Effect of *Aloe vacillans* Leaves Extract on CCl_4-induced Hepatotoxicity in Rats

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Received 01/02/2011
Accepted 02/04/2012

ABSTRACT

This Study was designed to evaluate the effects of *Aloe vacillans* leaves juice on carbon tetrachloride CCl_4- induced hepatotoxicity in rats.

Hepatotoxicity was induced in rats by intraperitoneal (i.p) injection of CCl_4 (1ml/kg) of body weight every 72h during ethanolic extract of *Aloe vacillans* leaves were administrated at dose 250 mg/kg and 500 mg/kg of body weight pass orally (p.o) daily for 14 days.

Twenty-four hours post-CCl4 treatment, blood samples were withdrawn through retro orbital sinus.The hepatotoxicity and its prevention was assessed by serum parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (T.P) and albumin (ALB).

In CCl4 treated rats, a significant decreasing in the relative body weight (212g) compared with normal group (231g), increasing in the liver enzymes levels ALT (254.6±6.6 IU/L), AST (322.9±4.42 IU/L) when compared with normal group (50.7±1.5 IU/L), (70.12±2.6 IU/L) respectively and decreasing the in T.P (4.5±0.17 g/dl) and albumin levels (2.65±0.12 g/dl) were shown ($p<0.05$) when compared with normal group (6.9±0.12 g/dl , 3.64±0.66g/dl) respectively.

Treatment with the ethanolic extract of *aloe vacillans* 250 mg/kg could significantly decrease in the liver enzymes levels ALT (185.9±6.2 IU/L), AST (242.5±4.2 IU/L) when compared with CCl_4- treated group (254.6±6.61,32 IU/L), (322..9±4.42 IU/L) respectively, and increased in the T.P (5.1±0.09 g/dl) and albumin level (2.28±0.01 g/dl) at $p<0.05$ when compared with CCl_4 –treated group (6.9±0.12 g/dl), (3.64±0.66 g/dl) respectively.

Treatment with the ethanolic extract of *aloe vacillans* 500 mg/kg could significantly decrease in the liver enzymes levels ALT (175.6±6.4 IU/L), AST(235±12.8 IU/L) when compared with CCl_4- treated group (254.6±6.61,32 IU/L), (322..9±4.42 IU/L respectively, and increased in the T.P (5.4±0.12 g/dl) and albumin level 2.86±0.10 g/dl at $p<0.05$ when compared with CCl_4- treated group(6.9±0.12 g/dl), (3.64±0.66 g/dl) respectively.

The body weight in the ethanolic extracts treated rats was reduced (209.39±0.27g), compared with the body weight in CCl_4- treated rats. (212.4±2.145 g).

The data suggested that oral administration of ethanolic extract of the leaves of *Aloe vacillans* significantly decreases the intensity of hepatic damage induced by CCl_4 in rats.

Key words: Alanine aminoatransferase (ALT), Aspartate aminotransferase (AST), Total Protein (T.P), Albumin (ALB), Hepatic injury, Rats.

تأثير مستخلص أوراق نبات الصبر (*Aloe Vacillans*) في التهاب الكبد المحدث برابع كلوريد الفحم (CCl_4) في الجرذان

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تاريخ الإيداع 2011/02/01
قبل للنشر في 2012/04/02

الملخص

هدفت هذه الدراسة إلى تقدير مدى تأثير مستخلص أوراق نبات الصبر من نوع *Aloe vacillans* في التهاب الكبد المحدث برابع كلوريد الكربون في الجرذان.

أُحدثت الأذية الكبدية في ثلاثين جرذاً عن طريق الحقن في البريتوان برابع كلوريد الكربون (1 مل/كغم) من وزن الجسم كل 72 ساعة في أثناء حقن المستخلص الكحولي للنبات في الجرذان عن طريق الفم بجرعتين مختلفتين (250 ملغ و500 ملغ/كغم) من وزن الجسم مرة في اليوم مدة 14 يوماً .

بعد 24 ساعة من آخر جرعة سُحب الدم من الوريد ألحجاجي للعين لمعرفة أثر الأذية الكبدية والحماية منها بقياس مستويات أنزيمات الكبد في المصل مثل الأنين امينو ترانسفيراز(ALT)،اسبارتات امينو ترانسفيراز(AST)، الألبومين (ALB) والبروتينات الكلية (T.P).

