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Hepatoprotective activity and antioxidant effects of avocado peels (*Persea americana*) on rats hepatotoxicity induced by carbon tetrachloride.

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Abstract

Liver is the largest internal organ in human body. Avocado peels have effectively improved liver function and protect against liver tissues damage induced by carbon tetrachlorid. This study aimed to evaluate the impact of avocado peel to reduce hepatotoxicity in rats. Twenty four adult male rats divided into two main groups and fed on standard diet. Group I: negative control (6 rats). Group 2: hepatotoxic groups (n=18), which were subjected to subcutaneous injection of a single dose of 0.3 ml/kg CC14 mixed with equal volume of corn oil on the 7th day. Hepatotoxic groups (n=18) were divided into (3) subgroups 6 rats per group (1) positive control fed on basal diet, group (2) fed on basal diet containing 2.5% of avocado peel, group (3) fed on basal diet containing 5% of avocado peel powder. Food intake was calculated daily and rats were weighed weekly. Feeding and growth performance were carried out by the determination of food intake and body weight gain. At the end of experimental (30 day) injection administration of a single dose of 0.3 ml/kg CC14 significantly increased levels of, liver function, , malonaldehyde, liver and kidney functions in positive control group. In contrast catalase and glutathione transferase were significantly decreased. Histopathological examination revealed degeneration of hepatocytes of rat livers treated with carbon tetrachlorid. Feeding rats with avocado peel at dose of 2.5 and 5%. resulted in reducing levels of hepatotoxic in serum liver, malonaldehyde, liver and kidney functions compared to positive control group. The study concluded. Avocado peels have effectively improved liver function and protect against liver tissues damage induced by carbon tetrachloride in experimental animals

Key words: hepatotoxicity, avocado peel, liver functions.

Introduction

Liver is the largest internal organ in human body. It processes and stores many of the nutrients absorbed from the intestine that are necessary for body function some of these major function include protein, carbohydrate and fat metabolism. It also secretes bile into the intestine to absorb nutrients (**Strauss, .2005**).The liver is the largest organ of the body.Constituting 2.5% of the adult body weight. It receives blood supply from two major blood vessels. The hepatic artery supplies oxygenated blood, whereas the portal vein, which provides 80% of the total blood supply, supplies nutrient-rich deoxygenated blood. The liver thus acts as a guard between the digestive tract and the rest of the body (**Mohan and Escott-stump, 2008**). Avocados are commercially valuable and are cultivated in tropical and Mediterranean climates throughout the world. They have a green-skinned, fleshy body that may be pear-shaped, egg-shaped, or spherical. Commercially, they ripen after harvesting. Trees are partially self-pollinating and often are propagated through grafting to maintain a predictable quality and quantity of the fruit (**Chen et al., 2008**).

Avocado peel is rich in flavonoids, proanthocyanidins, and hydronomic acids (**Kosinsk et al., 2012**). The avocado is high containing of antioxidant, vitamins C, A, D and E (**Sanjust et al, 2008**).

The avocado peel rich in total phenolics, flavonoids and carotenoids(**Aracibia-Avila et al., 2008**)

The freeze dried samples of the peel of the avocado were content of essential composition, minerals, total phenolic and antioxidant capacity. The peels pass larger total phenolic content and antioxidant activity in relation to the pulp (**Daiuto et al, 2014**). Avocado peels have concentration of vitamin C and E the highest value was found in the pulp (**Vinha et al , . 2013**). Avocado peels have effectively improved liver function and protect against liver tissues damage induced by toxic substances (**Irshad and Chaudhuri, 2002**). Feeding supplemented diet with the different concentration of avocado fruits 5,10and 15% on rats for 4 weeks and found that avocado fruit effectively improved liver function and protected against liver tissues damage (**Mohamed and Rrezq., 2013**), and reported that avocado caused significantly lower in serum concentration of AST, ALT, ALP, TP and total and direct bilirubin compared with that of the possative control rats. In addition to the improvement in liver tissues as indicated by slight hydropic degeneration of hepatocytes in treated rats with 5 and 10%of avocado fruit and apparent normal hepatocytes in some section of treated rats with 15% avocado. Avocado peel have effect on blood serum cholesterol level, it decrease total serum cholesterol levels, LDL and triglycerides and increase HDL in hypercholesterolemia patients (**Lopez et al., 2007**).

