Toxicological, Haemato-Biochemical, Oxidative and Histopathological Evaluation of Methanolic and Aqueous Extract of *Carica papaya* Seed and Leaf in Wister Rats

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ABSTRACT

The aim of this study is to evaluate active toxicity effects of methanolic and aqeous extracts of seeds and leaves of Carica papaya in Wistar ratsa and on Ascaris suum eggs. The study involved both in vitro and in vivo assays. The work demonstrated that both methanolic and aqueous extracts of Carica papaya leaf and seed have anthelminthic activity against larval stages, as they completely stopped the motility of Ascaris suum (P<0.05) The phytochemical screening of seeds and leaves of C. papaya comprised of alkaloids, sapronin, tannins, flavonoids, carbohydrates, phenols, steroids, anthraquinones, cardial glycosides and terpenoides respectively. The seed and leaf extracts of the Carica papaya plant exhibited to have medicinal value against ascariosis (Ascaris suum). Haematological parameters such as total white blood cell count, neutrophils, lymphocytes, hematocrit, total red blood cell count, haemoglobin count increased slightly within normal range during the experimental period and did not exhibit any discernible treatment-related variation in either the control or treated groups (P>0.5). Methanolic and Aqueous extracts of Carica papaya Leaf and Seed indicated that there was no visible lesion as evidenced by the presence of intact mucosa epithelium, lamina propria, submucosa, muscular and serosa tunical layers in the examined organs of Wister rats. The LD50 value of seed and leaf methanol and aqueous extracts were found to be greater than 5000mg/kg. Analysis of serum biochemical enzymes and electrolytes during and after treatment shows that all biochemical parameters such as Aspartate amino transferase, Alanine amino transferase, Alanine phosphatase, Total protein, Urea, Sodium, Chlorine and Potassium are within normal range during the experimental period and did not exhibit any significant variation in either the control or treated groups (P>0.05). The Histo-architectural structures of organs indicated unnoticeable differences between the control and test groups, according to the microscopic investigation, the intestine, spleen, and heart of the extract-treated rats did not exhibit any changes in cell structure or any negative consequences. The liver showed a marked diffuse hepatocellular degeneration (circular-outline) typified by cytoplasmic vacuolations and nuclear degeneration, the kidney showed moderate glomerular shrinkage, mild widening of urinary visible lesion and focal renal tubular distortion. The alveolar showed that there was moderate thickening of the alveolar septa and a mild presence of cellular debris in the alveolar spaces. Toxicological analysis demonstrated on the plant revealed less oxalate and alkaloids than other regularly consumed food products, and that theseeds and leaves are safe for ingestion Thus, this study support the use of Carica papaya, particularly the leaves and seeds as an anthelmintic agent in the management of infections by traditional or herbal practitioners and indigenous people.

Keywords: *Ascaris suum, Carica papaya, extracts,,Heamatological parameters, Wister rats, Herbal plants*

