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Effect of Vitamin C and Aqueous Crude Extract of Aloe Vacillans (Audhalica) Leaves on the Hepatotoxicity

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Abstract

Introduction: This study was designed to evaluate the effects of vitamin C and aqueous extract of Aloe vacillans leaves on carbon tetrachloride (CCl4) - induced hepatotxicity in rabbits.

Methods: Hepatotoxicity was induced in rabbits by intraperitoneal injection of CCl4 in the olive oil 1:1(v/v) at dose l ml/kg on day 13 and 14. Vitamin C was administrated at dose 0.02 gm/kg of body weight and the aqueous crude extract of Aloe vacillans leaves were administrated at dose 500 mg/kg of body weight pass orally (p.o) daily for 15 days. The hepatotoxicity and its prevention were assessed by serum parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (T.B) and total protein (T.P).

Results: In CCl4 treated rabbits, significant increase in ALT, AST, bilirubin and decrease in the total T.P levels were shown (p<0.05), due to liver damage, when compared with normal group. Treatment with the aqueous extract of Aloe vacillans could significantly decrease the ALT, AST and T.B, increased T.P in serum at (p<0.05) when compared with CCl4 –treated group. Treatment with vitamin C could significantly decrease the AST), ALT and T.B, increased T.P in serum at p<0.05 when compared with CCl4 –treated group.

Conclusion: The data concluded that oral administration of aqueous extract of the leaves of Aloe vacillans and vitamin C significantly decreases the intensity of hepatic damage induced by CCl4 in rabbits when compared with all CCl4 –treated groups.

Keywords: Carbon tetrachloride, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Total Protein, Total Bilirubin, Hepatic Damage, Rabbits.

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تأثير المستخلص المائي لخام أوراق نبات الصبر من نوع Aloe vacillans (audhalica) وفيتامين ج على السمية الكبدية

محمد علي قاسم

ملخص الدراسة

المقدمة: تهدف هذه الدراسة إلى تقدير مدى تأثير المستخلص المائي لخام أوراق نبات الصبر من نوع Aloe vacillans وفيتامين (ج) على التهاب الكبد المحدث برابع كلوريد الكربون في الأرانب.

المنهجية: أحدثت الأذية الكبدية في الأرانب عن طريق الحقن في البريتوان برابع كلوريد الكربون (1 مل/كغم) من وزن الجسم على اليوم الثالث عشر واليوم الرابع عشر في أثناء حقن المستخلص المائي للنبات وفيتامين ج في الأرانب عن طريق الفم بجرعة (500 ملغ/كغم) و (0,02غم/كغم) من وزن الجسم على التوالي مرة في اليوم لمدة خمسة عشر يوم. بعد 48 ساعة من آخر جرعة لرابع كلوريد الكربون ضحي بالحيوانات وجمع الدم لمعرفة اثر الأذية الكبدية والحماية منها بقياس مستويات أنزيمات الكبد في المصل مثل الانين امين ترانسفيراز(ALT) ، اسبارتات امين ترانسفيراز (AST)، البيليروبين والبروتينات الكلية.

النتائج: أظهرت النتائج في المجموعة المعالجة برابع كلوريد الكربون فقط ارتفاعا في مستويات أنزيمات الكبد (ALT,AST) والبيليروبين ونقصا في البروتينات الكلية عند$p<0.05$ مقارنة بالمجموعة الشاهدة. اما في المجموعة المعالجة بالمستخلص المائي فكان هناك نقصا في مستويات أنزيمات الكبد (ALT,AST) والبيليروبين الكلي وزيادة في البروتينات الكلية ذات دلالة معنوية عند $p<0.05$ مقارنة بالمجموعة المعالجة برابع كلوريد الكربون فقط. وفي المجموعة المعالجة بفيتامين (ج) ، كان هناك نقصا ذو دلالة معنوية عند $p<0.05$ في مستويات أنزيمات الكبد والبيليروبين وزيادة في البروتينات الكلية مقارنة بالمجموعة المعالجة برابع كلوريد الكربون فقط. كما أظهرت النتائج في المجموعة المعالجة بالمستخلص المائي وفيتامين ج نقصا ذو دلالة معنوية عند $p<0.05$ في مستويات أنزيمات الكبد والبيليروبين الكلي وزيادة في البروتينات الكلية مقارنة بالمجموعات الأخرى المعالجة برابع كلوريد الكربون.