. High avocado intake was shown in a preliminary study to lower blood cholesterol levels. Specifically, after seven days with a diet rich in avocado, mild hypercholesterolemia patients showed a 17% decrease in total serum cholesterol levels and a 22% decrease in LDL and triglyceride levels and a 11% increase in HDL (**Fulgoni et al., 2013**). Flavonoids, rutin, catechin and quercetin are widespread in nature and may act as powerful antioxidants. These findings and our results provide evidence for the importance of phenolic and flavonoid present in avocado peel (**Terpin et al., 2012**). The flavonoid luteolin has been shown to possess direct antioxidant activity; it is useful in the treatment of many chronic diseases associated with oxidative stress. Luteolin treatment involved changes in SOD activity, MDA content and expression of hemoxygenase-1 (HO-1) protein (**Guo, 2011**). Avocado fruits may contribute to eye health since they contain MUFA and lutein/zeaxanthin, and help improve carotenoid absorption from other fruits and vegetables (**Unlu et al., 2005**). Avocado peel contains a number of bioactive phytochemicals including carotenoids, terpenoids, D-mannoheptulose, prenyl caffeoyl and B, phenols and glutathione that have anti-carcinogenic properties (**Ding et al., 2009**). The aim of this study is to investigate the hepatoprotective activity and antioxidant effects of avocado peel (*Persea americana*) on rats with hepatotoxicity induced by carbon tetrachloride.

Materials and Methods

Materials

The fresh avocado collected from Beco company, Al Buhaiya, Egypt. Carbon tetrachloride (CCl₄), casein, cellulose, choline chloride, El-methionine, vitamin mixture and mineral mixture were obtained from Morgan Co. Cairo, Egypt. All chemical kits were purchased from El-Gomhoria Company for chemicals and Drugs El-America, Cairo, Egypt.

Adult male albino rats, Sprague Dawley strain, were obtained from Research Institute of Ophthalmology, Giza, Egypt.

Methods:

Preparation of dried avocado peel

The fresh avocado peel was cut into thin slices, then dried at 60°C for 1.5 hours using a vacuum oven, then kept in cold until use.

Chemical composition of dried avocado peel:

Moisture, fat, protein, fiber and ash contents were determined in avocado peel according to AOAC, (2010). The carbohydrate was calculated by difference. Total phenols were estimated according to Singleton and Rossi, (1965). Total carotenoids were determined according to Akin et al (2008).

Experimental design

Twenty four adult male rats SpragueDawley weighting (150 ± 5 g) were used in this study. The animals were housed individually in well aerated cages under hygienic laboratory condition and fed standard diet according to AIN-93 guidelines (Reeves et al., 1993) for 7 days as an adaptation period. Rats were randomly divided into two main groups and fed on

standard diet. Group I: negative control (6 rats). Group 2: hepatotoxic groups (n=18), which were subjected to subcutaneous injection of a single dose of 0.3 ml/kg CC14 mixed with equal volume of corn oil on the 7th day. (Saraswat et al., 1993).

Hepatotoxic groups (n=18) were divided into (3) subgroups 6 rats per group (1) positive control fed on basal diet, group (2) fed on basal diet containing 2.5% of avocado peel, group (3) fed on basal diet containing 5% of avocado peel powder. Food intake was calculated daily and rats were weighed weekly. Feeding and growth performance were carried out by the determination of food intake and body weight gain. At the end of experimental period (30 day), rats were anesthetized with diethyl ether after fasting for 12h and blood samples were collected and centrifuged to obtain serum and kept in frozen until analysis.

Biochemical analysis:

Alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) enzymes were measured according to the methods described by Bergmeyer and Harder (1986), Kachmar and Moss (1976) and Varley et al., (1980), respectively. urea and creatinine levels were determined in serum according to the method described by Houot (1985). Catalase, glutathione transferase (GTH) and malonaldehyde (MDA) were determined according to the methods described by Hu (1994), Aebi, (1974) and Jentzsch et al., (1996) respectively.

Histopathology examinations:

The liver organ was taken from each experimental group, fixed in neutral buffered formalin, dehydrated in ascending concentration of ethanol (70, 80 and 90%), cleared in xylene and embedded in paraffin. Histopathology examinations were described according to Bancroft and Stevens, (1996).

Statistical analysis:-

The results recorded as the mean \pm SD. The experimental data were subjected to an analysis of variance (ANOVA) for a completely randomized design using a statistical analysis system (SAS, 2000). Duncan's multiple range tests were used to determine the differences among means at the level of 5%.

