomodulator effects (2). Garlic is among the few herbs that have a universal usage and recognition. Its daily usage aids and supports the body in ways that no other herb does, the volatile oil is an effective agent of garlic consisting of sulphur-containing compounds, including allicin (=S-allyl-2-propenthiosulphinate), allyl-methyltrisulphide, diallyldisulphide, diallyltrisulphide, diallyltetrasulphide, allylpropyldisulphide, ajoene, 2-vinyl-4H-l, 3 dithiin, and alliin, which breaks down enzymatically to allicin; with citral, geraniol linalool and a- and b-phellandrene (3). Garlic oil is reported to increase the secretions of digestive juices in the stomach and aid digestion; it normalizes the peristaltic movement, help in relieving gas and distention, and as it is largely excreted via the lungs, it is used in infections of respiratory system such as chronic bronchitis, respiratory catarrh, recurrent colds and influenza. It may be helpful in the treatment of whooping cough and as part of a broader approach to bronchitic asthma. In general it may be used as a preventative for most infectious conditions, digestive as well as respiratory, for the digestive tract it has been found that garlic will support the development of the natural bacterial flora whilst killing pathogenic organisms (3, 4).

Material and Methods

Thirty healthy, local domestic rabbits weighing (1250-1500) g of both sexes (15 males & 15 females) were used in this study. They were put in the animal house of Veterinary Medicine College in Al-Qadisiya University; they were housed one per cage, which was provided with a wire mesh floor. All rabbits were fed standard diet and given water *ad libitum*. They were divided into 3 groups as follow:

1st group: received no treatment.

2nd group: received CCl_4 at a dose of 1.5 ml/kg/B.W orally at 10 a.m. by using of gastric tube. This single oral dose was found to be the most effect dose in producing severe liver damage (5).

3rd group: received 1ml/kg of garlic oil (Ranbaxy Laboratories Limited, India) at 9 a.m., then CCl_4 was given after one hour i.e. at 10 a.m. in the same dose and manner of the 2nd group. The treatment with garlic oil continues for three days, we double the dose used by (6) in rats.

The effects of CCl_4 and garlic oil were tested after 72 hours from induction of acute liver failure by CCl_4, on the basis of biochemical analysis of liver functions and histopathological studies of the liver. Blood samples were obtained from the heart of 5 animals from each group, left stand in serum tubes for 30 minutes to be coagulated, then centrifuged at 2500 rpm for 15 minutes, & then the separated serum transferred to another tubes for determination the following parameters:

1. AST estimation: a common colorimetric procedure is the method of Reitman and Frankle(7).

2. ALT estimation: the colorimetric ALT procedure is also the method of Reitman and Frankle & is similar to the AST procedure (7).
3. ALP estimation: the method devised by Bowers and McComb, which allows calculation of ALP activity basing on the molar absorptivity of P-nitrophenol (8).
4. Total serum bilirubin: Jendrassik-Grof method was used to determine total serum bilirubin (8).
5. Total serum protein: Biuret method was used to determine total serum protein (8).

Preparation method of liver sections.

At the end of experiment, the rabbits were subjected to liver resection under chloroform anesthesia. The abdomen was opened via a midline incision & the liver isolated & then put in formalin 10% for sectioning, stained with haematoxylin and eosin & subjected to histopathological examination.

Statistical analysis.

Data were expressed as mean ± standard error, significance between groups was determined by Complete Random Design, $p<0.05$ was used as a criterion for significance (9).

Results

The results revealed that 2nd group had a significant elevation ($p<0.05$) in the levels of serum AST, ALT, ALP, & Total serum bilirubin & significant reduction ($p<0.05$) in the level of total serum protein compared to the 1st & 3rd groups, In another side the results of the 3rd group revealed that the values of serum ALT, AST, ALP, & Total serum bilirubin decreased significantly, while the value of total serum protein increase significantly in comparison with the 2nd group, (Table 1).

Table (1) The serum ALT, AST, ALP, Total serum bilirubin (T.S.B.), Total serum protein (T.S.P.) levels (mean ± standard error) of the studied groups

Groups / Parameters	1st	2nd	3rd
ALT (U/ L)	27.76±5.01 a	113.42±4.67 b	45.97±6.79 c
AST (U/ L)	24.84±4.91 a	120.11±2.22 b	65.43±5.33 c
ALP (U/L)	41.27±8.57 a	196.72±7.89 b	83.77±5.91 c
T.S.B (µmol/L)	10.98±6.53 a	21.11±4.31 b	11.44±2.72 c
T.S.P (g / L)	53.72±3.36 a	43.51±1.89 b	49.11±2.22 c

The different letters refer to significant differences between the groups (P< 0.05)
1st = control
2nd = CCl_4
3rd = Garlic oil + CCl_4

In addition**,** Histopathological examination of the liver sections of the 2nd group showed severe congestion, fatty change, severe ballooning degeneration and necrosis of the portal area (Figure 2) in comparison with normal liver of the 1st group (Figure 1). In other hand treatment with garlic oil in the 3rd group reduced morphological changes produced by CCl_4 and greatly reverted the microanatomy of the liver to normal (Figure: 3, 4).