الاستنتاج: تشير نتائج المشاركة بين المستخلص المائي لخام أوراق نبات الصبر(Vacillans) وفيتامين ج إلى القدرة على تقليل شدة السمية الكبدية.

الكلمات المفتاحية: اسبارتات امينو ترنسفيراز (AST)، الانين امينو ترنسفيراز (ALT)، البروتين الكلي ، البيليروبين الكلي ، أذية كبدية، الأرانب.

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Introduction

Liver is the organ for metabolism and detoxification of various components enter into the body. It is involved in wide range of functions and hence it is exposed to toxic substances and drugs absorbed from the intestine [1]. Human and animal studies have shown that some drugs and chemical agents have potential hepatotoxic effects. In humans, hepatitis or liver injury is also caused by viruses, alcohol and autoimmune diseases [2-4].

The hepatotoxic effect of drugs and some chemical agents such as carbon tetrachloride (CCl_4) which often used to induce oxidation stress-related. The reactive metabolites such as trichloromethyl (CCl_3) and trichloromethyl peroxy (CCl_3OO) radical emanated from CCl_4 initiate per oxidation of membrane unsaturated fatty acid is reported to be associated with the generation of reactive oxygen species (ROS). These ROS are reported to be associated with lipid per oxidation of membrane seriously impairs its function and produces liver injury in the liver [5].

The most common reactive oxygen species are superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), peroxyl radical (ROO^-), and highly reactive hydroxyl radical(OH^-) Oxidative processes are the most important routes for producing free radicals in living systems. The liver, because of its strategic anatomical action and its large capacity for metabolic conversions, is expose to many kinds of xenobiotics and therapeutic agents. Due to these facts, efforts to find suitable curative agents for treatment of liver diseases in natural products of plant and mineral origin are being made [6].

Many different plant materials have recently become a major interest of scientific research as a result of naturally occurring antioxidants, which may protect cell constituents against oxidative damage and therefore, limit the risk of various degenerative diseases associated to oxidative stress [7]. Aloe species have been used for centuries for their various healing properties. Different plant parts including the leaves, roots, and gels from various Aloe species have been thoroughly investigated, affording several classes of secondary metabolites including alkaloids, pre-anthraquinones, anthraquinones, anthrones, chromones, flavones, coumarin derivatives and pyrones [8].

A number of reports are available on the biological activities of different extracts from Aloe species and isolated secondary metabolites. For example, anti-inflammatory [9] antioxidant [10]. The various species of Aloe have the same effective phenolic compounds (anthraquinones) such as aloe-modin, aloesin, barbaloin, aloenin, and isobarbaloin [11]. Anthraquinones may act as antioxidants and radical scavenger, reactive oxygen species and free-radical mediated reactions are involved in inflammatory response and can contribute to liver necrosis [12].

Aloe vacillans, (Syn. A. dhalensis Lavrans, and A. audhalica Lavrans and hardy) grows on rocky mountain slopes in Yemen and Saudi Arabia at an altitude of approximately 8000 ft. [13], two new anthrone

C-glycosides microdantin derivatives; vacillantin A (10) and B (11), together with nine known compounds belonging to the anthraquinone, anthrone and isocoumarin groups, Aloe emoden, a loin was isolated from the leaves of *Aloe vacillans* [14].

Vitamin C was discovered by Szent-Gyorgyi (1928) [15]. Vitamin C is a six-carbon compound structurally related to glucose, consisting of two inter-convertible compounds: L-ascorbic acid, which is a strong reducing agent, and its oxidized derivative, L dehydroascorbic acid. Among the antioxidants been evaluated is vitamin C which is a water soluble antioxidant. Reports have linked vitamin C with hepatoprotective property in animals [16], it is hydrophilic and is an important free radical scavenger in extracellular fluids, trapping radicals and protecting bio membranes from peroxide damage, Vitamin C effectively scavenges singlet oxygen, super oxide, hydroxyl, water soluble peroxyl radical and hypo chlorous acid [17]. Vitamin C has shown tremendous protective effect against drugs and chemical agents induced hepatotoxicity [18-19]. Vitamin C can be administered orally or intravenously [20].