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INTRODUCTION

Ascariosis is a *helminthic* disease which has a socioeconomic effect on pigs and humans (Parbin et al., 2020).It is caused by Ascaris lumbricoides and Ascaris suum respectively (*Urquhart et al.,* 1987). *Ascaris suum*, also called the large nematode of pigs or the large white pig worm occurs in the gastrointestinal tracts of domesticated pigs and feral boars worldwide *(Dold and Holland, 2011).* It has a high prevalence rates in pigs' populations (Dold and Holland, 2011) and the infection varies with geographical region and farm management practices but few swine herds are totally free of infection (Vismarra, *et al.,*2023). Adult worms are found in the small intestine and transitorily in the large intestine during expulsion of the worms (Soulsby, 1982). Ascariosis may occur in any age group depending on the housing and management system but piglets or growing pigs are mostly affected *(*Urquhart *et al.,* 1987). On the conventional pig farms ascariosis can be observed primarily in weaned and fattening pigs (Stewart and Hale 1988). The larvae are mostly responsible for causing the clinical diseases in the hosts. Some clinically healthy pigs may also harbour a large amount of adult ascarids, which cause production losses (Soulsby, 1982). T*he infection may have low to moderate* pathological and clinical changes caused by the different stages of the parasite (Thamsborg *et al.,* 2013), which includes, reduce feed conversion efficiency, compromise weight gain, meat quality, *liver condemnation incurring economic losses* to the meat industries associated with the condemnation due to lesions caused by migrating larvae 'milk-spot' livers, infected pigs in turn, shows symptoms like, coughing or thumping, liver damage, impaired growth, and increased susceptibility to other bacterial and viral infections due to the migratory and immune modulatory capacity of *Ascaris suum* (Roepstorff *et al.,*2011). For centuries now, indigenous medicinal plants have been used for the control of internal parasitesal though, there is no scientific validation of these traditional practices (Githiori *et al.,* 2005; Tolossa *et al.,* 2013). However, nowadays, phytotherapeutic studies on the effects of various plant extracts on pathogenic microorganisms are intensively developing because of the huge plant biodiversity (Băies *et al.,* 2022). Several natural plant compounds have been extracted by decoction or other simple procedures and they have some advantages like, low cost, integrated easily into local communities mostly when plants are locally available and it mitigates the problem of drug resistance (William *et al.,* 2016).

Carica papaya Linn (Family: caricaceae), is an herbaceous, perennial, single stemmed fruit tree plant also called pawpaw, readily available all year round with easy access for exploitation in tropical areas (Saumendu Deb Roy *et al.,* 2012; Fasae and Afolabi, 2016). Different parts of the plants have been used for the treatment of various ailments such as bacterial, helminthic and anticoccidial (Fasae and Afolabi, 2016). The plant contains many biologically active compounds like papain and chymopapain (Saumendu Deb Roy *et al.,* 2012). The concentration of the compounds varies in the fruit, latex, leaves and roots. It has been reported to possess high content of protein and good source of minerals with the presence of high fat and some ant nutritional factors such as phytic acid, tannins and oxalate (Dikibo *et al*, 2012), which if adequately harnessed can be beneficial to livestock. The aim of this study is to evaluate active toxicity effects of methanolic and aqeous extracts of seeds and leaves of *Carica papaya* in Wistar rats. Thus, the objectives was determined the safety of *Carica papaya* methanolic and aqueous extracts of seed and leaves and the dose-dependent toxicity in wistar rats measuring thep phytochemical, hematological, serum biochemical and histopathological analyses of the plant and the rats. These experiments will provide a basis for the subsequent application of *Carica papaya* seed and leaves extracts in managing parasitc infections.

MATERIALS AND METHODS

The present study was conducted in Plant Science Laboratory of the Faculty of Natural Sciences University of Jos, Nigeria.

Preparation of Plant Materials

Collection and preparation of the *Carica papaya* leaves and seeds

The seeds were collected freshly from ripe pawpaw fruits sold in a nearby fruit market, while the leaves were harvested directly from the tree around the surrounding areas within the campus and were washed with clean water to remove dirt and other debris. The seeds and leaves were air dried under shade at room temperature (25^{o}C) for two weeks and then pulverized into fine powder using a mortar and pestle. The powders were sieved and stored in air tight bottles until when needed.

Extraction of the Plant Material

The extraction was carried using cold maceration method using the ratio 1:10 grams to volume of methanol. 50g each of the powder was weighed using a top loading balance and it was then transferred into a large extracting flask (bottles) the content was soaked with 500ml of methanol and allowed to stand for three (3) days at room temperature with continuous shaking and stirring thoroughly with a sterile glass rod. The suspension was then filtered with a sterile muslin cloth and then filtered again using sterile Whatman No.1 filter paper inserted in a funnel. The plant residue was subjected to several parts of rinsing and filtration to attain an exhaustive level of extraction.