أظهرت النتائج في المجموعة المعالجة برابع كلوريد الفحم فقط نقصاً في أوزان الحيوانات عند $p<0.05$ (212g) مقارنة بالمجموعة الشاهدة (231g) وارتفاعاً في مستويات أنزيمات الكبد ALT (254.6±6.62IU/L)، AST (322.9±4.42IU/L) مقارنة بالمجموعة الشاهدة (50.7±1.5 IU/L) (70.1±2.6 IU/L) على التوالي ونقصا في البروتينات الكلية (4.57±0.17g/dl) والألبومين (2.6±0.12g/dl) عند $P<0.05$ مقارنة بالمجموعة الشاهدة (6.9±0.12g/dl) (3.64±0.66) على التوالي

أمَّا في المجموعة المعالجة بالمستخلص الكحولي 250 mg/kg فكان هناك نقص في مستويات أنزيمات الكبدALT (185.9±6.2 IU/L) AST (242.5±4.2 IU/L) مقارنة بالمجموعة المحقونة ب CCl_4 (254.6±6.62 IU/L) (322.9±4.42 IU/L) على التوالي وزيادة في البروتينات الكلية (5.17±0.09 g/dl) ومستوى الألبومين (2.78±0.01 g/dl) ذات دلالة معنوية عند $P<0.05$ مقارنة بالمجموعة المعالجة برابع كلوريد الكربون فقط (4.57±0.17g/dl) (2.6±0.1g/dl) على التوالي.

أمَّا في المجموعة المعالجة بالمستخلص الكحولي 500 mg/kg فكان هناك نقص في مستويات أنزيمات الكبدALT (175.6±6.4 IU/L) AST (235.1±12.8 IU/L) مقارنة بالمجموعة المحقونة بـ CCl_4 (254.6±6.62 IU/L) (322.9±4.42 IU/L) على التوالي وزيادة في البروتينات الكلية (5.4±0.12 g/dl) ومستوى الألبومين (2.86±0.10 g/dl) ذات دلالة معنوية عند $P<0.05$ مقارنة بالمجموعة المعالجة برابع كلوريد الكربون فقط (4.57±0.17g/dl) (2.6±0.1g/dl) على التوالي.

أمَّا بالنسبة إلى أوزان الحيوانات المعالجة بالمستخلص الكحولي فكان النقص في أوزانها 209.39 ±0.2g. مقارنة بالنقص في أوزان الحيوانات المعالجة بCCl_4 فقط 212.4±2.1g

توحي النتائج بأنَّ المستخلص الكحولي لعصير أوراق نبات الصبر(*Vacillans*) يملك القدرة على تقليل حدة الأذية الكبدية المحدثة في الجرذان بواسطة CCl_4.

الكلمات المفتاحية: اسبارتات امينو ترنسفيراز (AST)، الأنين امينو ترنسفيراز (ALT)، البروتين الكلي (PT)، الالبيومين (ALB)،أذية كبدية، الجرذان.

Introduction

Aloe plants grow in warm tropical areas and can not survive freezing temperature. The plant is native to south eastern africa and subsequently was 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 State of America and Venezuela (1). The various species of *aloe* have the same effective phenolic compounds (anthraquinones) such as Aloe-emodin, aloesin, barbaloin, aloenin, isobarbaloin (2). A number of investigators have previously demonstrated that antioxidants prevent CCl_4 toxicity particularly hepatotoxicity, by inhibiting lipid peroxidation(3), suppressing (ALT) and (AST) activities (4). The leaves juice of *Aloe* is used in eyes diseases and enlargement of spleen and Liver (5). 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).

Aloe-emodin a natural constituent of *Aloe* leaves significantly inhibited the growth of cells and nontoxic for normal cells (7). Antioxidant and radical scavenging activity of aloe-emodin appears to protect against hepactocyte death and the inflammatory response that occurs subsequent to lipid peroxidation(8). Also Aloe -emodin appears to have some protective effect not only against hepatocyte death but also on the inflammatory response subsequent to lipid peroxidation. Histological examination of the liver showed less marked lesions in the CCl_4+ aloe-emodin treated rats than in those treated with CCl_4 alone(9). Emodin and aloe-emodin also inhibit carbon tetrachloride or sodium taurocholated–induced necrosis production in vivo(10).

Anti inflammatory potential of *Aloe vera* leaves exudates was also demonstrated. This inflammatory activity is mediated partially via reduction of nitric oxide production in macrophages(11). The *Aloe vera* extract at the higher dose (500 mg/kg) significantly lowered the level of ALT, AST, alkaline phosphate(ALP) and bilirubin indicating a good level of protection against the toxicity of CCl_4 (12).

Materials and Methods

Plant material:

Aloe vacillans leaves were collected from Governorate Abyen (Yemen) in December –Feb 2009.

The dried leaves were finely grounded in an electrical grinder and extracted by soxhlet apparatus with ethanol (50-60%) until completely exhausted. Ethanol was evaporated under reduced pressure by a rotary evaporator (13).

The yield was determined as 5-6%(w/w).

Animals:

Forty male Albino rats weighing 200-250 gm were purchased from the Faculty of Science University of Aleppo and were used in these experiments.

