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Original Article

Effect of Methanolic Extract of *Carica papaya* Seed on Renal Function and Antioxidant Activities Following Ibuprofen-induced Toxicity on Male Wistar Rats

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ABSTRACT

In this study, the effect of methanolic seed extract of *Carica papaya* (MSECP) was investigated to ascertain its effect on the renal function and antioxidants activities following ibuprofen induced renal toxicity. Phytochemical screening of crude extract revealed the presence of alkaloids, flavonoids, glycosides, saponins and tannins while anthraquinone and phlobatannins were found to be absent. Thirty (30) healthy male Wistar rats were divided into 5 groups. Group A (received feed and water only); Group B received 80mg/kg of ibuprofen only; Group C received 80mg/kg of ibuprofen + 150mg/kg of MSECP; Group D received 80mg/kg of ibuprofen + 300mg/kg of MSECP; Group E (received 80mg/kg of ibuprofen and treated with 600mg/kg of (MSECP). The administration of the extract lasted for 21 days within hours of 7-8 am via oral gavage. After 21 days the animals were anesthetized with 25% Urethane and blood was collected using a heparinized capillary tube and transferred into a plain container and centrifuged. The serum retrieved was used to assay serum antioxidants Superoxide dismutase (SOD), Catalase activity (CAT), Glutathione (GSH), Glutathione peroxidase (GPX), Total Antioxidant capacity (TAC) and kidney enzymes (urea and creatinine). MSECP significantly reduced the plasma concentration of urea and creatinine ($p>0.05$) when compared to group A. The antioxidant enzymes reduced significantly ($p>0.05$) in groups that was administered with MSECP when compared to the group A and B. MSECP was discovered to have some therapeutic effect on renal function probably as a result of some of the antioxidants and phytonutrients present in *Carica papaya* which have the capability of increasing glomerular filtration of toxic substances from the kidney and hence recommend that *Carica papaya* seed could be included in our daily diet.

Keywords: Antioxidants, *Carica papaya*, Phytochemical, Oxidative stress.

INTRODUCTION

Carica papaya is an example of a medicinal plant with numerous therapeutic values. *C. papaya* (pawpaw) belongs to the family of *Caricaceae*. *Carica papaya* is not a tree but an herbaceous succulent plants that possess self-supporting stems.[1] With a rapid growth rate. They are usually short-lived but can produce fruit for more than 20 years. The plants are male, hermaphrodite, or female[2] These plants are self-pollinated.[3] *Carica papaya* leaf tea or extract has a reputation as a tumour-destroying agent.[4] The juice has been in use on meat to make it tender.[1] The high level of natural self-defence compounds in the plant makes it highly resistant to insect and disease infestation Several investigations have been done on the therapeutic values of *C. papaya*. However, there is still limited scientific information on the antioxidant and renal functions of *C. papaya* seed, due to the high rate of negligence in the consumption of the seed together with the pulp.[5] It is seen that most people cannot afford good food and health care, this has caused a lot of damages to people's health due to lack of balanced food nutrients and consumption of toxic substance.[6] Oxidative stress has been implicated in many pathological disorders like stroke, cancer, aging, Alzheimer's, diabetes, viral infections (that cause airway epithelial inflammation), neurodegenerative processes and infarction and brain edema. Oxidative stress is caused as a result of free radicals; reactive oxygen species (ROS) generated during normal cellular metabolic processes of the body; they can also be introduced from the environment. These molecules are highly reactive, they react with cellular molecules such as proteins, lipids and carbohydrates. They denature those thus vital cellular structures and functions of the body are lost and ultimately resulting to various pathological conditions. Antioxidant