Results and Discussion

Table (1) showed the chemical composition, total phenols and antioxidant activity of avocado peel. The chemical compositions of cocoa powder were 7.13, 57.82, 19.0, 0.95 and 14.65 mg/100g for moisture, carbohydrate, fat, protein, and ash respectively. These findings are in accordance with (Wang et al., 2010) reported that the chemical composition of avocado were 1.2, 20, 30 and 5.2 for protein, fat, moisture and ash respectively. Also the results indicated that avocado peel had total phenols (575.6 mg/100g), carotenoids (0.841 mg/100g) and antioxidant activity (47.0%). These results had the same trend of (Aracibia-Avila et al., 2008) reported that the skin of avocado contain total phenolics, antioxidant and carotenoids were 679, 815 and 44.3 mg/100g respectively.

Table (2): showed the effect of avocado peel on liver function in control and hepatotoxic group. Levels of (AST) aspartat amino transferase, (ALT) alanin amino transferase, (ALP) alkaninphosphat transferase, GGT: gama glotamattransferase, T-bill: total bilirubin, D.bil: direct bilirobin were significantly higher ($p \leq 0.05$) in hepatotoxic group than that in negative group while, Total protein and Albumen had opposite trend. Treatment diet rats with 2.5 and 5% avocado peel resulting in reducing the level o avocado peel and increasing t.pro and albumen. supplementation diet rats with 5% of avocado peel was more effecting ($p \leq 0.05$) in reducing AST, ALT, ALP, GGT, T-bill and D.bill than those supplementation rats diet with 2.5%. However there were no significant differences' ($p \geq 0.05$) in T.pro and albumen rats supplementation diet with 2.5 and 5% avocado peel. Carbon tetrachloride (CCl_4) is one of the most commonly used hepatotoxins in the e xperimental study o liver disease (fang and lin, 2008) these results were in accordance by (Niak and Panda, 2007) who mention that increased serum levels of AST, ALT, and ALP in CCl_4 - treated animals is an indicator of liver damage as these enzymes leak out from liver into blood at the instance of tissue damage, which is always associated with hepatonecrosis. (Al-Dosari, 2011) found that treatment with avocado peel (1 and 2 ml/rat/day) significant decrease in serum of GPT, GOT, GGT, ALP and bilirubin levels, while liver and heart MDA was also significantly decreased, However significant increase in Abumien and total protein.

Data in Table (3) showed that the effect of avocado peel on lipids profile in control and hepatotoxic groups. Values of serum total cholesterol (TC), triglyceride (GT), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were significantly higher ($p \leq 0.05$) in hepatotoxic groups compared with negative group while, high density lipoprotein (HDL) had opposite trend. Supplementation rat diets with 2.5

and 5 % of avocado peels were decreased the levels of TC, GT, LDL and VLDL by 5.80, 17.00, 8.60, 17.80% and 10.3, 25.00, 17.90 and 29.50% while, HDL were increased by 5 and 11.40% respectively. The highest improvement in lipids profile was found in hepatotoxic rats supplemented with 5% of avocado peels. These results agree with **(Lopez et al., 2007)** reported that avocado peel have effect on blood serum cholesterol level, it decrease total serum cholesterol levels, LDL and triglycerides and increase HDL in hypercholesterolemia patients. **(Serfaty et al., 2008)** found that avocado has rich in serotonin 5-hydroxytryptamine (5-HT) which is monoamine neurotransmitter. **Salgado et al. (2008)**. Reported that the effect of consumption of avocado peel on level of total cholesterol, HDL, LDL, T.G and hepatic with different concentration of avocado 15 and 25%. and found that the diet contain 15% avocado reduced the level of total cholesterol and HDL in comparison to the control, it was observed that for the excreted cholesterol, the best diet was 25% avocado peel. The diets contain 15% avocado peel also influenced the level of hepatic cholesterol. High avocado intake was show in on preliminary study to lower blood cholesterol levels. Specifically, after seven day with diet rich in avocado, mid hypercholesterolemia patients showed a 17% decrease in total serum cholesterol levels and a 22% decrease in LDL and triglyceride level and 11% increase in HDL **(Fulgoni et al., 2013)**.

Data in table (4) indicated that the untreated rats had significant increase $p \leq 0.05$ in serum Malondhyaldehyde (MDA) level compared with normal rats. Affected rats fed on supplemented diet with 2.5 and 5% avocado peel had significant decrease at $p \leq 0.05$ in serum level of MDA compared with positive control and supplanted rats with 5% was more effectively $p \leq 0.05$ decreasing in MDA while, Glutathione transferase (GST) and catalase (CAT) were significant higher $p \leq 0.05$ in hepatotoxic rats compared with negative group. Administration of avocado peel at two different levels 2.5 and 5% induced significantly higher in serum activity of GST and CAT enzyme compared with negative group. It obvious that the increasing serum activity of antioxidant enzymes GST and CAT were more detectable with increase avocado peels levels. **Augustinian et al, (2005)** found that CCl₄ initiates lipid peroxidation and reduces tissue CAT and SOD activities. These results were confirmed by **(Ruidong et al, 2001)** demonstrated that MDA levels in the CCl₄ treated group as indicated of lipid peroxidation were significantly higher than that in normal group **(Mohamed et al., 2013)** reported that fed on supplemented diet with different concentration of dried avocado fruits 5, 10 and 15% on rats and found that avocado caused significant decrease in serum concentration of MDA and significant increase the activity of

