International Journal of Science and Technology Research Archive

ISSN: 0799-6632 (Online)

Journal homepage: https://sciresjournals.com/ijstra/

(RESEARCH ARTICLE)

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Effect of leaf extracts of *Carica papaya* (Paw paw) and *Pakia biglobosa* (African locust bean) on some biochemical parameters of albino rats

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International Journal of Science and Technology Research Archive, 2021, 01(02), 014–021

Publication history: Received on 17 July 2021; revised on 08 September 2021; accepted on 10 September 2021

Article DOI: https://doi.org/10.53771/ijstra.2021.1.2.0033

Abstract

In spite of the advances made in orthodox medicine, there has been an increasing interest in herbal medicine. The leaves of *Carica papaya and Pakia biglobosa* have been reported to contain lots of beneficial medicinal compounds, hence their use in the traditional prevention, management and treatment of ailments/diseases. In this study, the effect of varied concentrations of the ethanol leaf extract of the plants on some biochemical parameters of albino rats was assessed. The phytochemical compositions of the leaves were determined using established standard laboratory methods. Fifty four male Albino rats weighing between 150g-200g were randomly distributed into nine groups of six animals each. A daily single dose of 500mg/kg, 1000mg/kg, 1500mg/kg and 2000mg/kg body weight of either of the extracts was respectively, administered to the eight test groups for fourteen days. The control group was given only feed and water. Biochemical parameters such as the serum activities of Aspartate Aminotransferase, Alanine Aminotranferase and Alkaline phosphatase as well as the serum concentrations of Bilirubin, Albumin, HCO_3^-, Urea, Creatinine and Na^+ were assessed. Result of the analyses showed that the administration of the extracts did not significantly raise the serum activity of ALT. The decreases in serum concentrations of Urea and increase in the concentration of HCO_3^- were directly proportional to the concentration of the extracts. It can be deduced from this study, that 500mg, 1000mg 1500mg and 2000mg/kg bw of either *C. papaya* or *P. biglobosa* did not elicit any marked hepatotoxicological or renotoxicological effect on the experimental animals

Keywords: *Carica papaya; Pakia biglobosa;* Aminotransferases; Hepatotoxicological; Renotoxicological; Biochemical parameters

1. Introduction

Toxicology is the study of poisons, or, more comprehensively, the identification and quantification of adverse outcomes associated with exposure to physical agents, chemical substances and other conditions. The fundamental goal of toxicology is to determine safe levels of exposure to potentially poisonous substances for humans and the environment [1]. Plants produce most of the world's oxygen, and are important in the food chain, as many organisms eat plants or eat organisms which eat plants. Plants that possess therapeutic properties or exert beneficial pharmacological effects on the human body are generally designated as medicinal plants. However, most of these plants used as traditional medicines by locals are not subjected to laboratory analysis or do undergo any purification processes apart from the washing and boiling and even extraction process. Dosage forms, side effects and efficacy of most of these medicinal plant preparations are usually not clearly defined, despite the common and frequent use for therapy, based on the belief that

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they are safe because they are natural [2]. Phytochemicals are found commonly in fruits, vegetables, nuts, legumes and grains. Sometimes phytochemials are confused with phytonutrients. Phytochemicals include plant compounds that are beneficial as well as those that are detrimental whereas phytonutrients specifically refers to compounds that have only positive nutritional effect(s).

Paw paw is an evergreen shrub or small tree that grows best in full sun to light shade and has the scientific name of *Carica papaya* linn, [3]. It belongs to the family, Caricaceae, and has local names such as Pepo, Cokia (Tripia), Ptega (Rahaing), Somphula (Khuml), Kanco (Bawan), Eww Ibep (Yoruba), Akpud (calabar), Gwanda (Hausa) [4] The plant, *Parkia biglobosa* is a dicotyledonous angiosperm belonging to the family *Fabaceae* (Caesalpinioideae - Mimosoid clade). It is categorized under spermatophytes, vascular plants [5] and has the common name, African locust bean.