This study was designed to evaluate the effects of vitamin C and aqueous extract of *Aloe vacillans* leaves on CCl4 - induced hepatotxicity in rabbits.

Methods

Plant Material and Extraction:
Aloe vacillans leaves were collected from Lauder region, Abyen Governorate, Yemen in December – Feb 2018. The leaves of the plant were washed with water, dried under shade and powder to fine grade by using laboratory scale mill, the powdered extracted with distilled water (250 g/4 liter) for 18 h with concomitant shaking. Filtrate was evaporated rotary to yield a brown powder, which was administer orally according to body weight (b.w) of animals [21].

Chemicals:
CCl4 where purchased from sigma chemical Co. (India). All other chemicals and reagent were purchased from Spinreact, S.A.U (Spain).

Animals:
Male rabbits weighing 1000-1200 gm were purchased from locally market (Abyen) and used in these experiments. The animals were housed at room temperature (28±2C) in standard cages with standard pellet food and kept under controlled environment following relative humidity (60±5%) with a 12h light/dark cycle.

CCl4- Induced Hepatic Damage in Rabbits:
About 30 male rabbits were divided into five groups of six animals each
Group I: received olive oil vehicle only at 5mL/kg day orally for fifteen days.
Group II: received CCl4 in olive oil only.
Group III: were administered with vitamin C (0.02mg/kg-day) orally for fifteen days.
Group IV: received 500 mg/kg-day Aloe extract orally for fifteen days.
Group V: received 500 mg/kg-day Aloe extract and vitamin C (0.02mg/kg) orally for fifteen days.

On the thirteenth and fourteenth day, animals from groups II–V were injected intra peritoneal with CCl4 in olive oil 1:1(v/v) at a dosage of 1ml/kg b.w.

Preparation of Serum from Blood:

After 48 hours of the second dose of CCl4, the rabbits were sacrificed, and the blood samples were collected by tubes from each animal. The blood allowed clotting for 30 min at room temperature. Serum was separated by centrifugation at 3000 r pm for 10 minutes [22], and analyzed for various biochemical parameters including serum aspartate aminotransferase (AST) serum alanine aminotransferase (ALT), total protein (T.P) and Total Bilirubin (T.B) by using (UV) spectrophotometer (model screen master plus RM 4040.

Statistics:

All the values are expressed as a mean± SEM. The data are evaluated using one way (ANOVA) test to determine the significance of difference between the normal group and the CCl4 treated group only. Differences between the CCl4-treated group alone and the CCl 4 groups treated with extract at 500mg/kg, and the CCl 4 groups treated with vitamin C (0.02 mg/kg) and the CCl 4 groups treated with extract and vitamin C at 500 mg/kg, 0.02 mg/kg respectively were compared for significance differences below (p<0.05) are considered as significant.

Results

The results of CCl4- induced hepatotoxicity are show in Figures 1 to 3.

The hepatic injury induced by CCl4 in rabbits caused significantly elevated the serum levels of ALT, AST and total bilirubin, total protein that whereas a significant decrease in level of the total protein in CCl4alone group compared with normal group.

The rabbits were treated with aqueous extract of *Aloe vacillans* at dose (500 mg/kg b.w, p.o) the rabbits treated with Vitamin C (0.02 g/kg b.w,p.o), showed a significant reduction in marker enzymes ALT, AST and serum bilirubin, and increase in the level of T.P as comparison to that of the CCl4.