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enzymes in the body are capable of stabilizing or deactivating free radicals before they attack cellular.[7] If the antioxidant defense of the body is lost, there would be oxidative stress, resulting to various pathological disorders. One of the therapeutic approach by which these disorders can be prevented is to increase the levels of antioxidant enzymes (SOD, CAT, GPX etc) in the body.[7] The kidneys play a vital role in excretion of waste products and toxins such as urea and creatinine, regulation of ECF fluid volume, serum osmolarity and electrolyte concentration as well as production of some hormones. There are several clinical laboratory tests that are useful in investigating and evaluating kidney function, but the most practical test to assess renal function is to estimate glomerular filtration rate (GFR). Creatinine is the most commonly used endogenous marker for the assessment of glomerular function. It is a byproduct of creatinine phosphate in muscle and it is produced at constant rate in the body. The kidneys are responsible for completely removing creatinine from the bloodstream. Decreased clearance by the kidney results in increased blood creatinine. Blood Urea Nitrogen (BUN) also known as urea is a nitrogen containing compound formed in the liver as the end product of protein metabolism and the urea cycle.[7] About 85% of urea is eliminated via kidneys; the rest is excreted via the GIT. Serum urea levels increases in conditions where renal clearance decreases (in acute and chronic renal functions). It may also increase in conditions not related to renal diseases such as upper Gastrointestinal (GI) bleeding, dehydration, catabolic States and high protein diets. For the purpose of this research, these two important markers were used to assess renal function to know how well the kidney is functioning by checking their glomerular filtration rate. Ibuprofen belongs to a class of drugs called nonsteroidal anti-inflammatory drugs (NSAIDS).Ibuprofen is prescribed commonly as an analgesic, anti-pyretic and anti-inflammatory agent in conditions like osteoarthritis, rheumatoid arthritis and acutely painful musculoskeletal conditions.[8] In cases of consumption of high doses of ibuprofen, it can become very toxic to the body system. Toxicity of ibuprofen can be discussed based on the organ of the body involved. For our research study, we will be focused on renal toxicity of ibuprofen. High doses of Ibuprofen have been discovered to elevate creatinine levels, cause microscopic hematuria with no proteins or casts in urine.[9] *Carica papaya* is a herbaceous plant cultivated in the tropics as a food and cash crop. *Carica papaya* seeds are black in colour and embedded in a fruit pulp. Therefore, this study assessed the renal function and antioxidant effect of methanolic seed extract of *Carica papaya* on male Wistar rats and the discovery from this study will help alleviate the problem of man towards actualizing a more and efficient functional kidney through the incorporation of seed extracts of *Carica papaya* in the manufacturing of some pharmaceutical products.

MATERIALS AND METHODS

Location of the Study

This study was carried out in the Department of Physiology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. Ethical approval consent was obtained for the progress of this study, from the Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus.

Materials

Thirty-(30) inbreed male wistar rats, *Carica Papaya* seed, Ibuprofen (200mg Capsules; Tabufen; was purchased from JUHEL Pharmaceutical Company, Awka, Anambra State), Absolute Ethanol (JHD Chemicals, Guangdong China), distilled water, Whatman qualitative filter paper no. 1, oral cannula, Automatic Water distiller (SZ-1 Search Tech Instrument), Randox reagent kit (Sigma Aldrich, USA), Rotary evaporator (Digital) TT-52 (Techmel and Techmel, USA), Thermostat Oven (DHG-9023A, PEC MEDICAL USA), UV-VIS 752N Spectrophotometer (Shanghai, Yoke Instrument Co., Ltd. China), Chloroform (Guangdong Guandgua Chemical Factory Co. Ltd. Shatou, Guondghuo, China), non-heparinized capillary tube, Electronic weighing balance (M-Metallar M311), 2ml hypodermic sterile syringe, animal weighing balance (Camry LB11), Normal laboratory chow (Standard Pellet) and standard cage.

Preparation of Methanolic Extract of *Carica papaya* Seed

The *Carica papaya* seeds were bought from Nkwo market, Nnewi, Anambra State, and was washed in running tap water to remove dirt, and air-dried under ambient temperature. The dried *Carica papaya* was milled into coarse form using Local grinder. 250g of the dried coarse form of *Carica papaya* was macerated in 1000mls of 95% absolute methanol for 48 hr. It was then filtered using a clean handkerchief and further filtration using Whatman No 1 filter paper. The filtrate was concentrated using a Rotatory Evaporator (TT-52 Techmel and Techmel USA) and was further dried using a Thermostat oven (DHG-9023A Pec Medicals USA) at 45 degree into a gel-like form. The extract was preserved in a refrigerator for further usage. This was done according to the method described[10] with modifications.