Yield Percentage of Extracts

After drying, the yield of each extraction was measured separately and the extraction efficiently was quantified by determining the weight of each of the extracts and the yield percentage was then calculated as dry weight/dry material x 100 (Parekh and Chanda, 2007a)

Phytochemical Determination

The plant fractions were screened for their phytochemical constituents to determine the presence of alkaloids, saponins, tannins, flavonoids, carbohydrates, steroids, anthraquinones, cardiac glycosides and terpenoids using standard phytochemical screening procedures.

Test for Alkaloids

6 ml of extract was mixed with 6 ml of 1% HCl in steam bath, and then it was filtered. 1 ml of Mayer's reagent was added. Presence of turbidity shows presence of alkaloids. Further addition of a few drops of olive oil to form an emulsion confirmed the presence of alkaloids.

Test for Saponins

0.5 g of the extract was dissolved in 5 ml distilled water. The mixture was shaken vigorously. Formation of stable persistent froth shows the presence of saponins. A further addition of 6 drops of olive oil while shaking forms an emulsion, confirming the presence of saponins.

Test for Tannins

0.5 g of the extract was dissolved in 10 ml of distilled water, then a few drops of 1% ferric chloride solution was added to obtain a brownish green or blue black precipitate, which confirms the presence of tannin.

Test for Anthraquinones

Borntrager's test was be used for the detection of anthraquinones, 0.5g of each extracts was taken into a dry test tube and 5ml of chloroform was added and shook for 5 minutes. The extract was filtered, and the filtrate shaken with an equal volume of 100% ammonia solution. A pink violet or red colour in the ammoniacal layer (lower layer) indicated the presence of free authraquinones.

Test for Cardiac Glycoside

100mg of the extract was dissolved in 70% alcohol and filtered. About 3 drops of lead sub- acetate was introduced into the filtrate and filtered. The filtrate was extracted with 10mls of chloroform in a separating funnel and concentrated to dryness. The resulting residue was dissolved in 1ml of glacial acetic acid containing one drop of Ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring obtained at the interphase indicates the presence of a deoxysugar characteristic of cardenolides.

Test for steriods

About 100mg of the extract will be dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interphase was indicative of the presence of steroidal ring.

Test for Terpenes

A little quantity of each extract was dissolved in chloroform, and 1ml of acetic anhydride was added, then two drops of concentrated Sulphuric acid was added. A pink colour which changes to bluish green on standing was indicative of the presence of steroid and terpenes.

Test for Flavonoids

5 ml dilute ammonia was added to 5 ml extract and then 5 ml concentrated sulfuric acid was added. Formation of yellow colour shows the presence of flavonoids.

Test for Carbohydrates

1 gm of the extract was dissolved in 10 ml of distilled water. This extract was boiled with Fehling solution A and B in test tube and colour changes were observed. Presence of brick red colour indicated the presence of reducing sugar.

Test for Phenols

2 ml of extract was dissolved in 4 ml of distilled water and added few drops of 10% FeCl3. Appearance of blue or green colour indicates presence of phenols.

Experimental design

Collection of eggs of *Ascaris suum*

At first,fresh intestine containing gravid *Ascaris suum* worms were collected from a local pig slaughter house in Dakace, a small village settlement in AngwanMangu, Zaria and brought immediately in a cooler to the Helminthology Laboratory of the Department of Parasitology and Entomology, ABU Zaria were the adult *A.Suum* were identified following a standard method (Parbin et al, 2020). Briefly, eggs were isolated from the uteri of the worms by opening up the uteri using forceps into a petri dish and washed with 0.5 KOH solution into a beaker. The eggs were then agitated gently in the 0.5M KOH solution for 30 minutes in order to dissolve the sticky albuminous layer and allow for uniform sampling (Andrew et al, 2016). The preparation was placed in centrifuge tubes and centrifuged at 1500 rpm for 3minutes to recover the eggs. The supernatant was decanted and the eggs

times in distilled water and embryonating fluid (0.1M sulphoric acid) for the same period and volumes of the sediment was adjusted to 20 mL in a graduated test tube.