The rabbits were treated with extract of *Aloe vacillans* at dose (500 mg/kg b.w, p.o) and Vitamin C (0.02 g/kg b.w, p.o) showed a significant reduction in marker enzymes ALT, AST and serum bilirubin, whereas increase in the level of total protein was shown in these groups in comparison to all treatment groups. Figures1 to 3

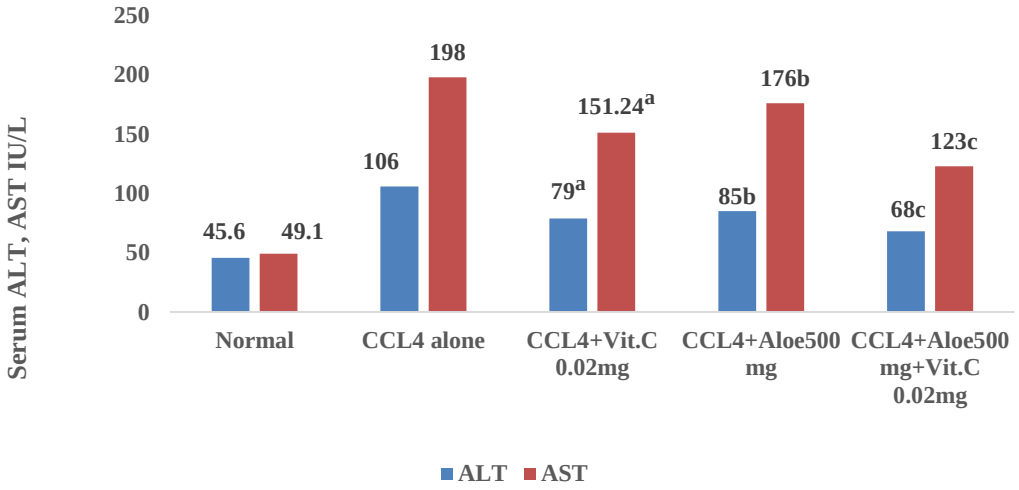

Figure 1: Values are Represented as Mean ±SEM (n=6)

[a,b,]significantly different from CCl_4 treatment only.
[c,]significantly different from all treated group.

ANOVA test (p<0.05) is used CCl_4 alone significantly different from normal control.

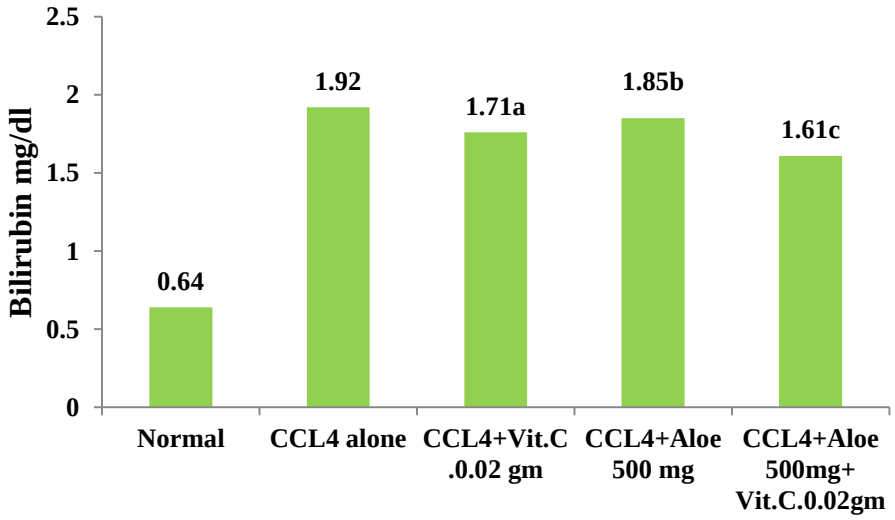

Figure 2: Values are Represented as Mean ±SEM (n=6)

ANOVA test (p<0.05) is used CCl_4 alone significantly different from normal control
[a,b,]significantly different from CCl_4 treatment only.
[c,]significantly different from all treated group.