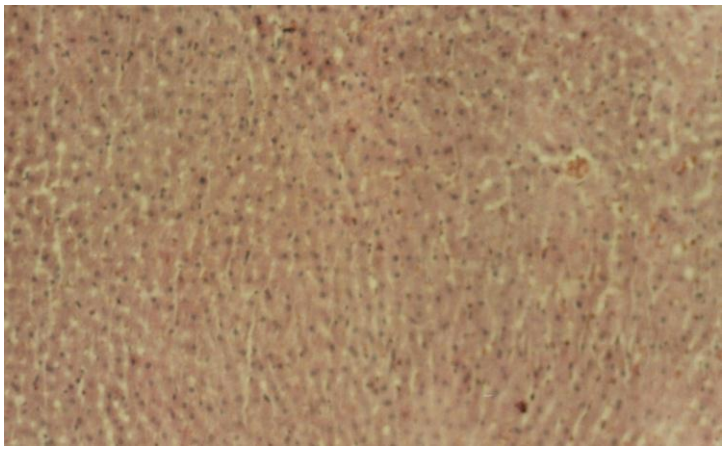

Figure (1) the normal liver tissue of the control group showing normal hepatocytes, H&E staining, (10 X)

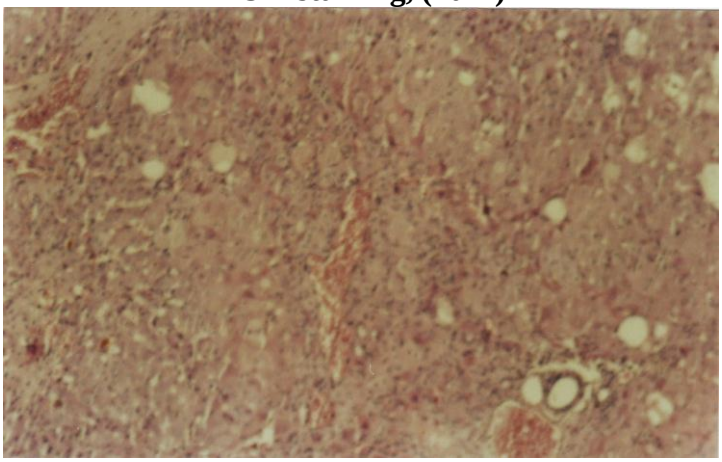

Figure (2) CCl4 treated liver revealed severe congestion, fatty change, severe ballooning degeneration and necrosis of the portal area, H&E staining, (10X)

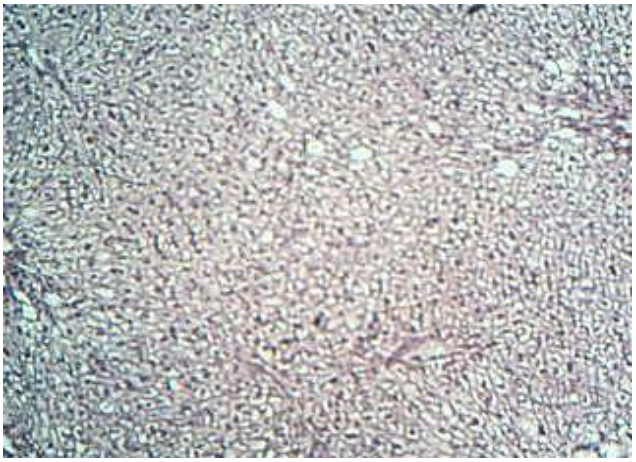

Figure (3) garlic oil treated liver nearly returns to normal state, H&E staining, (10X)

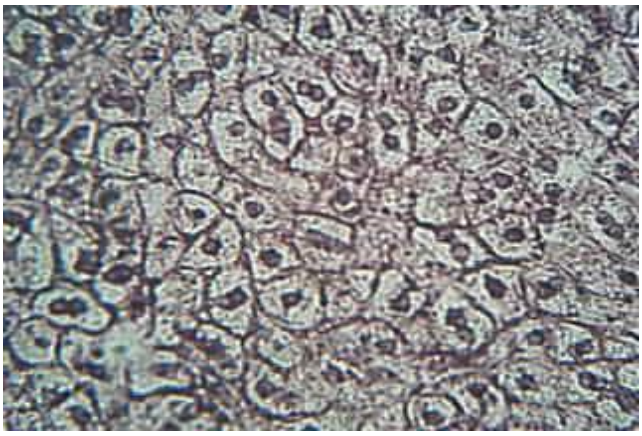

Figure (4) garlic oil treated liver nearly returns to normal state, mitotic divisions appear, H&E staining, (40X)

