PROTECTIVE EFFECT OF AVOCADO FRUIT (Persea americana) POWDER AND EXTRACT AGAINST HEPATIC INJURY IN EXPERIMENTAL RATS

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Abstract:

The purpose of this study was to investigate the protective efficacy of avocado fruit (*Persea americana*) powder and extract against paracetamol induced hepatic injury in rats. Forty male Sprague-Dawley rats weighing (115 ± 10) (8 rats), were divided into five groups: normal control group (-ve), paracetamol control single dose of (2 g/kg/b.wt/rat) as control (+ve), and the other three treated groups were with 5 g/kg/diet/day avocado powder, 0.5 ml /kg/ b.wt/ rats/day avocado extract by stomach tube and mixture with 5 g/kg/diet/day avocado powder and 0.5 ml /kg/ b.wt/ rats/day avocado extract. The result revealed that all of the treated groups showed significant increase in body weight gain, serum HLDL-C, total protein, total antioxidant capacity, SOD levels. Moreover, significant decrease serum TC, TG, LDL-C, VLDL-C, AST, ALT, alkaline phosphotase enzymes (Alk-Pho), total bilirubin and nitric oxide (NO) in comparing to positive control group. The current study suggests that avocado powder and extract may be content of the phytochemical such as tannins, saponins, glycoside, flavonoids and carbohydrates beneficial as a protective antioxidant supplement with promising antioxidant potential to combat paracetamol induced hepatic injury in rats.

Keywords: *Persea Americana,* Paracetamol, hepatotoxicity, phytochemical.

Introduction

The liver which is one of the most important organs of the body is responsible for metabolism and getting rid of toxic substances, it is

affected by many pollutants and drugs which cause damage and weaken it (**Krishna *et al.*, 2012**).

Paracetamol (acetaminophen or N-acetyl-p-aminophenol) which was presented to medicine as an analgesic and antipyretic by Von Mering in 1893. (**Eggins, 1996**), is the most consumed medicine as an effective and analgesic agent used for remedy headache, backache, arthritis and postoperative pain, when it is taken in over dose amounts, it may cause hepatotoxicity or death (**Rodenas *et al.*, 2000**). The effect of liver, and to a lesser extent the kidney and intestine, are the major organs implicated in the metabolism of paracetamol (acetaminophen) (**Toussaint *et al.*, 2010 and Bessems and Vermeulen, 2001**).

The avocado (*Persea americana*) or alligator pear. They have a green-skinned, fleshy body that may be pear-shaped, egg-shaped, or spherical and they are belonging to family Lauraceae. Avocado originated in Mexico, Central or South America, and was first cultivated in Mexico as early as 500 BC (**Wang *et al.*, 2015 and Fulgoni *et al.*, 2013**). Avocado contains minerals, phytochemicals ,phenolic acids, flavonoids, vitamins and antioxidants which are associated with many health benefits (**Stewart *et al.*, 2010**).

The antibiotic effect of aqueous extract of avocado seeds. Avocado's ethanoalic extract has demonstrated hypoglycemic properties both in normoglycaemic and alloxan-induced diabetic rats (**Nwozo *et al.*, 2004, Anita *et al.*, 2005 and Alhassan *et al.*, 2012**). Avocado leaf extract has powerful antioxidants capable of scavenging free radicals (**Owolabi *et al.*, 2007**). Another study in rats has shown the hypotensive (**Di' Stasi *et al.*, 2002 and Owalabi *et al.*, 2005)** and hypochlesterolemic properties (**Brai *et al.*, 2007**) of the extracts of avocado leaves. Also, avocado extracts which includes carotenoids and tocopherols inhibited the growth of prostrate cell lines in vitro (**Lu *et al.*, 2005**). Besides, Avocado extracts are claimed to have antifungal, anticancer and antioxidants activities (**Wang *et al.*, 2010**). In view of the antioxidant properties of avocado, it was thought to be worthwhile to investigate the beneficial effects on paracetamol induced hepatic injury in rats. Therefore, the present study was carried out to evaluate the effect of avocado fruit powder and extract with different levels on biochemical parameters and hepatotoxicity in paracetamol exposed rats.

Materials and Methods

Materials

Avocado fruit (*Persea Americana)*

Was obtained from local market, Mansoura.

Paracetamol drug

Was obtained from Kahira Pharm and Chem. Ind. Co., Cairo- Egypt.

