Protective effect of aqueous crude extract of leaves *Aloe vacillans* and Yemeni honey (*soumr*) against CCl_4 – induced hepatic damage in male Rabbits

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Abstract

The aim of this study is to investigate the effect of honey and Aqueous crude extract of leaves *Aloe vacillans* on carbon tetrachloride (CCl_4)- induced hepatotxicity in rabbits.
Hepatotoxicity was induced in rabbits by intraperitoneal injection of (CCl_4) 0.2 ml/kg of body weight on day 19, 20 and 21. The aqueous crude extract of *Aloe vacillans* leaves were administrated at dose 100 mg, 300 mg and 500 mg/kg of body weight pass orally (p.o) daily for 28 days. The hepatotoxicity was induced in rabbits by intraperitoneal injection of (CCl_4) 0.2 ml/kg of body weight on day 19, 20 and 21.
The honey were administrated at dose 500 mg/kg of body weight pass orally (p.o) daily for 28 days. The hepatotoxicity was induced in rabbits by intraperitoneal injection of (CCl_4) 0.2 ml/kg of body weight on day 19, 20 and 21.

The hepatotoxicity and its prevention was assessed by serum parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin (Bil) and total protein (T.P). In CCl_4 treated rabbits, a significant, increasing the in (ALT) ,(AST) , bilirubin and a decreasg the total protein levels were shown at ($p<0.05$), due to liver damage, when compared with normal group.

Treatment with the aqueous extract of *Aloe vacillans* could significantly decrease the (ALT),(AST) and bilirubin, increased T.P in serum at $p< 0.05$ when compared with CCl_4 –treated group.Treatment honey at dose (500 mg/kg) could significantly decrease the (ALT),(AST) and bilirubin, increased T.P in serum at $p< 0.05$ when compared with CCl_4 –treated group and the aqueous extract of *aloe* treated groups.

The data suggested that oral administration of Honey and aqueous extract of the leaves of *Aloe vacillans* at dose (500 mg/kg) significantly decreases the intensity of hepatic damage induced by CCl_4 in rabbits.

Keywords: Alanine aminatransferase (ALT), Aspartate aminotransferase (AST), Total Protein (T.P), Bilirubin (Bil), Hepatic damage, Rabbits.

Introduction

The liver being the largest internal glandular organ in the body is always in close contact with many harmful substances, the exposure to chemical substance in the occupational environment or through synthetic drugs consumed for pathological condition. thus generating interest in natural remedies that are particularly effective in either protecting the liver or curing it from many aliments caused by toxins. These compounds have many toxic manifestations or liver injury on the human liver. (15)

The liver diseases remain one of the serious health problems.

CCl_4 is a chemical agent widely used for experimental induction of fatty liver and liver fibrosis. It is considered that CCl_4 is metabolized by cytochrome P-450 (CYP) to unstable trichloromethyl free radicals (CCl_3, CCl_3O_2),which then bind covalently to membrane proteins, finally causing lipid peroxidation.The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in rat plasma rise remarkably with hepatocyte necrosis and lipid accumulation when CCl4 is

administered to rats and the rise in serum level of aspartate transminase (AST), alanine transminase (ALT) has been attributed to damage structural integrity of the liver because they are cytoplasmic in location and released into circulation after cellular damage.(17,19).

The plant is native to south eastern africa and subsequently introduced into northern Africa the Arabian Peninsula , China , the Mediterranean countries and West India. It is commercially cultivated in Aruba , Bonaire , Haiti, South Africa ,the United States of America and Venezuela.(2).The various species of *aloe* have the same effective phenolic compounds (anthraquinones) such as aloe-modin, aloesin, barbaloin, aloenin, and isobarbaloin (20,1).

A number of investigators have previously demonstrated that antioxidants prevent CCl_4 toxicity, particularly hepatotoxicity, by inhibiting lipid peroxidation (23)

The leaves juice of *Aloe* plant is used in eyes diseases and enlargement of spleen and liver (24). 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. (6).

Honey is a supersaturated sugar solution produced by honey bees from nectar of different plants. Fructose and glucose are the main components, with other chemical compounds in small quantities, including sucrose, glucose oxidase,hydrogen peroxide, phenolics, flavonoids, and terpenes. It has a long tradition of use for wound healing since ancient times. It was used alone or in combination with other substances externally or orally. Honey has bactericidal,bacteriostatic, antifungal, antiviral, scolicidal,antioxidant,antitumoral, hepatoprotective, and anti-inflammatory effects.(8)

Several types of honey from different countries have an antioxidant capacity that was dependent on the concentration of phenolic groups.(1)

A study done by (19) performed to assay the antioxidant capacity of honey.