SOD, GPX and CAT enzymes compared with that of the positive control rats

Terpinc *et al*, (2012) found that flavonoids, rutin, catechin and quercetin are widespread in nature and may act as powerful antioxidants. These finding and our results provide evidence for importance of phenolic and flavonoid present in avocado peel

Table(5) showed the effect of adding different levels of avocado peel to hepatotoxic rats diet on body weight gain rats. Before inducing hepatotoxicity, there was no significant difference in the body weight between groups. After the period of treatment it is clear that weight of normal group was increased significantly ($p \leq 0.05$) compared to the positive group, followed by the weight of 2.5 and 5% of avocado peel groups, the change weight were increased by 16.80 and 19.60% for 2.5 and 5% avocado peel respectively compared to the positive group which increased by 6.60%

Table (1): Chemical composition, total phenolic and total antioxidant activity of dry avocado peel

Parameter	Avocado peel
Moisture%	7.13 ±0.58
Carbohydrate%	57.82±0.02
Total fat %	19.50±0.50
Protein %	0.95±0.50
Ash %	14.65±0.14
carotenoids(mg/100g)	0.840±0.50
Total phenols(mg/100g)	675.0±20
Antioxidant activity (%)	47.00±5.00

Each value in the table is the mean ± standard deviation of three replicates. Means in the same row with different letters are significantly different ($p \leq 0.05$)

Table (2): Effect of avocado peel on liver functions in control and hepatotoxic groups

Variables	Negative (-)	Hepatotoxic groups		
		Control (+)	A Peel (2.5%)	A Peel (5%)
AST(u/l)	36.20 ^d ±0.90	79.00 ^a ±0.70	46.00 ^b ±1.20	39.60 ^c ±1.30
ALT(u/l)	30.70 ^d ±1.40	67.70 ^a ±1.60	35.60 ^b ±0.50	30.70 ^c ±1.40
ALP(u/l)	160.60 ^d ±2.80	280.00 ^a ±1.50	241.80 ^b ±2.10	210.60 ^c ±1.10
GGT(u/l)	3.90 ^d ±0.30	7.80 ^a ±0.26	6.96 ^b ±0.15	5.78 ^c ±0.10
T. Bill (u/l)	0.68 ^c ±0.10	1.40 ^a ±0.10	1.00 ^b ±0.10	0.93 ^b ±0.04
D. Bill (u/l)	0.19 ^d ±0.07	0.30 ^a ±0.01	0.27 ^b ±0.08	0.24 ^c ±0.8
T. protein(u/l)	7.40 ^a ±0.05	5.8 ^d ±.20	6.20 ^c ±0.21	6.70 ^b ±0.10
Albumin (u/l)	3.10 ^a ±0.10	2.40 ^c ±0.08	2.70 ^b ±0.07	2.80 ^b ±0.20

Means ± standard deviations in the same row with different letters are significantly difference (P ≤ 0.05)

.A.peel:avocadopeel,AST:aspartataminotransferaseALT:alanin amino transferase , ALP:alkalinphosphat transferase, GGT:gama glotamattransferase, T.Bill: tottal bilirubin, D.Dil: direct bilirobin. T.Pro:tottal proten

Table (3): Effect of avocado peel on liped profil in control and hepatotoxic groups

Variables	Negative (-)	Hepatotoxic groups		
		Control (+)	APeels(2.5%)	APeels(5%)
T.CHO(mg\dl)	109.80^d±1.30	130.20^a±0.80	122.60^b±1.10	116.60^c ±0.80
T.G(mg\dl)	71.70 ^d ±1.20	105.90 ^a ±0.80	87.80 ^b ±0.83	78.80 ^c ±0.80
HDL(mg\dl)	46.60 ^a ±1.10	35.80 ^d ±0.80	37.70 ^c ±0.67	39.90 ^b ±0.74
LDL(mg\dl)	51.9 ^d ±0.90	71.40 ^a ±1.05	65.20 ^b ±0.80	58.60 ^c ±0.14
VLDL(mg\dl)	14.50 ^d ±0.36	21.30 ^a ±0.20	17.56 ^b ±0.16	15.00 ^c ±0.34

