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Protective effects of alkaloids of *Cucumis metuliferus* isolated from the fruit pulp on some vital organs

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Abstract

The protective effects of the alkaloids of *Cucumis metuliferus* fruit pulp on carbon tetrachloride-induced hepatotoxicity and gentamicin induced nephrotoxicity in adult albino rats were investigated. The result showed a significant ($P<0.05$) decrease in the levels of alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST) in CCL_4 induced hepatotoxicity in rats. There was a significant ($P<0.05$) increase in the levels of the electrolytes (Na^+, K^+) which were dose dependent in gentamicin induced nephrotoxic rats compared to the controls. The dose dependent decrease in the level of urea was statistically significant ($P<0.05$), and there was a significant decrease in the levels of creatinine when 200 mg/kg of the alkaloid was administered to the rats alone. This result showed that alkaloids of C.metuliferus have protective effects on both the liver and kidney tissues.

Keywords: Alkaloids, *C. metuliferus*, Liver, kidney, Cabon tetrachloride, Gentamicin.

Introduction

Cucumis metuliferus E Meye belongs to the gourd family Curcubitaceae, which includes hundreds of vines bearing coiled, climbing tendrils.[1] It is monocious, annual herb with staminate flowers, it flowers and fruits from July to September and the fruits ripened from October to December.[2] The fruits have spiny thorns and are ovoid in shape, about 8-10cm long and 4-5cm in diameter, reddish-orange or yellow at maturity. The emerald green seeds are embedded in the mesocarp.[3] Phytochemical investigation of *C. metuliferus* fruit extract reveals presence of alkaloids, flavonoids, and cardiac glycosides in high concentration.[4]

The medicinal properties of *C. metuliferus* fruits have been widely reported[5]; in plateau state of Nigeria, it is generally believed that the fruit pulp is a remedy to all diseases, hence its local name “kanda” or ‘a local vaccine’.[6, 7]

Alkaloids are a group of naturally occurring chemical compounds that contain basic nitrogen atoms, with a wide range of pharmacological activities.[8, 9] The presence of various phytochemicals in a plant account for the diverse medicinal activities of that plant, hence the need to focus on the pharmacological activities of the phytochemicals present in this plant. This study aimed at investigating the protective effects of the alkaloids of *C. metuliferus*fruit pulp on the liver and kidney tissues integrity of albino rats.

Materials and Methods

Plant collection and authentication

The ripe fruit of *C. metuliferus* were harvested from Chong'Openg village of Jos south Local Government Area of Plateau State, Nigeria. The plant was identified by Professor C. O. Akueshi of the department of plant science of the University of Jos, Nigeria; further authentication was done by matching features and description obtained from the internet with those of the sample obtained.

Preparation of *C. metuliferus*

The mesocarp content of the ripened fruit of *C. metuliferus*was carefully scooped out from the pericarp with the aid of a spatula. The fleshy content was blended using electric blender and the fluidy product of blending was passed through a sieve size of 0.25mm to separate the seeds from the juicy contents. The smooth filtrate was evenly spread on an aluminium tray and dries in a drying cabinet, at about 55 ^{o}C until the liquid content had been evaporated. The resultant product was air dried for several hours and then pounded to powder using mortar and pestle and appropriately stored in an air-tight container.

Extraction of Alkaloids of the *C. metuliferus* Fruit Pulp

The alkaloids from *C. metuliferus* fruit pulp were isolated according to the method described by Agrawal and Paridhavi.[10] The pure alkaloid was stored in an air-tight container at room temperature prior to use.

Test Animals

Adult albino rats (wistar strain) bred in the animal house of the University of Jos, Nigeria, were used in the study. Proper handling and using of the animals were in accordance with the guidelines regulations, monitored and approved by the ethical committee on Animal use of the department of Pharmacology, University of Jos, Nigeria.