Thus, the current study has been aimed to evaluate the effects of aqueous extract of *Aloe vacillans* leaves juice and honey on CCl_4-induced hepatotoxicity in rabbits.

Material and methods

1- Plant Extraction:

Aloe vacillans leaves were collected from lauder region, Abyen Governorate, Yemen. 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 of animals. (21)

2-Chemicals:

CCl_4 where purchased from Sigma Chemical Co. India

All other chemicals and reagent were purchased from Spinreact, S.A.U (Aspin)

3- Animals:

Male rabbits weighing 800-1000 gm were purchased from local markets 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.

4-CCl_4- induced hepatic damage in rabbits:

About 36 male rabbits were divided into six groups of six animals each Group I. Rabbits receiving olive oil 0.2 ml/kg b.wt.p.o serve as a normal control.

Group II. Rabbits receiving equal mixture of carbon tetrachloride and olive oil (1:1) 0.2 ml/kg b.wt,i.p on days 19,20 and 21 serve as CCl_4 control.

Group III,IV and V were administered with aloe extract at 100,300, and 500 mg/kg b.wt.p.o respectively for 28 days on the day 19,20 and 21 receiving mixture carbon tetrachloride and olive oil 0.2 ml/kg b.wt, i.p.

Group VI received honey 500 mg /kg b.wt,p.o respectively for 28 days on the day 19, 20 and 21 received mixture carbon tetrachloride and olive oil 0.2 ml/kg b.wt, i.p.

5-Preparation of serum from blood:

After 24 hour of the second dose of CCl_4, 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 (27), and were 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 RM4040

6-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 CCl_4 treated group only. Differences between the CCl_4-treated group alone and the CCl_4 groups treated with extract at three different dose.(100 mg/kg ,300 mg/kg and 500mg/kg) and honey 500 mg/kg, were compared for significance, using student's t-test. Differences below ($p<0.05$) are considered as significant.

3- Results

The results of CCl_4- induced hepatotoxicity are shown in Fig. 1,2,3.

The hepatic injury induced by CCl_4 in rabbits caused significantly elevated the serum levels of ALT, AST and bilirubin, whereas there was a significant decrease in the level of the total protein that indicated acute heapatocellular damage.

The rabbits treated with aqueous extract of *aloe* at three dose levels (100,300 and 500 mg/kg b.w, p.o) and Honey at dose (500 mg/kg b.wt, p.o) showed a significant reduction in marker enzymes ALT,AST and serum bilirubin, whereas an increase in the level of total protein was observed in compared with of the CCl_4 alone group. (Fig 1, 2, 3).

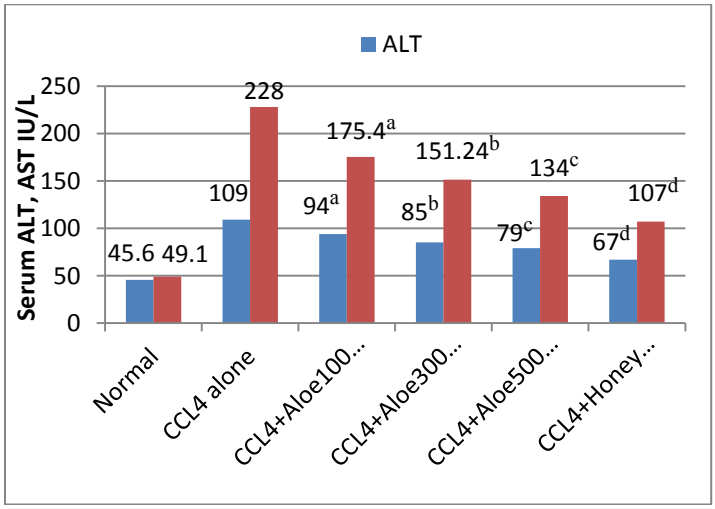

Fig.1 Values are represented as mean ±SEM (n=6)
Anova test used ($p<0.05$) is used, Student t-test ($p<0.05$) is used.
CCl_4 alone significantly different from normal control.
a, b, c significantly different from CCl_4 treatment only.
d significantly different from CCl_4 only and extract 100 ,300 and 500 mg/kg