Kits

For biochemical analysis were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

Animals

Forty male Sprague-Dawley rats weighing (115 ± 10gm.) were purchased from farm of experimental animals in Helwan, Egypt.

Basal Diet

The basal diet consisted of protein (13%), fat (4%), salt mixture (3.5%), vitamin mixture (1%), choline (0.2%), cellulose (5%) and the remainder was starch (**Reeves *et al.*, 1993**).

Methods:

Preparation of avocado fruit powder and extract:

The avocado fruit were dried at 60°C, and then crushed to powder. The avocado fruit powder was added to the diet as 10% of the diet. The other part was used for preparation of methanol extract. 100 g of avocado fruit powdered were soaked in 500 ml of 80% ethanol with frequent agitation. Clarification was then carried out using vacuum filtration through filter paper watman No.2. The resultant extract was concentrated to dryness in a rotary evaporator under reduced pressure at a temperature of 40°C according to (Irkin and Korukluoglu 2007)

Preliminary phytochemical screening of avocado fruit:

Detection of tannins:

Tannins was detected in the plant sample according to the method of (**Kahkonen *et al.*, 1999**).

Detection of saponins:

Saponins substances were detected in different crude extracts under investigation according to the method of (**Trease, 1961**).

Detection of flavonoids:

Flavonoids substances were detected in extracts of different samples using the method of (**Kahkonen *et al.*, 1999**).

Detection of carbohydrates and glycosides:

Carbohydrates and glycosides were treated by Molish test according to the method of (**Kahkonen *et al.*, 1999**).

Experimental design:

The experimental rats were divided into five groups after adaptation period (7 day). The first group which kept as normal control (-ve) group (8 rats) which fed on basal diet only. The rest of rats were administered paracetamol drug at a single dose of 2 g/kg (**Rafael *et al.*, 1999**) by stomach tube to induce liver injury then classified into four groups. One of them acted as control (+ve) and the other three treated groups were with 5 g/kg/diet/day avocado fruit powder, 0.5 ml /kg/ b.wt/ rats/day avocado extract by stomach tube and mixture with 5 g/kg/diet/day avocado fruit powder and 0.5 ml/kg/b.wt/rats/day avocado fruit extract. Feeding and growth performance were carried out by determination of daily food intake, body weight gain and feed efficiency ratio (FER) according to (Chapman et al., 1950).

Biochemical analysis:

Rats were sacrificed at the end of the experiment (60days) for collection of blood samples which centrifuged at 3000 rpm/ 15 minutes to obtain serum.

Determination of serum lipids:

Serum cholesterol, triglycerides (TG) and high density lipoprotein cholesterol (HDL-c) were determined by using enzymatic colorimetric methods **(Abell *et al.*, 1952, Buccolo and David, 1973 and Kostener, 1977)**. Low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c) and cholesterol/ HDL-c were calculated

according to (**Fruchart, 1982 and Castelli and levitar, 1977)**, a total lipid was determined according to (**Folch *et al.*, 1957**).

Determination of liver function parameters:

Rats liver were also collected for some biochemical analysis. Serum aspartate and alanine amino transferase, alkaline phosphatase and gamma glutamyle transferase (AST, ALT, AI'& yGT) enzymes activity, were estimated according to (**Reitman and Frankel, 1957; Draper and Hadley, 1990 and Kind and King 1954**) respectively. Also, serum total bilirubin and total protein were determined according to (**Jendrassik, 1938, and Weichselbaum, 1946**), respectively.

Assay of hepatic antioxidants parameters and nitric oxide:

Liver content of superoxide dismutase (SOD) activity and total antioxidants capacity (TAC), were determined according to (O**yanagui, 1984 and Cao *et al.*, 1993**) NO was measured by modified Griess reaction (**Nagi *et al.*, 2010**).

Statistical analysis:

The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, Statistical Soft-Ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test and $p<0.05$ was used to indicate significance between different groups according to (**Snedecor and Cochran, 1967**).

Reults and Discussion

Preliminary phytochemical screening of alcoholic aqueous extract of avocado fruit. The phytochemical screening of tannins, saponins, glycoside, flavonoids and carbohydrates of alcoholic extract of avocado fruit was detected and recorded in Table 1.

Table (1): Preliminary phytochemical screening of avocado fruit.