Experimental Animals

Thirty (30) inbreed male Wistar rats weighing 150-170g were used for the study. The experimental animals were housed in the Animal House Unit, Department of Physiology, College of Health Sciences and Nnamdi Azikiwe University Okofia Nnewi Campus. The animals were maintained in standard cages at ambient temperature (25±0.2°C) with standard pellets and distilled water *ad libitum*. The animals were acclimatized for two weeks before commencement of administration of the extract. Animals were maintained under 12 hr' light and dark cycles.

Acute toxicity test (LD_{50}) of *Carica papaya* seed extract

This was done using the method of[11] and was divided into stages, with the outcome from each stage determined the next step to taken (i.e, whether to terminate or proceed to the next stage).

Stage 1

This is the initial stage and it requires four animals. These animals were divided into four groups of one animal each. Then different doses of the test substance are administered to the different animals. The animals were observed for 1 hr post-administration and then 10 min every 2 hr interval for 24 hr. The behavioural signs of toxicity and also mortality were recorded. Where no mortality was recorded at this stage, the testing proceeded to stage 2.

Stage 2

This stage involved three animals, which were divided into three groups of one animal each. Different doses of the test substance (higher than those used in stage 1) were administered to the different animals and then observed for 1 hr after administration and periodically for 24 hr. Behavioural signs of was noted and mortality as well. As no mortality occurred, testing should proceed to stage 3.

Stage 3

This stage also required three animals which were distributed into three groups of one animal each. Various high doses of test substance (with 5000 mg/kg as the highest) were administered to the different animals. Observation is done for 1 hr after administration and then 10 min every 2 hr for 24 hr. Behavioural toxicity signs and also mortality were recorded. This was the final stage of testing and where no mortality was

recorded at this stage, the LD_{50} of the test substance is said to be greater than 5000 mg/kg and hence has a high degree of safety.

Furthermore, where the test did not show any mortality at 5000 mg/kg, a confirmatory test was also carried-out. This was done by administering 5000 mg/kg to two animals. Observation was done for 1 hr after administration and 10 min every 2 hr interval for 24 hr. The recording of no mortality showed confirmation of test result.

Method of qualitative analysis of *Carica papaya* seed

The phytochemical screening was carried out according to the method of[12] to determine the biological active, non-nutritive compound that contributed to the flavour, colour and other characteristics of plants such as alkaloids, tannin cardiac glycoside, saponins, flavonins among others

Preparation of Ibuprofen and MSECP Dosage

Approximately two hundred (200mg) milligram of Ibuprofen capsules were dissolved in 20mL of distilled water to obtain a stock of 10mg/ml. 5g of MSECP was dissolved in 50mL of distilled water to obtain a stock solution of 100mg/ml according to the standard method of.[13]

Experimental Design

The thirty (30) male Wistar rats of healthy status were divided into five (5) groups of 5 rats each.

Group A: received feed and distilled water only,

Group B: received 80mg/kg of ibuprofen only,

Group C: received 80mg/kg of ibuprofen + 150mg/kg of MSECP

Group D: received 80mg/kg of ibuprofen + 150mg/kg of MSECP,

Group E: received 80mg/kg of ibuprofen + 150mg/kg of MSECP,

The administration of the extract was done for 21 days, within the hours of 7:00 to 8:00 via oral garvage.

Sample Collection

Animals were anaesthetized with chloroform in an enclosed container 24 hr after the last administered dose of the test substances, blood was collected using heparinized capillary tube and put in a plain container as described by.[14] It was also centrifuge at 300 rpm for 20 min. The serum retrieved was used to assay serum antioxidants (SOD, CAT, GSH, GPx, and TAC) and kidney enzymes (urea and creatinine).

Estimation of Antioxidant Markers and Kidney Enzymes

Catalase (CAT) activity was assayed by measuring the degradation rate of H_2O_2 using[15] method. The rate of disappearance of H_2O_2 was monitored spectrophotometrically at 230 nm. The assay medium consisted of 50 Ml of 1 M Tris HCl buffer (pH 8), 930 µL 10 mM H_2O_2, 930 µL deionized water, and 20 µL serum sample. One unit of CAT activity is defined as the amount of enzyme causing about 90% destruction of the substrate in 1 min in a volume of 1 ml. CAT activity in the serum was expressed as U/ml.[15]

Superoxide-dismutase (SOD) activity was determined as described by.[16] This method employs xanthine and xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitro phenol-s-phenyl tetra-zolium chloride) to form a red formazon dye. SOD activity is then measured by the degree of inhibition of this reaction.