Means ± standard deviations in the same row with different letters are significantly difference (P ≤ 0.05). A P:avocado peel, T.CHO:tottal cholestrole, T.G: try glycraied, HDL: high denisty lipoprotein, LDL: low denisty lipo protein, VLDL:

Table (4): Effect of avocado peel on antioxidant in control and hepatotoxic groups

Variables	Negative (-)	Hepatotoxic groups		
		Control (+)	A P (2.5%)	A P (5%)
GST(u\l)	1348.00 ^a ±67.00	386.00 ^d ±24.00	525.00 ^c ±7.90	620.00 ^b ±15.00
CAT(u\l)	838.00 ^a ±30.0	453.00 ^d ±14.00	586.00 ^c ±20.00	636.00 ^b ±12.00
MDA(nmol\ml)	37.40 ^d ±2.40	90.00 ^a ±2.10	63.40 ^b ±2.70	52.80 ^c ±2.80

Means ± standard deviations in the same row with different letters are significantly difference

(P ≤ 0.05). A .Peel: avocado peel, GST:glotathione-s-transferase, CAT:catalase, MDA: malondialhyed

Table (5) :Effect of adding different portions of avocado peel to hepatotoxic rats diet on body weight in rat

Variables	Negative (-)	Hepatotoxic groups		
		Control (+)	A Peel (2.5%)	A Peel (5%)
Initial weight (gm)	152.60 ^d ±1.60	136.60 ^a ±2.07	141.20 ^b ±1.30	144.40 ^c ±1.14
Final weight (gm)	197.00 ^d ±1.50	153.00 ^a ±1.87	167.60 ^b ±1.14	176.60 ^c ±1.14
Changeweight	17.46	6.60	16.8	19.60

Means ± standard deviations in the same row with different letters are significantly difference (P ≤ 0.05). A .Peel: avocado peel

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التأثيرات الوقائية والمضادة للأكسدة لثمار الأفوكادو علي فئران التجارب المصابة بتسمم الكبد الناتج عن رابع كلوريد الكربون

يوسف عبد العزيز الحسانين , هبه عز الدين يوسف و زينب محمد منصور
قسم التغذية وعلوم الاطعمة, كلية الاقتصاد المنزلى جامعه المنوفية. مصر

الملخص العربى

يمثل الكبد اكبر غدة فى جسم الانسان حيث انه المكان الرئيسى الذى تتم فيه معظم عمليات الايض المختلفه كما انه له دور حيوى فى تنظيم العمليات الفسيولوجيه مثل الافراز والتمثيل الغذائى والتخزين. علاوة على ذلك فإنه يعمل على إزالة السموم والعقاقير المختلف التي تحدث داخل الجسم . يحتوى الافوكادو على مضادات الاكسده القويه النشطه وعلى المركبات الفينولية التي تحسن من وظائف الكبد وايضا نجد ان الافوكادو يجدد من نشاط خلايا الكبد ويمنع من تليفه وبالتالي يحدد من تكون الخلايا السرطانيه بالكبد. لذه هدفت هذه الدراسة الي معرفة التأثيرات الوقائية والمضادة للأكسدة لثمار الافوكادو علي فئران التجارب المصابة بتسمم الكبد الناتج عن رابع كلوريد الكربون. حيث تم استخدام اربع وعشرون من فئران ذكور الألبينو , قسمت الى مجموعتين رئيسيتين ،المجموعه الاولى :مجموعه ضابطة ساليه (6 فأر) , المجموعه الثانيه : الفئران المصابه بالتسمم الكبدى (18 فأر)،تم تقسيمها الى ثلاث مجموعات فرعيه متساويه , المجموعه الاولى :مجموعه ضابطة موجبه، المجموعه الثانيه والثالثه تناولتا الوجبه القياسيه باستبدال 2.5و5% من قشر الافوكادو المجفف على التوالي. وقد اشارت النتائج الى ان تناول الفئران لقشر الافوكادو أدى إلى انخفاض معنوي في انزيمات الكبد وتحسين وظائف الكبد ،، دهن الدم مقارنة بالمجموعه الضابطة الموجبة ومن ناحية أخرى وجد ان المعاملات أدت إلي زيادة معنوية في نشاط الجلوتاثيون والكتاليز مقارنة بالمجموعه الضابطة الموجبة ، لذا يمكن ان نستخلص من الدراسة كفاءة استخدام قشر الافوكادو في تحسين وظائف الكبد في الفئران المصابة بتسمم الكبد الناتج عن رابع كلوريد الكربون .

الكلمات المفتاحيه:تسمم كبدى, قشر افوكادو, وظائف كبد.