Sample	Tannins	Saponins	Flavonoids	Glycoside	Carbohydrates
Avocado	+	+	+	+	+

It was noticed that tannins, saponins, glycoside, flavonoids and carbohydrates were found in alcoholic extracts of avocado fruit. **Jaime *et***

al. **(2011)** reported that avocado is considered a healthy fruit for its monounsaturated oil similar to olive and content of polyphenols ,tocopherols and phytosterols.

Effect of treatment with avocado on feed intake and body weight gain of rats received paracetamol:

The results in Table 2 showed that normal control group had the higher body weight gain, in contrary we observed that the positive control group represented the lower body weight, however in the treated groups with avocado fruit powder, avocado fruit extract and mixture group we noted a significant increase in the body weight gain compared to positive control group. Concerning the feed intake, Table 2, the results showed that paracetamol induced a decrease in feed intake of exposed rats when compared to the control(-v) , whereas in the treated group with avocado fruit with different levels (5g, 0.5ml and mixture form 5g and 0.5ml), observed a significant increase compared with the intoxicated paracetamol group (+v), the result was in agreement with (**Amal *et al.*, 2013**).

Table (2): Mean values ± SD of body weight gain, food intake, FER and PER of the experimental rat groups

Groups / Variables	Normal control (-v)	Positive control (+v)	*Treated with avocado fruit*		
			powder	Extract	Mixture
Body weight gain (g)	95.72± 8.42 ^{a}	41.88± 5.1 ^{d}	63.43.0± 6.13 ^{c}	66.65± 5.57 ^{c}	73.21± 8.21 ^{b}
Feed intake g/day	16.85± 1.84 a	11.73± 1.17 ^{c}	15.99± 1.45 ^{b}	16.21± 1.22 ^{a}	16.81± 1.71 ^{a}
FER	0.0894± 0.001a	0.059± 0.003 ^{d}	0.065± 0.005 c	0.074± 0.004 ^{b}	0.073± 0.002 ^{b}

Effect of treatment with avocado on serum lipid profile of rats received paracetamol:

Serum lipid profile in the experimental animals is shown in Table3 paracetamol administration caused significant elevation in total cholesterol, triglycerides, LDL-c and VLDL-c levels in positive control group, whereas, a significant decrease in the HDL level was found in the positive control group compared to normal control rats. These levels were found to be restored in the treated group with avocado fruit mixture at (5 g/kg diet and 0.5 ml/kg/b.wt/rats) and avocado fruit extrcat treated group in a concentration dependent manner with 0.5 ml/kg/b.wt/rats to be the most effective concentration Table (3). Regarding the effects of paracetamol administration on triacylglycerols; our results showed significant increase in TG and VLDL levels. This finding was in agreement with the results of (**Gopi *et al.*, 2010 and Oyagbemi and Odetola 2010 and Ghebremeskel *et al.*, 2002**)

Table (3): Mean values ± SD of serum lipid profile of the experimental rat groups

Groups / Variables	Normal control (-v)	Positive control (+v)	*Treated with avocado fruit*		
			powder	Extract	Mixture
TC mg/dl	82.27± 4.16 d	128.57± 4.71 a	110.67± 7.26 b	97.80± 2.44 c	92.27± 2.89 c
TG mg/dl	68.13± 2.97 d	99.13± 2.97 d	89.60± 3.14 b	74.47± 5.22bc	70.27± 1.42cd
HDL-c mg/dl	39.50± 2.29a	28.23± 1.37c	31.17± 1.76b	33.53± 3.02b	37.70± 2.07a
LDL-c mg/dl	29.14± 5.99e	78.87± 3.55a	61.58± 5.76b	48.37± 1.34c	39.91± 3.66d
VLDL-c mg/dl	13.63± 0.59d	21.47± 2.13a	17.92± 0.63b	15.89± 1.04bc	14.65± 0.28cd

Mean values in each column having different superscript (a, b, c) denote significant difference.

Effect of treatment with avocado fruits on some liver functions of rats received paracetamol

The results of this study Table 4 showed that feeding diet containing (5, 0.5 and mixture from 5 and 0.5), avocado fruits powder or extract caused significant decrease in AST and ALT activities as compared to positive control rats. On the other hand the activities of AST and ALT enzymes decreased gradually with increasing level of avocado fruit. The 10% of avocado fruit were more effective in reducing the concentration of ALT. Treatment of paracetamol intoxicated rats with mixture, extract, powder of avocado fruit decreased AST enzyme by about 45.21, 46.14and 49.37 µ /ml respectively and ALT by about 14.13, 14.78and 15.61 µ /ml comparing with positive control group, from these results, it could be conculded that, treating intoxicated positive rats with avocado fruit mixture with powder and extract improved liver enzymes. Our results coincide with (**Murayama *et al.*, 2008, Yassin *et al.*, 2010, and Lee *et al.*, 2012**). Evaluation of the mechanisms of drug induced liver injury and release of soluble products including AST, ALT and ALP indicates that mitochondria are critical targets for drug toxicity, either directly or indirectly (**Tapsell *et al.*, 2006 and Murayama *et al.*, 2008**).