It has been reported [6] that the leaf of papaya contain many active components, including enzymes (Papain, Chymopapain, Caricain, Glycyl endopepetides), alkaloids (Carpaine, Pseudocarpaine, Dehydrocarpaine I and II, Nicotine, Choline, Bispiperidine, Carpinine), Flavonoids (Myricetin, Kaemferol, Quercetin), Phenolic compounds, (Caffeic acid, P-coumaric acid, Chlorogenic acid), Glycoside (Carposide), Cardiac glycoside (Cardenolids), Glucoside, (Cyanogenic glucoside, Benzylglucosinolate), Carotenoids (β-carotene, Lycopene, Lutein, Cryptoxanthin, Violoxanthin, Zeaxanthin), Vitamins (Thiamine, Riboflavin, Niacin, Ascorbic acid and α-tocopherol), Amino acids (Tryptophan, Methionine, Lysine), Electrolytes and Minerals (Ca, P, K, Mg, Zn, Mn, Fe) and other compounds (Cystatin, Saponins, Tannins, Anthraquinolones, Reducing sugars, Steroids). The intake of pharmacological substances by man has solely increased and this may be in the form of food, modern medicines and traditional medicines. These substances are capable of eliciting chronic and acute toxicity, which may be mild or severe, depending upon their nature.

The use of *C. papaya* leaves in the treatment/management of Dengue Fever, malaria, viral infection, Typhoid fever and Cancer [7]. It aids red blood cell production [8] and also ease menstrual pain, [9]. Komolafe [10] reported that *P. biglobosa* leaf did exhibit considerable antioxidant activity and ACE inhibition in vitro and also modulated mitochondrial functions by attenuating toxicant induced ROS generation, with slight mitochondrial membrane depolarization propensity. Leaves of *C. papaya* and *P. biglobosa* have been used for ages for medicinal purposes but their toxicological effects on living system have not been quite elucidated. Dosage forms, side effects and efficacy of most of these medicinal plant preparations has not clearly been defined, despite the common and frequent use for therapy, based on the belief that they are safe because they are natural [2]. However, The local use of these plants and their product in disease remediation is on the rise particularly in areas where access to modern health services is unavailable. Despite the wide and historical use of *C. papaya* in the traditional management of many diseases, the scientific validation of its use for therapy is lacking [11]. Although 5000mg/kg bw of java tea extract has been found to be safe [12], many of the medicinal plants may be toxic at lower concentrations. In order to boost the confidence of people who solely depend on herbal medicines; this research was aimed at determining the phytochemical constituents of the leaf extracts of these plants and their toxicological effect on the animals to which they were administered and give a clue to the determination of the dosage at which these plant leaves will be safe for usage with minimal toxicological effect on the living system.

2. Material and methods

The plant materials used in this study were *Carica papaya* and *Pakia biglobosa* leaves, while the animals used were albino rats.

2.1. Sample collection and preparation

Plant materials were harvested from the botanical garden of the Department of Plant Science and Biotechnology of the Nasarawa State University, Keffi-Nasarawa state, North Central Zone of Nigeria. The plants were identified at the same Department. The leaves were rinsed in water to remove dust and sand particles, and then dried under room temperature for fourteen (14) days. The dried leaves were then grounded into powder using electric blender. Ethanol was used to extract the bioactive ingredients from the leaves.

2.2. Preparation of extracts

Bioactive compounds were extracted by the method of Enemali *et al.* [13]; soaking 500 g of the plant materials in 1liter of absolute ethanol (that is, ratio 1:5; weight to volume) for 24 hours. The extracts were filtered using muslin cloth and then concentrated by freeze drying and stored in airtight containers at 4°C.

2.3. Quantitative phytochemical composition

The saponin and alkaloid contents were determined using the methods of Obadoni and Ochuko [14] and Mlozi *et al.* [15]. The flavonoids and total phenols contents were determined using the method described by Barros *et al.* [16]. The tannin contents of the samples were determined by the method of the AOAC [17].

2.4. Animal models

2.4.1. The LD_{50} Study

The LD_{50} test was an in vivo experiment whereby single doses of the extracts were administered to laboratory albino rats so as to evaluate doses causing deaths. The ethanol leaf extracts of *C. papaya* and *P. biglobosa* of doses 500, 1000, 1500, and 2000 mg/kg per kilogram body weight were administered to rats to determine the LD50 of the extracts within 72 hours with the exception of the control group (group 1) which was not treated with extracts. The same dose was administered to all six animals of each group. The leaf extracts either *C. papaya* or *P. biglobosa* doses 500, 1000, 1500, and 2000 mg/kg per kilogram body weight were administered to eight groups of rats respectively. The lethality was conducted followed by the toxicity assay.