In-vitro Assay of the anthelminthic properties of *Carica papaya* on the eggs of *Ascaris suum*

The seed extract (Methanolic and Aqueous) and levamisole standard reference for seed extract was made into different concentrations of 12.5, 25, 50 mg/ml by using normal saline as a solvent (Tarkang et al, 2012). For leaf extract (Methanolic and Aqueous) was made into 200, 400, 800 mg/ml concentrations, and the standard was levamisole, for both normal salines acted as a control (Zingare *et al.,* 2018). All the eggs were washed with normal saline and kept in a different concentration of extracts in a Petri dish. Then observed under the microscopy after 24 hours for the time required for the death or destruction of embryo. It was confirmed by mobility of the embryo as compared with a standard and control (Tarkang et al, 2012, Vismarra et al, 2023).

Biochemical Assays

The activity of some oxidative stress markers namely, Melondialdehyde (MDA), Superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were measured in wistar albino rats exposed to combine extracts of *Carica papaya* and the control which were not exposed.

Malondialdehyde (MDA) Assay

Malondialdehyde (MDA) formation was determine according to the ohkawa method (ohkawa et al., 1979) with modifications, 200µL of tissues homogeny ate was mixed with 1.5ml 20% acetic acid pH 3.5, with 1.5ml 0f 0.8% TBA (Thiobarbituric acid) and 200µL 8.1% SDS (Sodium dodecyl sulphate). The mixture was brought up to the volume of 4ml with water and heated in boiling water for 1 hour. After cooling and centrifugation (4000 RPM) at 4 ºc for 10 minutes, the MDA content in the supernatant was measured at 532nm with a UV 1650PC UV –VIS spectrophotometer. MDA levels were determined from the calibration curve and express in nmol/mg of liver protein.

Superoxide dismutase (SOD)

SOD activity was measured by using NBT (Nitro blue tetazolium) method (Beauchamp and Fidovich, 1971). Three mls reaction mixture was prepared by adding 50 Mm potassium phosphate buffer (pH 7.8), 13Mm methionine, 2µM riboflavin, 0.1mM EDTA (Ethylenediaminetetraacetic acid), 75 NBT amd 50 µL of enxymes extract. All the tubes were exposed to 400W bulbs for 15 minutes and their absorbance was read at 560nm.

The 50 % inhibition of the reaction between riboflavin and NBT in the presence of methionine was consider as one unit of SOD activity and it was express in units/mg of protein.

Catalase (CAT)

CAT activity was measured by following the standard protocol (Aebi, 1984). The activity was measured base on the quantity of the H_2O_2 substrate remaining often the action of CAT present in the enzymes extracts. To measure this, 0.4 ml of enzyme extract was mixed with 2.6ml of phosphate buffer along with 30% H_2O_2. The activity was measured nby determine the decomposition of H_2O_2 at 240 nm. CAT activity was calculated by using the milimolar extinction coefficient of 43.6 and expressed in terms of µm/min/mg of protein.

Reduced Glutathione (GSH)

The activity of GSH was measured by using DTNB (5, 5-Dithio-bis-(2-nitrobenzoic acid) method (Ellman, 1959). To measure the enzyme activity, liver cells were homogenized in ice-cold 10% TCA (Trichloroacetic acid) and 10mM EDTA solution (1:1). Then homogenate was centrifuged at 5000 rpm. Further enzyme reaction mixture was prepared by adding 200µL of supernatant, 0.2M tris-buffer (pH 8.0) and 50 µL of DTNB. The reaction mixture was incubated for 10 minutes at room temperature to get yellow colored complex. The absorbance was read at 412 nm, and the activity was expressed as µg/mg protein.

Estimation of Protein

Protein estimation was quantified as per the standard procedure (lowry, 1951). For this 20 of the homogenate was mixed with Lowry's reagent and folin-ciocalteaus solution. The optimal density was measured at 660 nm against blank after incubation. Then the amount of protein in each group was calculated with the BSA (Bovine serum Albumin) standard graph.

