Udom *et al.*, ***Journal of Herbal Drugs***, Vol. 8, No. 4: 219-225, 2018
doi: 10.14196/JHD. 2018. 219

Journal of Herbal Drug
journal homepage:www.jhd.iaushk.ac.ir

Hepatoprotective Properties of Ethanol Seed Extract of Citrus paradisi Macfad (Grape Fruit) Against Paracetamol-Induced Hepatotoxicity in Wistar Rats

Godswill J. Udom[1, 2*], Omoniyi K. Yemitan[3], Emem E. Umoh[1], H. O. C. Mbagwu[1], Ekemini E. Ukpe[1], Paul S. Thomas[4]

[1]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria;*
Email:udomgodswill@gmail.com*

[2]*Gimmex Health Consult, Suites B6 Real Towers Complex, 26 Ekukinam Street, Utako District, Abuja, Nigeria;*

[3]*Department of Pharmacology, Therapeutics and Toxicology, Lagos State University College of Medicine, Lagos, Nigeria;*

[4]*Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria;*

ARTICLE INFO

Type: *Original Research*
Topic: *Medicinal Plants*
Received *May 15th 2017*
Accepted *February20th 2018*

Key words:
- ✓ *Citrus paradisi*
- ✓ *Hepatocytes*
- ✓ *Paracetamol-induced Hepatotoxicity*
- ✓ *Hepatoprotection*

ABSTRACT

Background & Aim: The human body has the liver as one of its largest organs. It serves as the major site for metabolism and excretion. Injury to the liver or impairment of its functions may complicate one's health and therefore, constitutes one of the serious public health challenges. The ethanol seed extract of Citrus paradisi Macfad (CPE) was carried out to evaluate its protective usefulness on the liver against paracematol-induced liver injury.

Experimental: Thirty adult male Wistar rats were randomly allotted to five groups (6/group) and orally-treated daily with 100 mg/kg body weight of silymarin (positive Control), 10 ml/kg body weight of distilled water (negative control) and CPE (200, 400 and 600 mg/kg) body weight, respectively for 7 days. On the eighth day, all groups were administered 2 g/kg body weight of paracetamol. 24 h thereafter, animals were sacrificed under diethyl ether anesthesia and blood samples were collected by cardiac puncture for biochemical and haematological investigations.

Results: Compared to the negative control, extract (200 – 600 mg/kg) significantly ($p<0.05$) reduced the activities of ALP, ALT and AST dose-dependently. Extract significantly ($p<0.05$) elevated all blood parameters except for neutrophil differentials.

Recommended applications/industries: Grapefruit seed extract possesses hepatoprotective potential and can be used as an antidote against paracetamol-induced hepatotoxicity.

1. Introduction

The human body has the liver as one of its largest organs. It serves as the major site for metabolism and excretion (Ahsan *et al.*, 2009). The liver functions effectively in the detoxification as well as the excretion of many endogenous and exogenous substances. Thus, injury to the liver or impairment thereof its functions

may complicate one's health constituting one of the serious public health challenges. Liver damage is often presented with distortion of these metabolic functions as well as depletion of reduced glutathione levels, increase in tissue lipid peroxidation and optimal cellular necrosis. Additionally, serum levels of some biomarkers like alkaline phosphatase, transaminases, triglycerides, cholesterol and bilirubin are usually increased in liver disease (Subramaniam *et al.*, 2015). The liver plays a key role in transforming and clearing chemicals and is highly susceptible to the toxic effects of these agents. Certain medicinal compounds, when in overdose and sometimes even within therapeutic ranges, may injure the organ. Drug-induced liver injury is one of the main reasons behind the withdrawal of drugs from the market.

The plant *Citrus paradisi* Macfad (family: Rutaceae) is an important member of *Citrus* genus. It is a subtropical citrus tree originating from the Barbados (Carrington *et al.*, 2003). In many countries, various parts of the plant are employed for medicinal uses (Gupta *et al.*, 2011). It has been used as antiviral, antibacterial, anti-inflammatory, antifungal, antimicrobial, astringent, antioxidant, and preservative agent. The seed extract has been investigated for haematopoietic activities (Adeneye, 2008). Furthermore, some of the other traditional uses of the plant include prevention of cancer, immune boosting functions (Ionescu *et al.*, 1990), lupus nephritis, rheumatoid arthritis, cellular regeneration, lowering of cholesterol, detoxification, heart health maintenance and weight loss (Gupta *et al.*, 2013).