Table (4): Mean values ± SD of serum amino transferase (AST & ALT), alkaline phosphotase enzymes (Alk –Pho), total bilirubin and total protein of the experimental groups.

Groups / Variables	Normal control (-v)	Positive control (+v)	*Treated with avocado fruit*		
			Powder	Extract	Mixture
AST (µ /ml)	43.17± 5.81^{d}	67.39± 9.61^{a}	49.37± 6.01^{c}	46.14± 8.10^{b}	45.21± 6.15^{b}
ALT (µ /ml)	13.35± 1.12^{d}	25.55± 3.35^{a}	15.61± 1.81^{c}	14.78± 2.01^{b}	14.13± 3.51^{b}

Alk –Pho (µ /ml)	32.17± 5.66 [d]	50.38± 5.81 [a]	36.80± 4.11 [c]	34.13± 4.37 [b]	33.34± 5.01 [b]
total bilirubin (mg/dl)	0.72± 0.01 [d]	1.82± 0.11 [a]	0.99± 0.02 [b]	0.88± 0.12 [c]	0.75± 0.13 [d]
total protein (g/dl)	6.53 ±0.26 [a]	4.41 ±1.01 [d]	5.51± 0.81[c]	6.61± 0.77 [b]	6.67± 0.67 [b]

Mean values in each column having different superscript (a, b, c) denote significant difference.

A significant ($p<0.05$) increase in alkaline phosphotase enzymes (Alk- Pho) and total bilirubin were observed in positive control group (untreated+v) compared with normal rats. Treatment groups with avocado fruit powder or extract and mixture form (5g and 0.5ml) showed a significant decrease in serum alkaline phosphotase enzymes (Alk–Pho.) and total bilirubin levels in a dose dependent manner with respect to positive control group (+v) towards normalization and close to the normal control group ,Table 4.

Whereas, a significant decrease in the total protein level was found in the positive control group(+v) compared to normal control group(-v). These levels were found to be restored in the treated group with avocado fruit mixture group at (5g and 0.5ml) and avocado fruit extract treated group in a concentration dependent manner with 0.5 ml/kg to be the most effective concentration ,Table 4. These results are in consistence with (Parameshappa et al., 2012) impairment of the hepatic function by paracetamol. The Polyphenolic compounds and flavonoids present in avocado fruit as antioxidant and inflammatory or necrobiotic damage to the hepatocytes.

Effect of treatment with avocado on the activities of antioxidants parametes in serum liver and of rats received paracetamol:

The result of total activity antioxidant at Table 5, it can be observed that paracetamol poisoning induced depletion in the enzyme activity from 4.55 mmol/L in normal control group (-v) to 1.67 mmol/L in positive control group (+v). This may be explained as a body defense mechanism

against paracetamol -induced oxidative stress, increased total antioxidant capacity as compared to positive control group (+v) which given paracetamol only. These findings are consistent with those obtained by (**Umoh, 2011 and Sharma and Singh, 2012**).

Table (5): Mean values ± SD of serum liver Total antioxidants, superoxide dismutase (SOD) enzymes and nitric oxide (NO) of the experimental rats groups

Groups / Variables	Normal control (-v)	Positive control (+v)	*Treated with avocado fruit*		
			Powder	Extract	Mixture
Total antioxidants mmol/L	4.55± 0.22 a	1.67± 0.15 c	3.32± 0.15 b	3.94± 0.06 b	4.02± 0.06 b
Superoxide dismutase U/Ml	65.13± 5.22 a	21.25± 3.47d	54.14± 7.16 c	59.87± 6.35 b	64.29± 5.23 a
NO (µmol /l)	2.87± 0.33 ^{d}	13.99± 1.44 ^{a}	4.33± 1.11 ^{b}	3.22± 1.03 ^{c}	3.01± 1.05 ^{c}

Mean values in each column having different superscript (a, b, c) denote significant difference.