Effect of Alkaloids of *C. metuliferus* on Carbon Tetrachloride-Induced Hepatotoxicity

Thirty wistar strain albino rats were randomly divided into six groups of five animals each and were administered liquid paraffin (prepared in the ratio of 1ml of liquid paraffin in 2 ml of normal saline, ie 1:2v/v) on alternate days subscutaneously for seven days. CCL_4 was co-administered with liquid paraffin and treated as follows: Group 1 (control animals) were administered liquid paraffin 1ml/kg sc; group 2 were administered CCL_4 in liquid paraffin (1ml/kg sc); group 3

(Standard control) were administered phosphatydylcholine 1ml/kg daily p.o. and CCL_4 in liquid paraffin (1 ml/kg s.c.) on alternate days. Group 4, 5, and 6 were respectively administered 50, 100 and 200 mg/kg of the extract daily p.o.for seven days and liquid paraffin (1 ml/kg s.c.) on alternate days.[11]

Twenty four hours after the last administration, the animals were made unconscious using petroleum ether, and blood samples were collected through cardiac puncture using a sterile syringe for serum preparation. The collected blood was centrifuged for 10 minutes at 100 rpm to obtain the serum. Biochemical tests for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total protein were determined using Rantox test kits.

Effect of Alkaloids of *C. metuliferus* on Gentamicin-Induced Nephrototoxicity

Thirty animals were randomly divided into six groups of five animals each. The method according to Mitchell and Kline[12] was adopted and used in this study. The dailyurine volumes of the animals were noted, the electrolyte contents of the urine were also analysed. Serum urea and creatinine tests were carried out on the blood sample of the rats which was collected by cardiac puncture at the end of the administrations.

Statistical Analysis

All results were expressed in mean±SEM, and the data were analysed statistically using two way ANOVA and student's t-test and values of $P<0.05$ were considered significant.

Results and Discussion

Liver injury induced by CCL_4 is a common model for screening the hepatoprotective activity of drugs and it's used has been reported since 1936.[13]

The chemical is a potent hepatotoxin and is metabolized by cytochrome P-450 in the endoplasmic reticulum and mitochondria with the formation of CCl_3O^- which is a

reactive oxidative free radical, which initiates lipid peroxidation.[14]

A single exposure can rapidly lead to severe hepatic necrosis and steatosis.[15] When the cell membrane of hepatocytes is damaged, a variety of enzymes, such as SGOT, SGPT, ALP normally located in the cytosol, are released into the blood [16] and these authors further emphasized the widely used of serum transaminase activity as an index of hepatic damage.

This study showed that alkaloid of *C. metuliferus* fruit havehepatoprotective activity; due to the fact that the parameters used in the study were low, indicating decreased in toxicity in the study groups pretreated with 50 mg/kg and 100 mg/kg *C. metuliferus* compare to the control. The decrease in the levels of ALP in the groupprетreated with 50, 100 and 200 mg/kg alkaloid of *C. metuliferus* were statistically significant ($P<0.05$) while those of ALT and AST were only statistically significant in groups pretreated with 200 mg/kg alkaloids (Table 1). The observed dosed- dependent decrease in the levels of enzymes concentrations is suggestive of the hepatoprotective effect of the administered alkaloids in the animals as reported by Abdullah and Al-Assaf.[14]

Table 2 showed a significant ($P<0.05$) increase in the Na^+, K^+ and CL^-ion concentrations in the urine of the rats treated with gentamicin 100 mg/kg plus 50, 100, 200 mg/kg of the alkaloids, suggesting the protective effect of the administered extract; this is similar to previous Studies that have shown nephroprotective effect of herbs on Carbon tetr*a* chloride renal injured rats.[17, 18] Table 3 showed the Urea, Creatinine and Creatinine clearance level in albino rats.