Glutathione reductase (GSH) activity was determined by the method of.[17] A total of 200µL of standards and samples were added to the cuvette. Then 200µL of chromogens was added to each cuvette, and 200µL of the enzymes was added to each of the cuvette, mixed, and then incubated at room temperature for 5 min. A total of 200µL of NADPH was added to each cuvette. Changes in absorbance at 412nm for 3 min were recorded and observed.

Glutathione Peroxidase (GPx) activity was determined by the method of.[18] 0.2mL each of EDTA, sodium azide, GSH, H_20_2, serum sample were mixed and incubated at 37°C for 10-min. The reaction was arrested by addition of 0.5mL of TCA and tubes were centrifuged. To 0.5mL of supernatant, 3mL of phosphate solution, and 1 mL of DTNB were added and the colour developed was read at 420nm immediately using spectrophotometer. GPx activity was expressed as U/ml.

Total antioxidant capacity (TAC) activity was estimated according to the method described by.[19]

Serum urea estimation was done according to the method described by.[18] Serum creatinine was estimated using enzymatic method as described by.[20-21]

This method involves two underlying principles of catalyzed steps of reaction that results in the formation of hydrogen peroxide. The final step involves a trinder indicator system involving sequential reactions to give an intense red colour with maximum absorbance at wavelength of 510nm.

Table 1: Qualitative analysis *of the* Phytochemical components of *Carica papaya* seed.

Sl. No.	Phytonutrient	Concentration
1	Alkaloids	+
2	Anthraquinone	-
3	flavonoids	+
4	Phlobatamines	+
5	Phenols	+
6	Glycosides	+
7	Saponins	+
8	Tannins	+

Values with + sign indicates the presence of the Phytochemical and sign (-) means absence of the Phytochemical.

Table 2: Effect of methanolic seed extract of *Carica papaya* on urea and creatinine level on Ibuprofen impaired renal function.

Groups	Urea concentration (mg/dL)	Creatinine concentration (mg/dL)
	MEAN±STD	MEAN±STD
Group A (Positive control)	53.59±5.45	3.32±0.44***
Group B (Ibuprofen only)	33.17±0.46	1.76±0.71
Group C (Ibuprofen + 150mg/kg of MSECP)	53.58±4.13***	3.04±0.17**
Group D (Ibuprofen + 300mg/kg of MSECP)	48.27±3.06**	2.96±0.18*
Group E (Ibuprofen + 600mg/kg of MSECP)	37.26±0.08	1.34±0.38

Values were considered extremely significant, moderately and significant at ***$p<0.001$, **$p<0.01$, and *$p<0.05$ respectively.

Statistical Analysis

Data obtained were subjected to SPSS version 25. ANOVA was used to analyze the serum urea, creatinine, SOD, CAT, GSH, GPx, and TAC followed by multiple comparism using post HOC Tukey HSD. Values were presented as Mean ± Std and were considered significant at $p<0.05$.

RESULTS

Table 2 revealed an extremely significant ($p<0.05$) increase in urea level in-group C and D compared to group A while group E had a no significant ($p>0.05$) increase as compared to group A. Group B had a decrease in urea concentration ($p>0.05$) decrease when compared to group A. The result of the Creatinine concentration revealed an extremely and moderately significant ($p<0.05$) increase in group C and D compared to group A while there was no significant decrease in group E ($p>0.05$) compared to group A.

Table 3 Showed a significant ($p<0.05$) decrease in glutathione reductase and superoxide dismutase activity in group C, D, and E as compared to group B. Group B had a significant ($p>0.05$) increase when compared to group A.

Glutathione peroxidase activity showed a significant ($p<0.05$) decrease in-group C and D; and group E had a non-significant ($p>0.05$) decrease as compared to group B. Group B when compared to group A revealed a non-significant ($p>0.05$) decrease.

Table 4 revealed a significant ($p<0.05$) decrease in-group C and D; and group E had a non-significant ($p>0.05$) decrease as compared to group B. Group B when compared to group A revealed a non-significant ($p>0.05$) increase in catalase activity. Total antioxidant activities revealed a significant ($p<0.05$) decrease in-group C, D, and group E as compared to group B. Group B when compared to group A revealed a significant ($p<0.05$) decrease.