Hence, non-significant difference of SOD was observed after treatment of rats with avocado fruit mixture group (5g and 0.5ml) as compared to normal control group (-v), therefore increase in the level of SOD in treated group avocado fruit mixture to 64.29 U/mL compared to positive control (+v) 21.25 U/mL. The results was in agreement with previous work by (**Jaeschke *et al.*, 2000**) who observed drug toxicity can also induce an inflammatory response with the formation of reactive oxygen species by Kupffer cells and neutrophils. Table 5, showed the concentration of NO in plasma of all groups. NO is a marker of nitric oxide, after 5 consecutive weeks significant increase in MDA concentration was observed in intoxicated group +v (13.99 umol/L) compared to normal control group-v (2.87 umol/L). Hence, the MDA concentration decreased from 4.33 umol/L for intoxicated group to 3.01 mmol/L in treated group with avocado fruit mixture group. A significant reduction of MDA in groups treated with avocado fruit powder or extract

and avocado fruit mixture group comparatively to intoxicated group+v (paracetamol alone). This finding is consistent with (**Sabina *et al.*, 2011**). Avocado fruit, prevents the production of free radicals, neutralizes, and scavenges free radicals produced in the body. Saponins, tannins and glycoside are the major bioactive flavonoids present in avocado fruit, they suppress the accumulation of reactive oxygen and nitrogen species in the cells.

Conclusion:

The results have shown that, avocado fruit can improve the likelihood of subsequent hepatic changes following administration of high dose of paracetamol. It can ameliorate biochemical hepatic alterations. Avocado hepatic protection, in major part, caused by decreasing oxidative stress and by increasing total antioxidant capacity. The therapeutic efficacy of avocado fruit was of powder and extract. From this point of view, combination regimens containing suitable doses of avocado fruit and paracetamol could be advantageous, particularly in treating patients who are susceptible to liver function disorders or when high doses of paracetamol.

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التأثير الوقائي لفاكهة الأفوكادو (مسحوق ومستخلص) ضد الإصابة الكبدية في فئران التجارب

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الملخص

الغرض من هذه الدراسة التعرف على فعالية التأثير الوقائي لفاكهة الأفوكادو مسحوق ومستخلص ضد الإصابة الكبدية الناجمة من تناول عقار الباراسيتامول في فئران التجارب. وقد استخدم فى هذه التجربة أربعون فأرا من نوع سبراغ داولي أوزانهم (115جم ± 10) كل مجموعة تتكون من عدد 8 فأر، و تم تقسيم الفئران إلى خمس مجموعات: المجموعة الأولى: الضابطة السالبة (v–) والمجموعة الثانية : الضابطة الموجبة (v+) التى تناولت عقار الباراسيتامول بجرعة واحدة (2 جم / كجم من وزن الفار) لإحداث الإصابة الكبدية، أما المجموعات الثلاث الأخرى تمت وقايتهم بالمعالجة بمسحوق فاكهة الافوكاد وبنسبة 5 جم/كجم/الوجبة/يوم، ومستخلص 0.5 ملجم/كجم من وزن الفأر/يوم, بواسطة الأنبوبة المعدية, وخليط من مسحوق ومستخلص فاكهة الأفوكادو، وأظهرت النتائج إن كل المجموعات المعالجة: أظهرت زيادة ملحوظة في وزن الجسم, وفي مستوى كل من (اللبيوبروتينات عالية الكثافية HLDL–C – البروتين الكلي– مضادة للأكسدة – SOD). وفى المقابل لوحظ انخفاضا معنويا فى مستوى كل من الكوليسترول الكلى (TC)، الجليسريدات الثلاثية (TG)، اللبيوبروتينات منخفضة الكثافية LDL–C، و VLDL–C، وإنزيمات الكبد AST، ALT، والإنزيمات القلوية (Alk), والبيليروبين الكلي وأكسيد النيتريك (NO) بالمقارنة مع المجموعة الضابطة الموجبة (v +) ، ولذا توصى الدراسة بتناول فاكهة الافوكادو فى صورة مسحوق أو مستخلص لاحتوائه على مركبات كيميائية فعالة كالتانينات tannins والسابونين saponins والجليكوسيدات glycoside, والفلافونيدات والكربوهيدرات ذات التأثيرالوقائي والمضاد للأكسدة الذى قلل من حدة الإصابة الكبدية الناجمة من تناول عقار الباراسيتامول في فئران التجارب.