Discussion

The results of the 2nd group had a significant elevation ($p<0.05$) in the levels of the ALT, AST, ALP and TSB and & significant reduction ($p<0.05$) in the level of T.S.P compared to the 1st & 3rd groups, and this may return to the treatment with CCl_4, which is considered as a potent hepatotoxic agent, and these enzymes are highly sensitive to liver injury & they are released in significant quantities in the serum after any oxidative stress performed on the liver (10). In addition the significant increase ($p<0.05$) in total serum bilirubin also may be due to treatment with CCl_4 which can rapidly produce lipid peroxidation of hepatocytes membranes by the action of free radicals (11) and the action of CCl_4 in diminish of antioxidant enzymes activities which act as free radical scavengers (12). Serum ALP also increased significantly for the hepatocellular injury & cholestasis so large quantities of this enzyme were released (13). The reduction in the level of T.S.P in the 2nd group indicated the effect of CCl_4 on liver functions because protein fractions are synthesized in the liver (1), this result is in agreement with (14) who found significant decrease in serum protein & albumin in ethanol administered rats.

These results supported by (15) who indicated that chronic alcohol (used as toxic substance as CCl_4) intake lead to many cellular and tissue abnormalities such as alteration in liver enzymes (ALT, AST, & ALP) which indicated the increase permeability, damage, and / necrosis of hepatocytes.

In another side the results of the 3rd group revealed that the values of serum ALT, AST, ALP & Total serum bilirubin decreased significantly, while there was a significant elevation in the level of total serum protein in comparison with the 2nd group, this may be due to treatment with garlic oil which preserve the structural integrity of the liver from the toxic effects (1), so the significant decrease in these enzymes indicated antihepatotoxic effect of this oil because it contains sulfur compounds which are good antioxidants & hypolipidemic agents (16), in addition the garlic poly sulfides in the oil can react with thiol groups of enzymes and other macromolecules in the body and regulate their actions (2), also garlic oil can eliminate electrophilic intermediates and free radicals through conjugation and reduction reactions, therefore it protect the liver from toxic doses of different toxic substances (17).

In the current study, significant increase in total serum protein level in the 3rd group indicate the ability of garlic oil to stimulate the regeneration of hepatic tissue which increase protein synthesis in damaged liver and improve the functional status of the liver cells (1).

Histopathological examination of the liver sections of the 2nd group compared to the control may be return to the hepatotoxic effects of CCl_4 which are largely com from its

active metabolites, trichloromethyl radical. These activated radicals bind to micromolecules and reduce lipids peroxidative degradation of polyunsaturated fatty acids (18), this leads to the formation of lipid peroxides which in turn gives products like malonylaldehyde that cause damage to membranes (19). This lipid peroxidative degradation of biomembranes is one of the main causes of hepatotoxicity by CCl_4 & this is evidenced by a rise in the serum marker enzymes, this result is agree with the results found by (20)

Treatment with garlic oil in the 3rd group reduced morphological changes produced by CCl_4 and greatly reverted the microanatomy of the liver to normal which can be explained by containing of garlic oil certain compounds such as germanium and selenium that play an important role in normalizing the oxygen utilization in the cells(1).

For this garlic oil lowered the high levels of liver enzymes especially ALP, also garlic oil may have protective effects against liver injury through restoration of glutathione level & enhancement the activities of antioxidant enzymes (21).

In conclusion, CCl_4 significantly impairs the antioxidant defense system and in consequence may lead to significant increase in oxidative stress mainly in the liver, garlic oil can influence on normalization of cellular metabolism and functions by its powerful antioxidant effect against oxidative damage caused by CCl_4.