Studies have shown a number of plants to possess hepatoprotective properties especially via an improved antioxidant status. Traditional or herbal medicines are effective in the management of certain disorders and are based on the use of medicinal plant wholly, and/or their derivatives in the amelioration of common illnesses. Therapeutic options for common liver diseases are very limited, and there may be a lack in efficacy with modern medicine. Besides being expensive, the efficacy of treatments of liver conditions with corticosteroids and interferons are often inconsistent, with the risk of adverse events (Stickel and Schuppan, 2007). More recently, following toxicological reports on some synthetic antioxidants, herbal remedies rich in natural antioxidants have gained considerable attention (Ramalakshmi *et al.*, 2007). Therefore, it is imperative that effective therapeutic agents for the treatment of liver diseases, but with low toxicity profile be developed. The present study assayed the antidotal properties of ethanol seed extract of *Citrus paradisi* Macfad against paracetamol-induced liver injury.

2. Materials and Methods

2.1. Plant Material

A fresh part (a branch with flowers) of *Citrusparadisi* was collected from the farmland of the Department of Agriculture, Use-offot, University of Uyo, for botanical identification. The plant was identified and authenticated as *Citrusparadisi* Macfad by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Nigeria. The voucher specimen for the sample is conserved at the Faculty of Pharmacy Herbarium under the reference number DPTU 33.

2.2. Extraction

About 200 ripe grapefruits were harvested from the farm, washed and cut so as to separate their seeds. The seeds were thoroughly but gently rinsed with distilled water. The seeds were shade-dried at room temperature (28 ± 2 ^{0}C) for 2 weeks, protected from direct sunlight. The dried seeds were then pulverized using a laboratory hand mill.

About 580 g of the pulverized sample of *C. paradisi* seeds was macerated in 70 % ethanol (Sigma, USA) for 72 hours. It was vigorously shaken for 15 minutes prior to its rapid filtration using Whatman filter paper. The filtrate was concentrated *in vacuo* using rotary evaporator and evaporated to dryness in a water bath (Griffin, Britain) regulated at 40 ^{0}C until a yellow-to-brown aromatic/oily residue was obtained. The residue was allowed to cool and was stored in a tight cap-fitted container in a refrigerator set at - 4 ^{0}C. The extraction was repeated once more and the percentage yield of the extract was calculated [35.07 ± 1.2 % ($^W/_W$)].

2.3. Phytochemical Screening

Phytochemical screening of the crude extract was done using standard procedures and tests (Trease and Evans, 1989; Sofowora, 1993), to reveal the presence of phytochemical constituents like flavonoids,

monoterpenes, alkaloids, terpenes, tannins, cardiac glycosides, carbohydrate and fixed oil/fats.

2.4. Experimental Animals

The animals (male Wistar albino rats and Swiss albino mice) used for this study were obtained from and kept at the Department of Pharmacology and Toxicology Animal House of the University of Uyo, Uyo, Nigeria. The animals were maintained under standard environmental conditions, being fed with standard rodent feed obtained from Livestock Feeds, Nigeria Ltd. Food and water was given water *adlibitum*. All animals were kept at room temperature in cross-ventilated rooms, without illumination at night to achieve 12 h light/ 12 h dark period.

The animals were acclimatized to the laboratory condition for at least 7 days prior to the experiment, during which they were given free access to food and water *ad libitum*. The care and use of animals was conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH, 1996). Moreover, Ethical approval for animal use was obtained from the Experimental Ethics Committee on Animal Use of the College of Health Sciences, University of Uyo, Uyo, Nigeria.