2.4.2. Procurement and preparation of the animals for the study

Fifty-four male Albino rats weighing between 150g-200g were used for the study. These rats were purchased from the animal house of the National Veterinary Research Institute (NVRI), Vom in Plateau state. They were housed in clean, well ventilated metal cages in the animal house of the Department of Biological Sciences (Zoology unit), Nasarawa State University Keffi. The animals were kept under 12-hour light/dark cycling. They were allowed access to unlimited food and water supply and allowed to acclimatize for two (2) weeks before the commencement of the study. All the animals were marked for identification, and their respective weights recorded. The animals were first fed with the chow (feeds) and intubated with the plant material.

2.5. Administration of extracts

The fifty-four male Albino rats were randomly distributed into nine groups of six animals each. A daily single dose of 500mg/kg, 1000mg/kg, 1500mg/kg and 2000mg/kg body weight of either of the extracts was respectively, administered to the eight test groups for fourteen days. The group 1 (the control group) was given only feed and water. Group 2 received 500mg/kg bw of *C. papaya*. Group 3 received 1000mg/kg bw of *C. papaya.* While groups 4 and 5 received 500mg/kg bw and 1000mg/kg bw of *P. biglobosa* respectively, Groups 6 and 7 received 15000mg/kg bw and 2000mg/kg bw of *C, papaya* respectively. Lastly, groups 8 and 9 were respectively given 15000mg/kg bw and 2000mg/kg bw of *P. biglobosa* the administration of extracts to the animals in groups 2 to 9 was in addition to the normal feeding with chow and water. On the fifteenth (15th) day, the animals were fasted for up to seven hours, and sacrificed.

2.6. Animal's sacrifice, collection and preparation of samples

The animals were anaesthetized in an enclosed container and incisions were quickly made into the animals' cervical region with the aid of sterile blades and blood samples collected into plain and heparinized tubes as described by Enemali, *et al.* (2018). Serum was collected as supernatant after centrifuging the clotted blood in a HSC (1000 to 4000rpm) bench centrifuge at 3000 rpm for 10 min. This was kept for further analyses.

2.7. Biochemical analyses

The biochemical analyses were carried out using standard methods. Portions of the concentrated extracts were used for phytochemical screening using standard methods [19, 20, 21, and 22]. Serum biochemical markers of hepatic and renal injury such as creatinine (CRE), urea (UR), Carbonate ion (HCO-3), sodium ion (Na+), chloride ion (Cl-), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin (Alb), and Bilirubin (BIL) were estimated using Biolabo commercial kits (Biolabo S.A., Paris, France) according to the manufacturer's manual and URIT 8021 Automated analyser (URIT Medical Electronic Group Co., Ltd).

2.8. Statistical analysis

Data were expressed as means ± standard deviation (SD). The statistical tools used for the analysis was one way analysis of variance (ANOVA) and the post hoc Newmann Keul's multiple comparisons test. The computer software utilized were Microsoft excel 2016 edition and statistical package for social sciences (SPSS) 16.0 for windows. Differences between means were considered significant at $p<0.05$.

3. Results

The ethanol leaf extracts of *C. papaya* and *P. biglobosa* were found to contain varying types of phytochemicals in varying quantities. Both extracts had the presence of alkaloids, tannins, flavonoids, phenols and saponins with *P. biglobosa* having higher concentrations of the bioactive compounds than the *C. papaya* extract as shown in Table 1.

The administration of 500mg, 1000mg, 1500mg or 2000mg per kilogram body weight of either the ethanol leaf extract of *C. papaya or P. biglobosa* elicited no clinically observable toxicological effect on the experimental rats within the period of 72 hours.