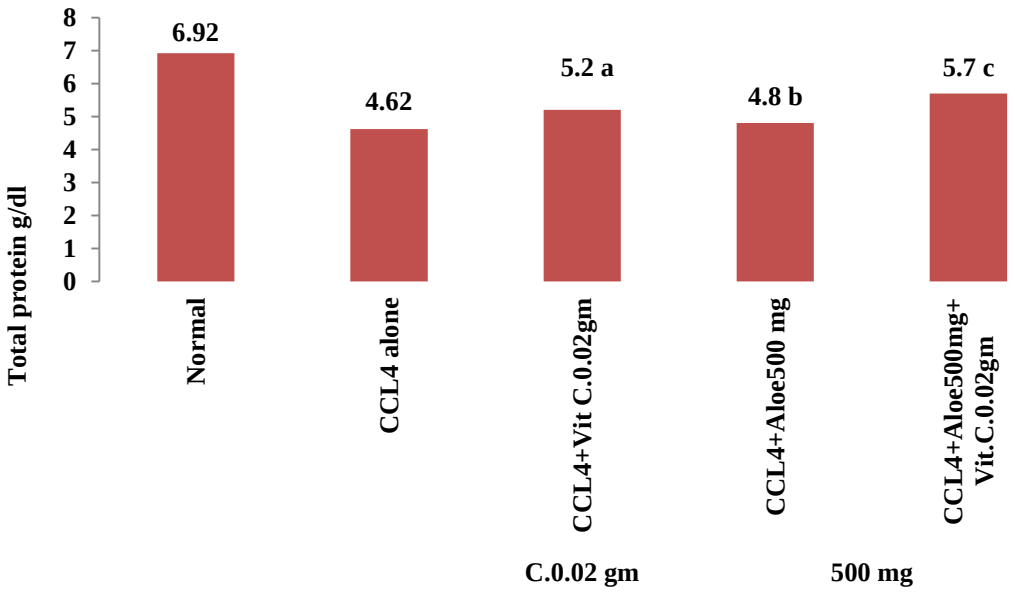

Figure 3: Values are Represented as mean ±SEM (n=6)

ANOVA test (p<0.05) is used CCl_4 alone significantly different from normal control
[a,b,]significantly different from CCl_4 treatment only.
[c,]significantly different from all treated group.

Discussion

In this study, rabbit's treatment with dose of CCl_4 developed significant hepatic damage, which was observed from a substantial increase in activities of serum ALT, AST and bilirubin, decreasetotal protein significantly p<0.05 compared with normal group.

CCl_4 is bio transformed by the cytochrome p450 system in endoplasmic reticulum to produced trichloromethyl free radical. Trichloromethyl free radical then combined with cellular lipids and proteins in the presence of oxygen to form trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl radical. Thus, trichloromethyl peroxyl radical lead to elicit lipids peroxidation, and finally results in cells death [23]. The hepatic cells consist of higher concentrations of AST and ALT in cytoplasm and AST in particular exists in mitochondria, due to the damage caused to hepatic cells, the leakage of plasma causing an increased level of hepatospecific enzymes in serum [1].

Increase of levels enzymes ALT, AST metabolic activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation indicative of cellular leakage and loss of functional integrity of the cell membranes in liver [24,25].

Reduction in the levels of the ALT, AST, and bilirubin, and increase in the level of total proteins by Vitamin C at dose (0.02g/kg b.w,p.o) was significant $p<0.05$ compared to alone CCl_4 group. These findings are in agreement with results of previous studies [26-29].

Reduction in the levels of the ALT, AST, and bilirubin, increase in the level of total proteins by plant aqueous extract at dose (500 mg/kg b.w, p.o) significantly $p<0.05$ compared to alone CCl_4 group, according to previous reports [30-32]

Studies indicated stabilization of plasma membranes as well as repair of hepatic tissue damage caused by CCl_4 [33-35]. This effect shows that serum levels transaminase return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes [36,37].

Aloe is the only supplement to increase the absorption of vitamin in the intestine and should be considered as a complement it, the *Aloe vacillans* aqueous extract at dose (500 mg/kg) and vitamin C at dose 0.02 gm/kg, significantly lowered the levels of ALT, AST, and bilirubin and increased the T.P when compared with all treated groups at $p<0.05$ according to previous evidence [38,39].

Conclusion

Treatment with aqueous extract of *Aloe vacillans* and vitamin C reduced the degree of hepatic-cellular injury as evidenced with improved biochemical parameters. The improvement can be explained by the possibility that *Aloe vacillans* is a supplement to increase the absorption of vitamin C in the intestine, and the extract that contains phenolic compounds, might had scavenged the free radicals offering hepatoprotection.

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