2.5. Acute Toxicity Test

The median lethal dose (LD_{50}) was determined using the limit dose test of the Up-and-Down Method – statistical programme AOT425StatPPgm, also known as OECD Test Guideline 425 (OECD, 2001). This method allows the dosing of one animal at a time, observation for signs of toxicity within 24 h and further supervision for a period of 14 days for occurrence of toxic symptoms and mortality (OECD, 2001).

2.6. Biochemical Analysis

Using a centrifuge (Nikon optical Co., Japan), whole blood of each sacrificed rat collected through cardiac puncture into different plain sample bottles was centrifuged at 4000 rpm for 10 min at 10 ^{0}C to separate the serum. The activity of serum alkaline phosphatase (ALP) was determined at 405 nm using a standard method (Bessey *et al.*, 1946), serum alanine aminotransferase (ALT) and aspartate amino transferase (AST) were determined at 340 nm (Reitman and Frankel, 1957). Also, serum levels of sodium (Na) and chloride (Cl) ions were measured. These determinations were done spectrophotometrically at Effective Medical Laboratory, Uyo using Randox analytical kits according to standard procedures of manufacturer's protocols.

2.7. Haematological Analysis

Blood samples were collected through cardiac puncture from each diethyl ether anaesthetized/sacrificed rat using 21 gauge (21G) needles mounted on a 5 ml syringe (Hindustan Syringes and Medical Devices Ltd., Faridabad, India) into different Ethylene Diamine Tetra-acetic Acid (EDTA)-coated sample bottles.

The blood samples were analyzed for red blood cells (RBC) count, haemoglobin (Hb), haematocrit or packed cell volume (PCV), white blood cells (WBC) and differential WBC. These parameters were analyzed using automated Haematology analyser according to manufacturer's protocols (Sysmex Haematology-Coagulation Systems®, Model KX-21N, Sysmex Incorporation, Kobe, Japan) at Effective Medical Laboratory, Uyo.

2.8. Statistical Analysis

Data obtained from the study were statistically analyzed using SPSS statistical package (version 17.00). Statistical significance between the groups was analyzed by means of two-way analysis of variance (ANOVA) followed by Fisher's *post-hoc* PLSD multiple comparison tests. Results are presented as Mean ± S.E.M. *P* values less than 0.05 ($p<0.05$) were considered significant.

3. Results and discussion

3.1. Phytochemical Screening

From the phytochemical screening, the extract tested positive to alkaloids, tannins, saponins, flavonoids, polyphenols, terpenoids, carbohydrate, cardiac glycosides as well as fixed oil and fat (Table 1).

3.2. Acute Toxicity Test

From the preliminary limit dose test of 2000 mg/kg of the Up-and-Down (OECD 425) procedure of the seed extract, no death was recorded among the five sequentially treated animals. Also, 3800 mg/kg body weight of extract was well tolerated in mice even after 72 h post administration. However, at a high dose of

4000 mg/kg, mortality was recorded among another set of five sequentially treated animals (Table 2). The LD_{50} values were estimated to be as follows: > 2000 mg/kg (p.o); < 4000 mg/kg (i.p).

Table 1. Result of the phytochemical screening of ethanol seed extract of Citrus paradisi.

S/n	Tests	Inference
1	Alkaloids	+++
2	Tannins	+++
4	Flavonoids	+++
5	Terpenoids	+++
6	Polyphenols	+++
7	Cardiac Glycosides	++
8	Carbohydrates	+++
9	Fixed oil and fat	+++
10	Free and combined anthraquinones	-

KEY:+Trace, ++ Positive, +++Strongly positive, - Negative

Table 2. Result of the up-and-down procedure of acute toxicity testing in mice.

Test Sequence	Animal ID.	Weight (Kg)	Doses (mg/kg)	Short term result (24 hours)	Long term result (14 days)
1	T_1	0.023	2000	O	O
2	T_2	0.026	2000	O	O
3	T_3	0.024	2000	O	O
4	T_4	0.023	2000	O	O
5	T_5	0.023	2000	O	O
6	T_6	0.024	3000	O	O
7	T_7	0.026	3200	O	O
8	T_8	0.021	3400	O	O
9	T_9	0.025	3800	O	O
10	T_{10}	0.023	4000	X	-

**O = survival; X = death.* Sequence 1 – 5 received the test dose orally while 6 – 10 received the test dose intraperitoneally.