Laboratory animals

The present study was carried out following the internationally accepted principles for laboratory animal use and care and complies with ARRIVE (Animal Research: Reporting of *in vivo* Experiments) guidelines carried out with the UK Animal (Scientific Procedures) Act, 1987 with regards to EU Directive 2010/63/EU for animal experiments (Kilkenny et al., 2010).Thirty Wistar Rats, of 113.5-186 lb were acquired from National Veterinary Institute Vom and were acclimatized in the laboratory under standard conditions for two weeks, feed and water were provided Ad Lib. Three rats of both sexes were randomly assigned to 2 groups of three rats each which served as the control group they received distilled water at 5 mL kg^{-1} b.wt.

Determination of Acute toxicity (LD50) / In vivo assay of the methanolic and aqueous extracts of seed and leaf of *C. papaya*

The method of Lorke (1983) was used in the LD50 determination. Three groups of three rats each were orally administered with the methanolic and aqueous extracts of leaf and seed at doses of 10mg/kg, 100mg/kg and 1000mg/kg body weight and were observed for 24hours. In the second phase, four groups of one rat each were administered orally with methanolic and aqueous extracts at doses of 1600mg/kg, 2900mg/kg and 5000mg/kg respectively. They were observed for 24hours and number of mortality was recorded.

Hematological and Biochemical analysis

After the experiment, one rat from each group was anesthetized with ether, blood was taken from posterior vena cava through a cardiac puncture. The blood was placed into well labelled EDTA bottles for hematological assay and in plain bottle for clinical biochemistry determination. The blood for hematological assay was immediately analyzed using a hematological analyzer (KX-21N Sysmex Cooperation, Japan). White blood cell (WBC),lymphocyte, Neutrophils, red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), The blood collected in plain bottles was allowed to stand for a minimum of 24 h for complete clotting. The serums were collected and transferred into well labelled tubes which were centrifuged at 4 000 rpm at 4°C for 10 min. The serums were then analyzed for clinical biochemistry parameters using the Vitalab Selecta, E-series, Netherlands. Clinical Biochemistry values and liver enzymes (alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). total protein, Renal profile parameters measured was urea and electrolytes like Na, K+ and Cl- were all measured and recorded appropriately.

RESULTS

The methanolic and aqueous extracts of *Carica papaya* leaf and seed have anthelminthic activity against larval stages, as they completely stopped the motility of *Ascaris suum* indicating that it is significantly important in the treatment ascariosis (P<0-05). The phytochemical screening of seeds and leaves of *C. papaya* comprised of alkaloids, sapronin, tannins, flavonoids, carbohydrates, phenols, steroids, anthraquinones, cardial glycosides and terpenoides respectively (Appendix 1). Tables 1-4 demonstrate broad behavioral alterations, toxicology signs, and post-treatment death. The oral administration of leaf and seed methanolic and aqueous extracts at a dose of 5000 mg/kg did not result in any mortality or clinically obvious harmful effects in rats. As neither of the extracts tested showed any mortality or clinical symptoms of toxicity. The LD50 value of seed and leaf methanol and aqueous extracts were found to be greater than 5000mg/kg

Table 5, shows the hematological profile of the treated and control groups. Analysis of blood parameters after treatment shows that all haematological parameters such as total white blood cell count, neutrophils, lymphocytes, hematocrit, total red blood cell count, haemoglobin count are increased slightly though, within normalrangeduring the experimental period and did not exhibit any discernible treatment-related variation in either the control or treated groups (P<0.5).

Table 6, shows the serum biochemical profile of the treated and control groups. Analysis of serum biochemical enzymes and electrolytes during and after treatment shows that all biochemical parameters such as AST, ALT, ALP, TP, Urea, Sodium, Chlorine and Potassium are within normal range during the experimental period and did not exhibit any significant variation in either the control or treated groups.