The animals were housed at room temperature (28±2C) in standard cages and then had free access standard dry pellet food and tap water and kept under controlled environment following relative humidity (60±5%) with a 12h light/dark cycle.

Behavioral and Toxic Effects:

The acute toxicity study was evaluated in rats according to the method of (14).

Five groups of ten animals were administrated with 125, 250, 500, 1000, 2000 mg/kg of the *Aloe vacillans* extracts orally, while one group with the same number of rats kept as a control group.

The animals were observed continuously for 72 h, and then after every 24 h for 15 days for any gross behavioral change, symptoms of toxicity or mortality.

Induction of Hepatic Injury :

The model was described in (15)

The animals were divided into four groups of ten animals.

Group I received a single dose of liquid paraffin (1ml/kg) of body weight passed orally (p.o).

Group II (negative control) received carbon tetrachloride (CCl_4) (1m/kg) of body weight intraperitoneal (i.p) every 72h for 14 days as 1:1 dilution with paraffin.(16)

Group III, IV (test groups) were administered *Aloe* ethanol extract of 250 mg/ kg and 500 mg/kg of body weight passed orally (p.o) daily for 14 days, simultaneously with CCl_4 as in group II.

All animals, were anaesthetized with ether, and blood was withdrawn from the orbital vein 24 h after the last dose.

The blood was centrifuged at 3000 rpm for 10 min to obtain serum. ALT, AST, T.P and albumin activities were measured with biochemical detectors (17).

The dose and timing were selected based on previous reports (18,12,14).

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 two different dose.(250mg/kg and

500mg/kg) were compared for significance using student's t-test. Differences below ($p<0.05$) are considered as significant.

Results and Discussion :

In the toxicity study, the rats when fed with *Aloe vacillans* up to 2000 mg/kg, p.o exhibited no mortality or any sign of gross behavioral changes when observed for 72 h and then after every 24 h for 15 days. The LD50 was greater than 2000 mg/kg p.o., it may be considered relatively safe.

The effect of ethanolic extract of *Aloe vacillans* on serum enzymes were studied and the results were given in Fig .1 and Fig. 2.

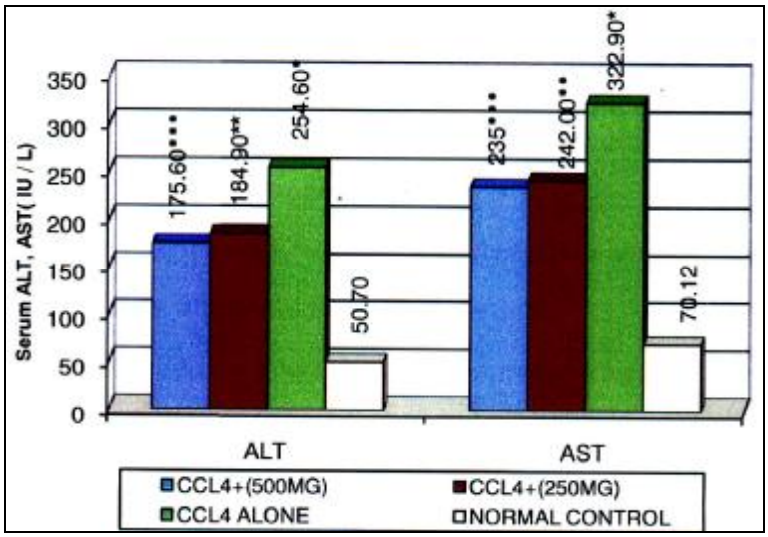

Fig. 1. Values are represented as mean ±SEM (n=10) Anova test used ($p<0.05$) is used, Student test ($p<0.05$) is used.

* significantly different from normal control.

,*significantly different from CCl_4 treatment only.

***significantly different from CCl_4 only + extract 250 mg/kg.

Administration of carbon tetrachloride to rats produced hepatotoxicity showed by the significant increase of serum transaminases (ALT, AST) $p<0.05$ due to hepatocyte damage as a results (19), (20). This is indicative of cellular leakage, and loss of functional integrity of cell membrane in the liver (oxidative stress) according to (21) and (22).

The groups of animals treated with ethanolic extract of *Aloe vacillans* exhibited significant reduction in levels of ALT, AST and increasing in T.P and Albumin ($p<0.05$) at the lower dose (250mg/kg)

as in the higher dose (500mg/kg) when compared with CCl_4 treated group only. The higher dose (500mg/kg) of *Aloe* extract significantly lowered the levels of ALT, AST and raised the T.P and albumin when compared with lower dose (250mg/kg) at ($p<0.05$) according to results (12). Reduction in the levels of ALT and AST by plant extract is an indication of stabilization of plasma membranes as well as against effects of free radicals, it also reduced lipid peroxidation damage caused by CCl_4 according to (23), (24), in addition the *Aloe* aqueous extract showed significant hepatoprotective activity against CCl_4 induced as evident by restoration of serum transaminases (ALT, AST) and protein towards their near normal levels (14), this result similar to our results, but there is no other report indicating hepatoprotective activity in *Aloe* aqueous extract.