3.3. Effect of Extract on Biochemical Parameters

The three extract-treated groups showed significant ($p<0.005$) reduction in AST, ALT and ALP levels when compared with the negative control. There was no significant ($p>0.005$) difference in serum electrolytes levels measured (Table 3).

3.4. Effect of Extract on Haematological Indices

Results showed that oral treatment with graded doses of ethanol extract of *Citrus paradisi* significantly ($p<0.05$) increased red blood cell count (RBC), white blood cell count (WBC), haematocrit (PCV), haemoglobin (Hb), mean cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and lymphocytes differential dose-dependently compared to the negative control. However, a reversed effect was recorded for neutrophil differentials (Table 4).

Table 3. Effects of Extract on Liver Enzymes and Electrolytes

Parameters	AST (U/L)	ALT (U/L)	ALP (U/L)	Na^+(mmol/L)	Cl^- (mmol/L)
Silymarin+PCM	105±4.16	83.7±2.08	122±3.44	103±25.7	70.1±19.1
Distilled H_2O+PCM*	138±5.51	150±5.12	357±10.1	127±11.1	87.1±8.11
200mg/kg CPE+PCM	124±2.90[b]	139±0.59[b]	216±11.6[b]	145±1.45	101±0.99
400mg/kg CPE+PCM	119±1.39[b]	121±0.73[b]	193±4.05[b]	147±0.62	103±0.75
600mg/kg CPE+PCM	111±1.76[b]	110±2.78[b]	147±1.57[b]	148±0.85	102±0.32

Result presented as Mean ± S.E.M;[b]represent significant decreases at P <0.05 respectively, when compared to control values; *represent negative control, PCM = Paracematol, CPE = Citrus paradisi extract; n = 6

Table4. Effect of ethanol seed extract of Citrus paradisiMacfadon blood parameters of Wistar rats

Treatment	Neutrophils	Lymphocytes	WBC ($\times10^9$/L)	PCV (%)	Haemoglobin (g/dL)	RBC ($\times10^6$/µL)	MCHC (g/dL)	MCV (fL)
Silymarin+PCM	41.0±1.65	62.8±2.10	9.6±1.38	43.9±1.41	11.4±0.43	5.77±0.17	29.1±0.58	58.5±0.85
Distilled H_2O+PCM*	42.8±4.10	64.3±3.09	10.8±2.35	45.2±1.01	13.0±0.23	7.40±0.16	29.0±0.26	66.9±1.55
200mg/kg CPE+PCM	40.5±0.65	72.0±1.68[a]	12.34±1.35	48.3±0.66	17.0±0.39[a]	8.02±0.05	30.8±0.49	70.8±0.84[a]
400mg/kg CPE+PCM	34.8±1.55[b]	75.8±1.31[a]	14.0±3.09[a]	54.5±0.83[a]	20.8±0.33[a]	9.18±0.19[a]	33.7±1.26[a]	77.4±0.88[a]
600mg/kg CPE+PCM	28.8±3.68[b]	80.0±3.19[a]	18.78±1.95[a]	67.3±1.29[a]	25.1±1.07[a]	10.4±1.17[a]	38.5±0.20[a]	82.2±0.95[a]

Result presented as Mean ± S.E.M;a represent significant increases at p<0.05, while brepresent significant decreases at p<0.05 respectively, when compared to control values; *represent negative control, PCM = Paracematol, CPE = Citrus paradisi extract; n = 6

The ethanol seed extract of *Citrus paradisi* Macfad presented acute toxic effects with LD_{50}> 2000 mg/kg (p.o) and < 4000 mg/kg (i.p). This suggests that the extract may be relatively safe on acute oral exposure.