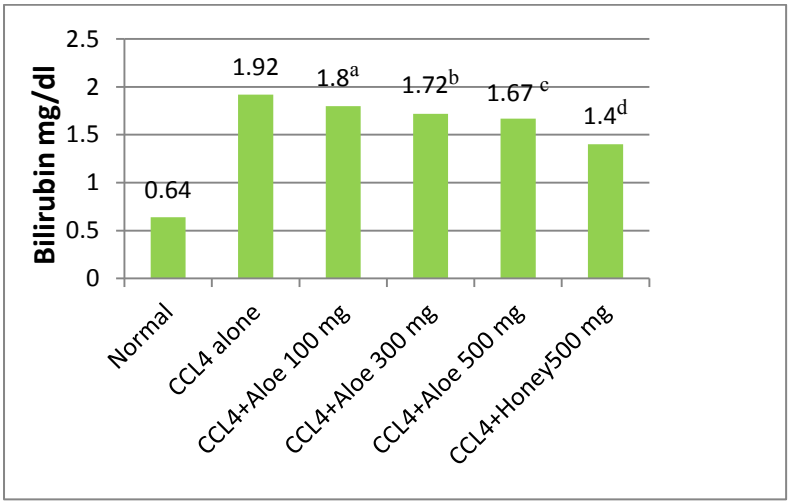

Fig. 2 Values are represented as mean ±SEM (n=6)
Anova test was ($p<0.05$) was used , Student t-test ($p<0.05$) was used.
CCl_4 alone were significantly different from normal control.
a, b, c significantly different from CCl_4 treatment only.
d was significantly different from CCl_4 Only and it was also significant with extracts 100 ,300 and 500 mg/kg

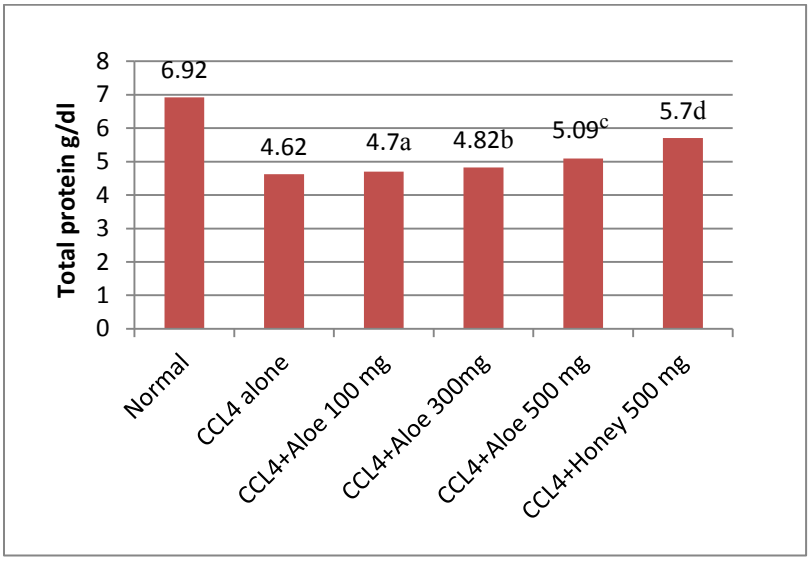

Fig. 3 Values are represented as mean ±SEM (n=6)
Anova test ($p<0.05$) was used , Student t-test ($p<0.05$) was used.
CCl_4 alone was significantly different from normal control.
a, b, c significantly were different from CCl_4 treatment only.
d significantly was different from CCl_4 only and extract 100 ,300 and 500 mg/kg

Discussion

In this study, rabbit's treatment with dose of CCl_4developed a significant hepatic damage, which was observed from a substantial increase in the activities of serum ALT,AST and bilirubin, as well as the decrease of the total protein which were significantly $p< 0.05$, compared with normal group ,these findings in current results were in agreement with the results of (4,28,7)

(Azri, etal) found that CCl_4 is bio transformed by the cytochrome p450 system in endoplasmic reticulum to produce trichloromethyl free radical. trichloromethyl free radical then combined with cellular lipids and proteins in the presence of oxygen to form trichloromethyle peroxyl radical ,which may attack lipids on the membrance of endoplasmic reticulum faster than trichloromethyl radical Thus, trichloromethyl peroxyl radical lead to elicit lipids peroxidation, and finally results in cells death, 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. (12,13) reduction in the levels of the ALT, AST, and biliurubin, and an increase in level of total proteins by plant aqueous extract groups at three different dose (100 ,300 , 500 mg/kg b.w,p.o) were significantly at $p<0.05$ compared to alone CCl_4 group, according to results (18)

The *Aloe vacillans* aqueous extract at highest dose (500 mg/kg) significantly lowered the levels of ALT,AST, and bilirubin ,increased the T.P when compared with lower dose (100,300 mg/kg) at $p<0.05$and This findings were in agreement the results of (2,5).