The Histo-architectural structures of organs shown in (Figures 1-7) indicate unnoticeable differences between the control and test groups, according to the microscopic investigation,the intestine, spleen, and heart of the extract-treated rats did not exhibit any changes in cell structure or any negative consequences. The liver showed a marked diffuse hepatocellular degeneration (circular-outline) typified by cytoplasmic vacuolations (Figure 5) and nuclear degeneration (Figure 2); the kidney showed is moderate glomerular shrinkage, mild widening of urinary visible lesion and focal renal tubular distortion (Figure 3); The alveolar showed that there is moderate thickening of the alveolar septa and a mild presence of cellular debris in the alveolar spaces.

As shown in the (Figure1), there was no visible lesion as evidenced by the presence of intact mucosa epithelium, lamina propria, submucosa, muscular and serosa tunical layers. Magnification: Main (x100) and Inset (x400); Stain: Haematoxylin-eosin (Figure 2). There was moderate glomerular shrinkage, mild widening of urinary visible lesion and focal renal tubular distortion. Magnification: x400; Stain: Haematoxylin-eosin (Figures 3 and 4).

There is moderate thickening of the alveolar septa (arrowhead) and mild presence of cellular debris in the alveolar spaces (black arrow). Magnification: A. Main (x100) and Inset (x400); Stain: Haematoxylin-eosin (Figures 4 and 5). There was no visible histo-architectural distortion in the heart parenchyma as shown by intact cardiac muscle nuclei (black arrowhead), presence of numerous muscle fibre branching (black arrow), distinct intercalated disc (red arrowhead) and numerous fibroblasts in the muscle fibre interstices (red arrow). Magnification: x400; Stain: Haematoxylin-eosin (Figures 6 and 7). There was marked diffuse hepatocellular degeneration (circular-outline) typified by cytoplasmic vacuolations (red arrow) and nuclear degeneration. Magnification: x400; Stain: Haematoxylin-eosin (Figures 8

Table 1: Acute toxicity table for leaf extracts Lethal Dose (LD50) determination for (a) Phase a.

S/No	Label/Mark	Weight	Dose (Mg/Kg)	Conc. (Mg/mL)	Volume mL	Mortality	Remark
1	Head	186.5	10	10	0.19	0/3	Calm
2	Back	116.4	10	10	0.12	0/3	Calm
3	Tail	136.5	10	10	0.14	0/3	Calm
Control							Restless
1	Head	136,5	100	100	0.14	0/3	Scraching

Table 2: Acute toxicity table for Leaves extracts Lethal Dose (LD50) determination for (a) phase a.

S/No	Label/Mark	Weight	Dose (Mg/Kg)	Conc. (Mg/mL)	Volume mL	Mortality	Remark
1	Head	164.0	1600	500	0.43	0/3	Calm
2	Back	128.4	2900	500	0.74	0/3	Calm
3	Tail	113.5	5000	500	1.14	0/3	Calm

Table 3: Acute toxicity table for seed extracts Lethal Dose (LD50) determination for (a) Phase b.

S/No	Label/Mark	Weight	Dose (Mg/Kg)	Conc. (Mg/mL)	Volume mL	Mortality	Remark
1	Head	144.3	10	10	0.14	0/3	Calm
2	Back	162.1	10	10	0.16	0/3	Calm
3	Tail	141.6	10	10	0.14	0/3	Calm
1	Head	129.1	100	100	0.13	0/3	Scraching Body
2	Back	114.2	100	100	0.11	0/3	Very Active
3	Tail	128.4	100	100	0.3	0/3	Very Active
1	Head	171.3	1000	100	1.34	0/3	More Active
2	Back	180.0	1000	100	1.5	0/3	More Active
3	Tail	173.0	1000	100	1.0	0/3	More Active

Table 4: Acute toxicity table for Seed extracts Lethal Dose (LD50) determination for (a) Phase b.