Table 3: Effect of methanolic seed extract of *Carica papaya* on glutathione, superoxide-dismutase, and glutathione peroxidase activity on Ibuprofen induced oxidative stress in Wistar rats.

Groups	Glutathione reductase (U/ml)	Superoxide-dismutase (U/ml)	Glutathione Peroxidase (U/ml)
Group A (Positive control)	9.17±0.17**	10.63±0.42***	1.21±0.30
Group B (Ibuprofen only)	10.96±0.92	17.84±0.98	1.16±0.04
Group C (Ibuprofen + 150mg/kg of MSECP)	5.38±0.06***	7.42±1.35***	0.72±0.03**
Group D (Ibuprofen + 300mg/kg of MSECP)	6.17±0.01***	7.35±0.38***	0.80±0.01*
Group E (Ibuprofen + 600mg/kg of MSECP)	8.07±0.38***	12.53±0.51***	0.93±0.04

Values were considered extremely significant, moderately and significant at ***$p<0.001$, **$p<0.01$, and *$p<0.05$ respectively.

Table 4: Effect of methanolic seed extract of *Carica papaya* on catalase and total antioxidant capacity activity on Ibuprofen induced oxidative stress.

Groups	Catalase (U/ml)	Total antioxidant capacity (U/ml)
	MEAN±STD	MEAN±STD
Group A (Positive control)	73.18±3.03$_{NS}$	772.66±0.35...
Group B (Ibuprofen only)	73.55±5.71	836.81±3.16
Group C (Ibuprofen + 150mg/kg of MSECP)	54.78±3.44...	773.89±0.62...
Group D (Ibuprofen + 300mg/kg of MSECP)	61.11±1.00..	775.78±0.34***
Group E (Ibuprofen + 600mg/kg of MSECP)	69.73±1.55$_{NS}$	698.51±1.50***

Values were considered extremely significant, moderately and significant at ***$p<0.001$, **$p<0.01$, and *$p<0.05$ respectively.

DISCUSSION

The presence of alkaloids, flavonoids, tannins, saponins, glycosides and phenols has been revealed by the study as the phytochemical components in the *Carica papaya* seed. The various Phytochemical detected in *Carica papaya* seeds are known to have beneficial uses and exhibit physiological activity.[21] From the result of the study carried out, MSECP showed a significant positive effect on renal function by decreasing the plasma concentration of urea and creatinine in the blood after an induced toxicity of ibuprofen. Urea is a byproduct of protein metabolism and it's excreted from the body through urine. From the study, MSECP caused a significant effect in plasma urea level at low and moderate dosages (group C and D), this shows that MSECP is good for renal function as it increases the glomerular filtration rate of urea from blood plasma at low and moderate dosages. There was no significant effect when MSECP was administered at high dosage. From the result above, plasma creatinine levels increased significantly in the positive control group and this shows that the blood contains high concentration of creatinine in normal body activities. When MSECP was administered, the plasma concentration of creatinine reduced significantly especially in group C and D where MSECP was administered in low and moderate doses. But at high dosage, MSECP showed no significant effect on plasma creatinine levels. From the result, there was an increase in the plasma concentration of antioxidant enzymes in group B where toxicity was induced. This shows that toxicity increases the activity of antioxidant enzymes in the body. When MSECP was administered, there was a significant decrease in the concentration of antioxidant enzymes except superoxide Dismutase (SOD). This shows that MSECP has antioxidant properties that works in synergy with antioxidant enzymes and helps to reduce oxidative stress thus, the concentration of antioxidant enzymes reduced significantly especially in group E where high dose of MCPS was administered. Total antioxidant capacity is the measure of the amount of free radicals scavenged by a test solution being used to evaluate the antioxidant capacity of biological samples. From the result, TAC decreased significantly in group C, D and E. This suggests that MCPS has high antioxidant properties and has therapeutic effect that can ameliorate the activities of free radicals in the body.

CONCLUSION

Our study confirmed the effect of Phytochemical extracted from *Carica papaya* seeds to have antioxidant effect and can facilitate the removal of toxins from the body by increasing its glomerular filtration rate from the body at low and moderate doses. The effect is dose dependent hence it is okay to say that *Carica papaya* seeds are safe to consume in cases of toxicity in the body.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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