The ALT,AST and Bilirubin levels in honey treated rabbits (500 mg/kg) were significantly decreased, total protein level is significantly increased when compared with CCl_4 treated group, and with *aloe* treated groups.

The results (16.25.10) indication of that stabilization of plasma membranes as well as repair of hepatic tissue damage caused by CCl_4 .

This effect view that serum levels transminase return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (12). Honey contains many compounds that can act as antioxidants such as polyphenolics, The phenolic compounds which are widely distributed in leaves of *aloe* plants, such as aloin, aloe-emodin enthrone, emodin enthrone , the have been considered to play an important antioxidant role as indicated by protection against increased lipid peroxidation and scavenged the free radicals offering.

Conclusion

We have concluded that Simultaneous treatment with honey and aqueous extract of *Aloe vacillans* reduces the degree of hepato-cellular injury as evidenced by improved biochemical parameters.
Honey and extract at dose (500mg/kg) are more hepatoprotective.

Acknowledgment

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التأثير الواقي للمستخلص المائي الخام لأوراق نبات الصبر والعسل اليمني (السُمر) ضد التهاب الكبد المستحدث برابع كلوريد الكربون في الأرانب

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الملخص

تهدف هذه الدراسة إلى تقدير مدى تأثير المستخلص المائي الخام لأوراق نبات الصبر من نوع *Aloe vacillans* والعسل اليمني (السُمر) على التهاب الكبد المحدث برابع كلوريد الكربون في الأرانب.
أحدثت السمية الكبدية في الأرانب بواسطة حقن (CCl4) في البريتوان (0.2 مل/كغ) من وزن الجسم على اليوم التاسع عشر، واليوم العشرين واليوم الحادي والعشرين أثناء فترة التجربة.
أحدثت الأذية الكبدية في الأرانب عن طريق الحقن في البريتوان برابع كلوريد الكربون (0.2 مل/كغم) من وزن الجسم على اليوم التاسع عشر، واليوم العشرين واليوم الحادي والعشرين أثناء حقن المستخلص المائي للنبات في الأرانب عن طريق الفم بثلاث جرعات مختلفة (100 ملغ ، 300 ملغ و500 ملغ/كغم) من وزن الجسم مرة في اليوم لمدة 28 يوم .
أحدثت الأذية الكبدية في الأرانب عن طريق الحقن في البريتوان برابع كلوريد الكربون (0.2 مل/كغم) من وزن الجسم على اليوم التاسع عشر، واليوم العشرين واليوم الحادي والعشرين أثناء حقن العسل في الأرانب عن طريق الفم بجرعة (500 ملغ/كغم) من وزن الجسم مرة في اليوم لمدة 28 يوم. وفي اليوم التاسع والعشرين تم سحب الدم لمعرفة أثر الأذية الكبدية والحماية منها بقياس مستويات أنزيمات الكبد في المصل مثل الأنين امين ترانسفيراز(ALT),اسبارتات امين ترانسفيراز(AST), البيليروبين والبروتينات الكلية.
أظهرت النتائج في المجموعة المعالجة برابع كلوريد الكربون فقط ارتفاعًا في مستويات أنزيمات الكبد (ALT,AST) والبيليروبين ونقصًا في البروتينات الكلية عند $P<0.05$ مقارنة بالمجموعة الشاهدة.
أما في المجموعات المعالجة بالمستخلص المائي لنبات الصبر كان هناك نقصًا في مستويات أنزيمات الكبد (ALT,AST) والبيليروبين وزيادة في البروتينات الكلية ذات دلالة معنوية عند $P<0.05$ مقارنة بالمجموعة المعالجة برابع كلوريد الكربون فقط .
أما في المجموعة المعالجة بالعسل (500 ملغ/كغ) فكان هناك نقصًا في مستويات أنزيمات الكبد (ALT,AST) والبيليروبين وزيادة في البروتينات الكلية ذات دلالة معنوية عند $P<0.05$ مقارنة بالمجموعة المعالجة برابع كلوريد الكربون والمجموعات المعالجة بالمستخلص المائي للصبر.
توحي النتائج بأن العسل و المستخلص المائي لأوراق نبات الصبر(*Aloe Vacillans*) (500 ملغ/كغ) له القدرة على تقليل شدة الأذية الكبدية المحدثة في الأرانب بواسطة CCl_4.

الكلمات المفتاحيه: أسبارتات أمينو ترنسفيراز (AST)، الآنين أمينو ترنسفيراز (AST)، البروتين الكلي (T.P) ،البيليروبين (Bil) ، أذية كبدية، الأرانب.