The administration of 500mg/kg bw of extracts of both *C. papaya* and *P. biglobosa* significantly reduced the activity of ALP while that of 1000mg/kg bw of these extracts increased the activity of the enzyme. 1500mg/kg bw of *C. papaya* decreased the activity of ALP and that of *P. biglobosa* significantly increased the activity of the enzyme, while both extracts increased the activity of AST. At a dosage of 2000mg/kg bw, both ethanol leaf extract of *C. papaya* and *P. biglobosa* elicited significant decrease of ALP activity and increase in AST activity. All the doses administered showed either slight increase or slight decrease in the activity of ALT when compared to the control (see Table 3)

Table 1 The phytochemical compositions of ethanol leaf extracts of *C. papaya* and *P. biglobosa*

Parameter	*C. papaya*		*P. biglobosa*	
	Qualitative	**Quantitative (mg/dl)**	**Qualitative**	**Quantitative (mg/dl)**
Alkaloids	+	0.05	+	0.32
Tannins	+	2.32	+	2.10
Flavonoids	+	0.51	+	0.55
Phenols	+	0.06	+	0.14
Saponins	+	9.44	+	10.66

The administration of 500mg/kg bw and 1000mg/kg bw of ethanol leaf extract of *C. papaya* and *P. biglobosa* led to significant decrease in the serum concentration of Urea of the test animals when compared to the control group. 1500mg/kg bw of *C. papaya* also decreased the serum Urea concentrations, significantly ($p < 0.05$). Other parameters (HCO_3^-, Creatinine and Na^+) were mildly affected by the varying doses of the extracts used in this study.

Table 2 Observations recorded for 1-72 hours during the Lethality (LD_{50}) test of *C. papaya* and *P. biglobosa*

Parameters for assessment	Dose and extract administered								
	500mg/kg (*C. papaya*)	500mg/kg (*P.biglobosa*)	1000mg/kg (*C.papaya*)	10000mg/kg (*P.biglobosa*)	1500mg/kg (*C. papaya*)	1500mg/kg (*P. biglobosa*)	2000mg/kg (*C. papaya*)	2000mg/kg (*P. biglobosa*)	Control
Feeding	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Fur condition	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Eye colour	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Convulsion	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Locomotion	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Sedation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mortality	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table 3 Effect of Ethanol leaf Extract of *C. papaya* and *P. biglobosa* on some Liver Function indices of Albino rats

Group	ALP (U/l)	ALT (U/l)	AST (U/l)	BIL (µmol/l)	ALB (g/dl)
Control	214.17±11.28	31.92±6.42	31.83±5.59	6.17±2.97	3.17±0.59
500mg/kg (*C. papaya*)	104.00±21.48*	36.30±9.83	33.47±11.29	4.27±2.55	3.60±0.36
500mg/kg (*P. biglobosa*)	151.00±31.23*	33.06±6.47	30.67±13.64	10.00±7.98	3.60±0.12
1000mg/kg (*C. papaya*)	385.67±99.79**	26.47±8.49	125.50±11.48**	13.10±5.92**	3.67±1.06
1000mg/kg (*P. biglobosa*)	694.00±28.69**	38.85±1.48	33.70±5.23	7.98±2.60	3.30±0.30
1500mg/kg (*C. papaya*)	72.0±19.16*	24.90±7.48	122.93±13.25**	3.30±1.90*	3.90±0.27
1500mg/kg (*P. biglobosa*)	247.00±14.9**	25.33±9.64	70.63±51.16**	5.70±1.65	3.53±0.21
2000mg/kg (*C. papaya*)	67.0±7.88*	25.13±3.32	134.27±10.79**	8.57±1.33	4.00±0.96
2000mg/kg (*P. biglobosa*)	172.33±19*	25.75±8.17	69.60±20.46**	9.57±1.20	4.03±0.50

Values are mean ± SD of six (6) results; values with superscripts ** and * within a column (for the same parameter) are significantly higher and lower respectively, compared to the control.

Table 4 Effect of ethanol leaf extract of *C. papaya* and *P. biglobosa* on some kidney function parameters of Albino Rats

Group	HCO_3^- (mmol/l)	Urea (mg/dl)	Creatinine (µmol/l)	Na^+ (mmol/l)
Control	11.57±5.64	19.45±8.56	39.5±2.75	56.17±8.19
500mg/kg (*C. papaya*)	11.27±5.33	5.27±4.97--	44.70±7.02	52.00±9.17--
500mg/kg (*P. biglobosa*)	14.10±5.45	09.00±3.94--	35.70±3.51	66.33±12.93
1000mg/kg (*C. papaya*)	11.47±5.75	9.01±4.54--	38.20±2.31	66.67±19.83
1000mg/kg (*P. biglobosa*)	9.93±3.20	13.53±2.91--	35.30±3.52	55.33±8.03
1500mg/kg (*C. papaya*)	16.13±7.65	3.94±2.62--	39.70±8.35	58.00±10.52
1500mg/kg (*P. biglobosa*)	8.24±3.48	19.17±10.42	37.17±4.27	54.53±6.96
2000mg/kg (*C. papaya*)	15.47±7.46	18.65±1.99	44.53±4.19	53.67±38.14
2000mg/kg (*P. biglobosa*)	7.29±6.23	17.13±60.57	10.77±3.02--	52.33±25.54