The *Aloe vacillans* extract might contains phenolic compounds such as aloe-emodin, emodin, barbaloin, antioxidant activity as indicated by protection against increased lipid peroxidation.

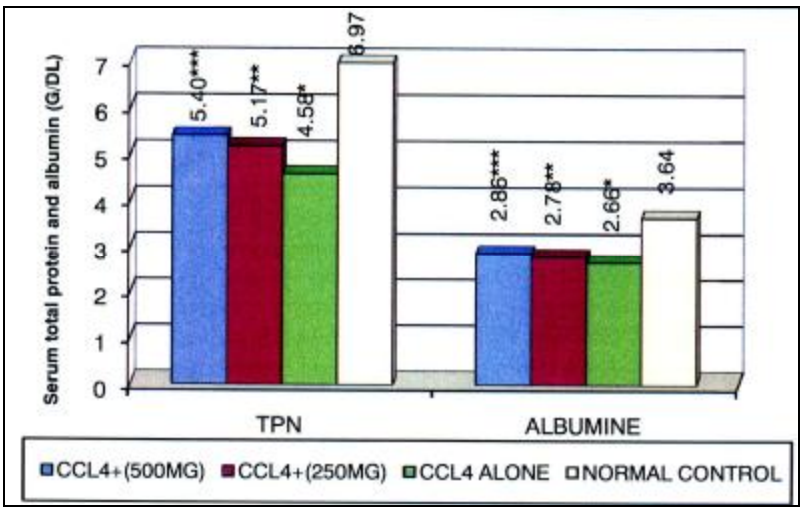

Fig. 2. Values are represented as mean ±SEM (n=10) Student test($p<0.05$) is used, * significantly different from normal control.

,*significantly different from CCl_4 treatment only.

***significantly different from CCl_4 only and extract 250 mg/kg.

Significant reduction in body weight was noticed in the CCl_4 treated rats at ($p<0.05$) when compared with normal control group, due to hepatic injury and fibrosis caused by the toxicant (25), which lead to abstention the animals off the eating.

Simultaneous treatment with the *Aloe* extract at two different dose 250-500 mg/kg restored the body weight significantly ($p<0.05$) when compared with CCl_4 treated group only. Table. 1

Table. 1. Effect of ethanolic extract of *Aloe vacillans* on the body weight

	BODY WIGHT	
	DAY1/gram	**DAY14/gram**
NORMAL CONTROL Group I	230.900 ± 4.23071	231.900 ± 3.7607
CCL4 ALONE Group II	216.100 ± 2.16769	212.400 ± 2.1458*
CCL4+(250mg/kg) Group III	212.700 ± 1.64688	209.396 ± 0.275**
CCL4+(500mg/kg) Group IV	233.500 ± 4.13185	230.700 ± 4.0580***

Values are represented as mean ± SEM (n=10)

Anova test ($p<0.05$) is used - Student test($P<0.05$) is used.

*significantly different from normal control.

,*significantly different from CCl_4 only.

Conclusion

The free radicals thus generated after the injection of animals by carbon tetrachloride alone which can bind with polyunsaturated fatty acid formation alkoxy (R) and peroxy radicals (ROO) that can generate lipid peroxide which cause cellular leakage, and loss of functional integrity of cell membrane in liver, change enzyme activity and finally induce hepatic injury or necrosis. The phenolic compounds which are widely distributed in leaves of *aloe* plants, such as Aloin, aloe-emodin enthrone, emodin enthrone have been considered to play an important antioxidant role. Antioxidant and radical scavenging activity of aloe-emodin appears to protect against hepatocyte death and the inflammatory response that occurs subsequent to lipid peroxidantion. Emodin and aloe-emodin also inhibits carbon tetrachloride –induced necrosis production in vivo. The results of the present study demonstrated that treatment of rats with *Aloe vacillans* had protective effect against CCL_4-induced hepatotoxicity in rats, as evidenced by decreased serum ALT, AST and increased T.P and albumin activities. Simultaneous treatment with ethanol extract of *Aloe vacillans* reduced the degree of hepato- cellular injury as evidenced by improved biochemical parameters. The reason for this improvement may be that *Aloe vacillans* extract contains phenolic compound which might have scavenged the free radicals offering hepatoprotection.

The extract at a higher dose (500mgkg-1) is more hepatoprotective.

Acknowledgment

I thank all for valuable help which was given to me during this work.
This research was partially supported by the Faculty of Pharmacy.

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