Paracetamol readily induces hepatotoxicity through the formation of reactive oxygen species like hydrogen peroxide, superoxide anion, hydroxyl radical as well as reactive nitrogen species like peroxynitrite and nitric oxide (James *et al.*, 2003; Reid *et al.*, 2005; Bessems and Vermeulen, 2011). Paracetamol is metabolized in the liver by hepatic phase II drug metabolizing systems via glucuronidation, sulphation and conjugation (Henderson *et al.*, 2000). However, a small portion of paracetamol biotransformation is mediated by cytochrome P-450 system to a highly reactive electrophilic intermediate called N-acetyl-p-benzoquinone imine (NAPQI) (Dahlin *et al.*, 1984). NAPQI owing to its highly reactive nature generates reactive oxygen species (ROS), depletes the body's antioxidant system, thereby inducing oxidative stress (OS) and cellular injuries. Paracetamol-induced hepatotoxicity is manifested biochemically by significant increase in the serum levels of non-specific biomarkers such as ALP, ALT and AST. The measurement of enzyme activity is particularly important as it gives insight to the site of cellular damage following assault by drugs or other chemicals. Drug-induced (paracetamol and other hepatotoxins) liver damage is often assessed by the determination of enzyme levels such as ALT and AST (Dobbs *et al.*, 2003). These enzymes are predominantly found in high concentrations in the liver. In the event of liver damage, these non-specific biomarkers leaks into blood. Therefore, the high concentration of these biomarkers observed in the serum of the control (negative) group is interpreted to be suggestive of a

possible damage to the liver. The significant reduction of these said biomarkers in the three (3) extract–treated groups is indicative of the hepatoprotective potentials of grapefruit seed extract. This observed effect is attributed to the extract's phytochemical constituents especially as the plant extract is known to have antioxidant properties. The significant increase in haematological parameters suggests that grapefruit seed extract contains active biological principle(s) that stimulates the synthesis or release of haematopoeitins (erythropoietin, thrombopoetin). The haematopoietic effect observed in this study is therefore attributed to the said active biological principle(s). As reported by Marcus and Coulston (2001), grapefruit is a known natural source of ascorbic acid. Nonetheless, ascorbic acid (vitamin C) is very necessary for body tissue formation and maintenance (Osilesi *et al*., 1997). The result of this study corroborate with that of earlier studies done on the haematopoietic effect of grapefruit seed extract (Adeneye, 2008). Following this observed effect, grapefruit seed extract may be exploited in the phytotherapeutic management of certain types of blood deficiencies.

4. Conclusion

From the findings of this study, it can be said that grapefruit seeds extract possess hepatoprotective property as well as haematinic properties. Therefore, it may be exploited as an adjuvant therapy in the management of paracetamol-induced liver injury and blood deficiencies.

5. Acknowledgements

The authors are very grateful to Mr. Nsikan Malachy Udo of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria, for his technical assistance.

6. References

Adeneye, A. A. 2008. Hematopoetic effect of methanol seed extract of *Citrus paradisi* Macfad (grapefruit) in wistar rats. *Biomedical Research*, 19(1): 23-26.

Ahsan, M. R., Islam, K. M. and Bulbul, I. J. 2009. Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. *Global Journal of Pharmacology*, 3:116-122.

Bessems, J.G.M. and Vermeulen, N.P.E. 2001. Paracetamol (acetaminophen)-induced toxicity: Molecular and Biochemical Mechanisms, Analogues and Protective Approaches. *Critical Reviews in Toxicology*, 31: 55-138.

Bessey, O. A., Lowry, O. H. and Brock, M. J. 1946. A method for the rapid determination of alkaline phosphate with few cubic millimeters of serum. *Journal of Biological Chemistry*, 164:321-329.

Carrington, S., Fraser, and Henry, C. 2003. "Grapefruit". A~Z of Barbados Heritage. Macmillan Caribbean. pp. 90–91.

Dahlin, D. C., Miwa, G. T., Lu, A.Y. and Nelson, S. D. 1984. N-acetyl-p-benzoquinone imine: A cytochrome P-450 mediated oxidation product of acetaminophen. Proceedings of the National Academy of Science, USA. 81: 1327–1331.