Values are mean ± SD of six (6) results; values with superscripts-- within a column (for the same parameter) are significantly lower than the control.

4. Discussion

The ethanol leaf extract was found to be rich in phytochemicals, containing alkaloids, tannins, flavonoids, phenols and saponins. These bioactive compounds have been found to be of health benefits by researchers. Alkaloids have established broad-spectrum antibacterial activity [22] and anti-inflammatory action [24]. Flavonoids and phenols found in the extracts are suggestive of their antioxidant property. Flavonoids are reported to be antioxidants [25] and their presence in the extracts is an indication of the plants' potent antioxidant properties. Flavonoids are considered as the most active antioxidant phenolic compounds due to their chemical structure [26].

The administration of 500mg, 1000mg, 1500mg or 2000mg/kg bw of the ethanol leaf extract of *C. papaya* or *P. biglobosa* did not lead to the death of any of the experimental animals.

Neither did they lead to any clinically observable abnormality in the behavior of the animals. Their feeding was normal, so also were their fur condition, eye colour and locomotion. There was none that was convulsive and no sedation was noticed. The graded doses (500mg, 1000g, 1500mg and 2000mg), of these extracts, (*C. papaya* and *P. biglobosa*) orally administered to the rats did not lead to significant change in the ALT activity of the animals. Although, there were changes in the activities of AST and ALP; in some cases significant, it does not signify liver damage. The amount of ALT, AST and ALP in the blood is directly related to the extent of the tissue damage. Huang *et al*. [27] reported that after severe damage and that AST levels rises 10 to 20 times greater than normal, whereas ALT can reach higher levels (up to 50 times greater than normal range). On the other hand, the ratio of AST to ALT (AST/ALT) sometimes can help determine whether the liver or another organ has been damaged [28]. But in this study such rises in AST ad ALT activities were not experienced and the changes in both the bilirubin and albumin concentrations of the serum of these animals were not seen to be adverse. This finding is in consonance with the report of Achini *et al.* [29] which stated that administration of high dose of mature leaf concentrate of *Carica papaya* on 3 consecutive days showed neither provocation of overt signs of toxicity nor stress, where hepatotoxicity, renotoxicity, hematotoxicity and neurotoxicity were also ruled out.

Of the four kidney function parameters (HCO_3^-, Urea, Creatinine and Na^+) assayed, only Urea at the concentration of (*C. papaya*) of 500mg, 1000mg and 1500mg and (*P. biglobosa*) at the concentrations of 500mg and 1000mg with *C. papaya* at 500mg showed significant decrease in their serum concentrations when compared to the control. These decreases are has been adjudged to be beneficial rather than being detrimental to the animals (Enemali *et al.,* 2018).

5. Conclusion

This study found that oral ingestion of 500mg, 1000mg 1500mg and 2000mg/kg bw of either *C. papaya* or *P. biglobosa* is found in this study not to elicit any marked toxicological effect on the experimental animals rather, it showed some beneficial effects like the reduction of the serum Urea concentration of the animals. Though not significant at $p< 0.05$, it

also reduced the serum activity of ALT of the animals, therefore justifying the use of *C. papaya* and *P. biglobosa* in the treatment of various diseases especially, liver and kidney related ailments.

Compliance with ethical standards

Acknowledgments

The Authors acknowledge the assistance rendered by the Technologists in the Department of Biochemistry, NSUK; Mr. Sunday Alaku and Mr. Ezekiel Alama in ensuring the success of this research.

Disclosure of conflict of interest

The Authors declare that there are no conflicts of interest in connection with this paper.

Statement of ethical approval

Ethical approval for the use of animals in this study was obtained from the animals research and Ethics Committee of the Nnamdi Azikiwe University, Awka.

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