References

1. Hussein, J. S.; Oraby, F. S. & El-Shafey, N. (2007). Antihepatotoxic effect of garlic and onion oils on ethanol-induced liver injury in rats. J. Applied Sciences Research, 3 (11): 1527-1533.
2. Augusti, K. T.; Ashankumari, C.; Daniel, G.; Jaya, S.; Mathew, S. K.; Sreerekha & Joseph, P. K. (2008). The beneficial effect garlic cake on coconut oil fed rats. J. Medical Plants Res.,2 (2): 034-038.
3. Hoffmann, D. L. (2001).Garlic. Herbal Medicine Materia Medica. Healthy.net.
4. He, N.; Li, Q.; Sun, D. & Ling, X. (2007). Isolation, purification and characterization of superoxide dismutase from garlic. Bio. Eng. J., 38 (1): 33-38.
5. Ademuyiwa, O.; Onitilo, O.; Dosumu, O.; Ayannuga, O.; Baker, A.; Akinlatun, W. & Ogunyemi, E. O. (2002). Zinc in CCl_4 toxicity. Biomed. Environ. Sci., 15 (3): 187-195.
6. Fukao, T.; Hosono,T.; Misawa, S.; Seki, T. & Ariga, T. (2009). Chemoprotective effect of diallyl trisulfide from garlic against carbon tetrachloride-induced acute liver injury of rats, Bio. Factors, (21) 4: 171-174.
7. Anderson, S. C. & Cockayne, S. (1993). Clinical chemistry: Concepts and Applications. Philadelphia; W. B. Saunders Company, PP. 248-264.
8. Bishop, M. L.; Fody, E. P. & Schoeff, L. (2005). Clinical chemistry: Principles, Procedures, Correlations. 5th ed., Baltimore: Lippincott Williams: 250-253, 476-486, 172-174.
9. Niazi, A. D. (2004). Statistical analysis in medical research. 2nd ed. Dep. of Community Medicine, Iraq.
10. Uskokovic- Marcovic, S.; Milencovic, M.; Topic, A.; Kotur-Stivuljevic, J.; Stefanovic, A. & Antic- Stancovic, J. (2007). Protective effect of tungstophosphoric acid and sodium tungstate on chemically induced liver necrosis in wistar rats. J. Pharm. Phamaceut. Sci.,10(3): 340-349.
11. Lee, K. J.; Terada, K.; Oyadomari, S.; Inomate, Y.; Mori, M. & Gotoh, (2004). Induction of molecular chaperones in carbon tetrachloride-treated

rat liver: implications in protection against liver damage. Cell –stress chaperon., 9 (1): 58-68.

12. Jung, S. H.; Lee, Y. S.; Lim, S. S.; Lee, S.; Shin, K. H. & Kim, Y. S. (2004). Antioxidant activities of isoflavones from the rhizomes Blamcanada chinensis on carbon tetrachloride-induced hepatic injury in rats. Pharm. Res., 27 (2): 184-188.
13. Hewawasam, R. P.; Jayatilaka, K.; Pathirana, C. & Medduwa, A. (2004). Hepatoprotective effect of Epaltes divericata extract on carbon tetrachloride induced hepatotoxicity in mice. Indian J. Med. Res., 120 (1): 30-34.
14. Ahmed, B., T. Alam, M. Varshey, & Khan, A. S. (2002). Hepatoprotective activity of two plants belonging to the Apiaceae and the Euphorbiaceae family. J. of Ethnopharmacology, 79 (3): 313-316.
15. Pari, L. & Karthikesan, K. (2007). Protective role of caffeic acid against alcohol-induced biochemical changes in rats. Fundamental & Cli. Pharmacol., 21 (4): 355-361.
16. Stajner, D. & Varga, I. S. (2003). An evaluation of the antioxidant abilities of Allium species. Acta Biologica Szegediensis, 47: 103-106.
17. Heybatuallah, K. & Majeed, S. (2001). The protective effect of garlic oil on hepatotoxicity induced by acetaminophen in mice and comparison with N-acetylcysteine. Saudi Med. J., (22) 12: 1080-1084.
18. Adefolaju, G. A.; Ajao, M. S.; Olatunji, L. A.; Enaibe, B. U. & Musa, M. G. (2009). Hepatoprotective Effect of Aqueous Extract of Water Leaf (*Talinum Triangulare*) on Carbon tetrachloride (CCl_4) Induced Liver Damage in Wistar Rats. The Int. J. of Pathol., 8 (1): 205- 215.
19. Ulicna, O.; Greskshek, M.; Vancovao, O.; Zlator, I. & Bocek, P. (2003). Hepatoprotective effect of Rooibos tea (Aspalathus linearis) on CCl4 induced liver damage Physiol. Res., 52: 461-466.
20. Das, S.; Sarma, G. & Barman, S. (2008). Hepatoprotective Activity Of Aqueous Extract of Fruit Pulp Of Cassia Fistula (AFCF) Against Carbon Tetrachloride (CCL_4) Induced Liver Damage In Albino Rats. J. of Cli. & Diag. Res., (12) 5: 1133-1138.
21. Tao, Z.; Cui-Li, Z.; Guang-Bing, P.; Sheng, Z.; Dan-Dan, D.; Xing, X. & Ke-Qin, X. (2008). The protective effects of garlic oil on acute ethanol-induced oxidative stress in the liver of mice. J. Sci. Food & Agric., 88 (13): 2238-2243.