Table 1: Effect of isolated alkaloids of *C. metuliferus* on CCL_4 induced Hepatotoxicity in albino rats

Treatment (mg/kg)	Total protein (g/L)	Albumin (g/L)	ALP (u/L)	ALT (u/L)	AST (u/L)	Total acid phosphatase(u/L)
CCL_4	78.00±0.00	45.00±0.00	276.00±34.00	33.00±6.00	31.00±0.00	10.00±0.00
CCL_4 + Phosphatylcholine	74.00±2.00	45.00±3.00	455.50±16.50	56.50±1.50	64.20±2.00	18.50±1.50
CCL_4 + 50 alkaloids	71.50±4.50*	36.50±9.00*	261.00±25.00	38.00±9.00	46.50±4.50	23.50±2.50
CCL_4 + 100 alkaloids	68.50±4.50	39.00±5.50*	378.50±58.50*	27.00±7.00 *	22.50±4.50	15.50±2.50
CCL_4 + 200 alkaloids	68.50±4.50	45.00±3.00*	174.50±43.50*	26.00±0.50*	8.00±0.50*	6.00±0.50

n = 5, *P<0.05

Table 2: Effect of *C. metuliferus* alkaloid extract on electrolyte excretion on Gentamicin induced Nephrotoxicity in albino rats

Treatment (mg/kg)	Na^+ (mmol/L)	K^+ (mmol/L)	CL^- (mmol/L)	HCO_3^- (mmol/L)
Normal saline	87.00±7.00	77.50±22.00	185.00±15.00	17.50±2.50
Gent 100	145.00± 8.00	42.50±7.00	111.50± 16.50	14.00±1.50
Gent 100 + alkaloid 50	201.50±1.50*	100.50±6.20*	190.00±25.00	20.00±2.00
Gent 100 + alkaloids 100	192.10± 4.00*	102.00±1.00*	210.00±8.60	16.50±4.00
Gent 100+ alkaloids 200	195.50±5.50*	97.50±12.50*	156.50±23.50	15.00±5.00
Alkaloids 200 alone	*230.50±6.00*	110.00± 3.00*	243.50±6.50*	24.50±0.50

n = 5, *P<0.05, Na+ = sodium ion, K+ = potassium ion, CL- = chloride ion, HCO3 = carbonic ion

Table 3: Effect of alkaloids of *C. metuliferus* fruit on Gentamicin induced Nephrotoxicity in albino rats

Treatment (mg/kg)	Urea (mmol/L)	Creatinine (mmol/L)	Creatinine clearance (mmol/L)
Normal	8.04±1.08	84.80±7.70	0.36±0.24
Gent 100	4.42± 2.40	96.80 ±6.18	0.11±0.03
Gent 100 + alkaloid 50	6.74±1.06*	99.80±11.39	0.19±0.03
Gent 100 + alkaloids 100	7.32±0.89*	102.76±8.89	0.24±0.01
Gent 100+ alkaloids 200	7.68±0.46*	91.50±8.22	0.12±0.04
Alkaloids 200 alone	6.18± 0.74*	69.20±3.56	0.12±0.01

n = 5, *P<0.05

Tubular damage and tubular dysfunction are the main cause of renal insufficiency observed in nephrotoxicity of gentamicin which reduced glomerular filtration rate.[19] Studies have also shown that an increase or decrease in the values of Creatinine, urea, uric acid and electrolytes is often indicative of kidney dysfunction,[20] hence the usefulness of these parameters as routine diagnostic markers for renal function.

Conclusion

The present study showed that pretreatment with the alkaloid of *C. metuliferus* fruit suppressed CCl_4 and gentamicin-induced hepatic and nephroticinjury.

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Reference

1. Noel N W, Simeon O, Steven S G, Nanloh S J, Maxwell L D P, Elekwechi I, and Lohya N. Evaluation of the antiviral properties of the ethanolic extract of the fruit pulp of *Cucumis metuliferus* E. Meye (Curcubitaceae). Nigerian Journal of Scientific Research. 2009; 8:55-59.

2. Bates, D.M., Robinson, R.W. and Jeffrey C. Biology and Utilization ofCucurbitaceae. Cornell Publication, 1990. Electronic Version Retrieved June, 2011.