Dobbs, N. A., Twelves, C. J., Gregory, W., Cruickshanka, C., Richards, M. A. and Rubens, R. D. 2003. Epirubicin in patients with liver dysfunction. Development and evaluation of a novel dose modification scheme. *European Journal of Cancer*, 39:580-586.

Gupta, V., Kohli, K., Ghaiye, P., Bansal, P. and Lather, A. 2011. Pharmacological potentials of citrus paradisi- an overview. *International Journal of Phytotherapy Research*, 1(1): 8-17.

Gupta, M., Dalia, B. and Arup, M. 2013. Studies of anti-inflammatory, anti-pyretic and analgesic effects of aqueous extract of traditional herbal drug on rodents. *International Research Journal of Pharmacy*, 4(3): 113-120.

Henderson, C. J., Wolf, C. R., Kitteringham, N., Powell, H., Otto, D. and Park, B. K. 2000. Increased resistance to acetaminophen hepatotoxicity in mice lacking glutathione S-transferase. Proceedings of the National. *Academy of Science*, USA. 97: 12741–12745.

Ionescu, G., Kiehl, R., Wichmann-Kunz, F., Williams, C., Ba, L. and Levine, S. 1990. Oral Citrus Seed Extract in Atopic Eczema: *In Vitro* and *In Vivo* Studies on Intestinal Microflora. *Journal of Orthomolecular Medicine*, 5(3): 155–157.

James, L. P., Mayeux, P. R. and Hinson, J.A. 2003. "Acetaminophen-induced Hepatotoxicity". *Drug Metabolism and Disposition*, 31(12): 1499–1506.

Marcus, R. and Coulston, A.M. 2001. Water-soluble vitamins: the Vitamin B Complex and Ascorbic Acid. In: Goodman and Gilman's, The

Pharmacological Basis of Therapeutics (Hardman JG and Limbird LE eds.). 10th ed., New York: McGraw-Hill Medical Publishing Division, pp. 1753-1791.

NIH [National Institutes of Health].1996. Revised Guide for the Care and Use of Laboratory Animals. In: NIH Guide, 25: 28.

OECD. 2001. Acute Oral Toxicity (AOT, Test Guideline 425) Statistical Programme (AOT425StatPPgm), version 1.0. Available online at http://www. oecd. org/ OECD/ pages/home/displaygeneral/ 0,3380, EN-document-524-nodirectorate-no-24-6775-8,FF.html.

Osilesi, O., Adeiyi, A., Ogunyemi, E. O. and Fakunle, J. B. 1997. The glycaemic response to selected fruits and vegetables in Nigerian diabetics. *African Journal of Medicine and Pharmaceutical Sciences*, 1: 1-6

Ramalakshmi, K., Rahath, K.I. and Rao, L.J. 2007. Antioxidant potential of low-grade coffee beans. *Journal of Food Science*, 41: 96–103.

Reid, A. B., Kurten, R. C., McCullough, S. S, Brock, R. W. and Hinson, J. A. 2005. Mechanisms of Acetaminophen induced hepatotoxicity: Role of Oxidative Stress and Mitochondrial Permeability Transition in freshly Isolated Muse Hepatocytes. *Journal of Pharmacology and Experimental Therapeutics*, 312:509-516.

Reitman, S. and Frankel, S. 1957. Glutamic-pryuvate Transaminase Assay by Colorimetric Method. *American Journal of Clinical Pathology*, 28:56.

Sofowora, A. 1993. Medicinal plants and Traditional Medicine in Africa. 2nd ed., Ibadan, Spectrum Ltd., p.152.

Stickel, F. and Schuppan, D. 2007. Herbal Medicine in the Treatment of Liver Diseases. *Digestive and Liver Disease*, 39: 293–304.

Subramaniam, S., Khan, H. B. H., Elumalai, N. and Lakshmi S. Y. S. 2015. Hepatoprotective Effect of Ethanolic Extract of Whole Plant of Andrographis paniculata against CCl4–induced Hepatotoxicity in Rats. *Comparative Clinical Pathology*, 24: 1–7.

Trease, G.E. and Evans, W.C. 1989. A Textbook of Pharmacognosy. 11th ed., London: Balliere Tindal and Cox, p.475.