3. Wannang N.N., Jimam S.N., Omale S., Dapar D.L.P., Gyang S.S., Aguiyi J.C. Effects of *C. metuliferus* (Cucurbitaceae) fruits on enzymes and haematological parameters in albino rats. African J. of Biotech. 2007; 6(22):2515-2518.

4. Jimam N.S., Wannang N. N., Anuka J. A., Omale S., Falang K. D., Adolong A. A. Histopathologic Effects of *C. metuliferus* E Mey (Cucurbitaceae) fruits in albino rats. International J of Pharm Science and Research. 2011; 2(8):2190-2194.

5. Chiej R. Encyclopaedia of medicinal plants. 1984, MacDonald ISBNO-356-10541-5.

6. Wannang N.N., Kwanashe H.O., and Ede S. Antiviral activity of the fruit extract of *Cucumis metuliferus* E Meye (Curcubitaceae) in Chicks. African Journal of Basic and Applied Sciences. 2010; 2(3-4):89-93.

7. Shirwaiker A, Sreenivasan KK, Krishnanand BR, Kumar AV. Chemical investigation and anti-hepatotoxic activity of the root bark of Capparisspinosa. Fitoterapia. 1996; 67(3):200-204.

8. Michael, R. and Irwin, Z. Chemistry of herbal medicines - evidence based herbal medicine. Hanley and Belfus, Inc., Philadelphia. 2002; 1:29- 43.

9. Abonyi D.O., Adikwu M.U., Esimone C.O. and Ibezim E.C. Plants as Sources of Antiviral Agents. African Journal of Biotechnology. 2009; 899(17): 3989-3994.

10. Agrawal S.S. and Paridhavi M. Herbal drug technology: extraction, isolation and analysis of phytopharmaceuticals. Universities Press (India) Private Limited, 20007, 354-439.

11. Bacon B.R., O'Glagy J.S. and Dibisceglie A.M. Comprehensive clinical hepatology. Elsevier London, 2005, 11 - 109.

12. Mitchell H.R.,and Kline W. Core curriculum in nephrology, renal function testing. American Journal of Kidney Diseases. 2006; 47:174-183.

13. Nanji AA, Jokelainen K, Foutouhinia M, Rahemtulla A, Thomas P, Tipoe GL. Increased severity of alcoholic liver injury in female rats: role of oxidative stress, endotoxin and chemokines. Am J PhysiolGastrointest Liver Physiol. 2002; 281(6):G1348-56.

14. Abdullah, H and Al-Assaf. Hepatoprotective and antioxidant effect of corosolic acid on carbon tetrachloride induced hepatotoxicity. African Journal of Pharmacy and Pharmacology. 2013; 7(12):673-678.

15. Brautbar N. and Williams J. Industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms, Int. J. Hyg. Environ. Health. 2002; 205:479–491.

16. Varadharajan Madhavan, Anita Murali, Sunkam Yoganarsimhan, and Ajay Shankar Pandey. Protective effect of the roots of CapparissepiariaLinn (Himsra) on carbon tetrachloride-induced hepatotoxicity. Asian Journal of Traditional Medicines, 2012, 7(1): 19 - 28

17. Subal Debnath, Nilesh Babre, Manjunath Y.S., Mallareddy., Pabba Parameshwar and Hariprasath K. Nephroprotective evaluation of ethanolic extract of the seeds of papaya and pumpkin fruit in cisplatin-induced nephrotoxicity. Journal of Pharmaceutical Science and Technology. 2010; 2 (6):241-246.

18. Foote, J., & Cohen, B. Medicinal herb use and the renal patient.Journal of Renal Nutrition. 1998; 8(1):40-42.

19. KacewS, and Bergeron MG. Pathogenic factors in aminoglycoside induced nephrotoxicity. ToxicolLett. 1990; 51: 241–259.

20. Gowda S., Desai B.P., Kulkarni S.S., Hull V., Math A.K. and Vernekar N.S. Markers of renal function tests. North American Journal of medical Sciences. 2010; 2(4):14-17.