S/No	Label/Mark	Weight	Dose (Mg/Kg)	Conc. (Mg/mL)	Volume mL	Mortality	Remark
1	Head	164.0	1600	500	0.63	0/3	Calm
2	Back	138.4	2900	500	0.79	0/3	Calm
3	Tail	113.5	5000	500	1.14	0/3	Calm

Table 5: The effect of methanolic and *aqueous* extracts of leaf and seed of *Carica papaya* on red blood cell and white blood cell indices.

Treatment	WBC	Neut	Lymph	HCT	RBC	HBG	MCV	MCH	MCHC
LEA	7.35±3.32	27.25±16.32	72.75±16.32	53.69±2.86	9.05±0.50	174.75±11.76	59.50±1.92	19.45±1.12	325.00±9.05
LEM	7.08±1.15	28.50±14.80	71.50±14.80	53.89±2.15	8.83±0.73	177.50±12.40	61.50±3.42	20.10±0.55	329.00±14.99
NC	2.28±0.00	26.00±0.00	74.00±0.00	52.96±0.00	9.92±0.00	191.00±0.00	53.00±3.85	19.20±0.00	360.00±0.00
SEA	9.80±4.36	20.75±6.19	78.50±6.14	54.89±3.37	9.09±0.50	179.25±5.68	60.75±5.90	19.80±0.81	329.25±22.57
SEM	6.98±2.51	30.75±2.22	71.00±5.29	54.94±2.33	8.93±0.54	178.00±14.54	61.75±1.71	19.10±0.63	323.50±17.82

LEA: Leaf aqueous; LEM: Leaf methanolic; NC: Normal control; SEA: Seed aqueous; SEM: Seed methanolic; HCT: Hematocrit; HGB: Hemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration; MCV: Mean Corpuscular Volume, P>0.05.

Table 6: Effect of methanolic and aqueous extracts of leaf and seed of *Carica papaya* on serum biochemical indices.

Treatment	AST(µ/l)	ALT(µ/l)	ALP(µ/l)	TP(g/L)	Urea (mg/dl)	Na (mEq/l)	Cl-(mEq/l)	K+
LEA	66.33±24.83	20.00±4.36	41.19±2.19	67.41±3.35	40.12±14.14	139.90±4.59	87.15±1.19	3.54±0.48
LEM	55.50±10.54	20.25±2.22	43.99±1.89	62.74±4.54	41.73±12.39	142.13±2.08	88.63±3.64	3.87±0.12
NC	52.00±0.00	17.00±0.00	39.41±3.79	70.85±0.00	62.46±0.00	132.80±0.00	89.72±0.00	4.02±0.00
SEA	62.00±5.35	21.00±3.27	41.16±1.89	67.07±2.20	42.10±4.61	139.25±4.92	90.18±1.60	3.97±0.0.07
SEM	60.00±4.02	19.50±5.20	40.55±1.89	59.40±10.18	40.54±5.37	140.80±2.93	90.57±4.04	3.89±0.04

LEA: Leaf aqueous; LEM: Leaf methanolic; NC: Normal control; SEA: Seed aqueous; SEM: Seed methanolic; AST: Aspartate amino transferase; ALT: Alanine amino transferase; ALP: Alanine phosphatase; TP: Total protein; Urea; Na: Sodium; Cl-: Chlorine; K+: Potassium,p>0.05.

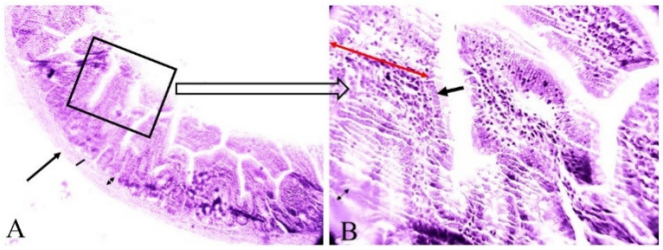

Figure1: Photomicrograph of the intestine of rat exposed to Methanolic and Aqueous extracts of *Carica papaya* Leaf and Seed.

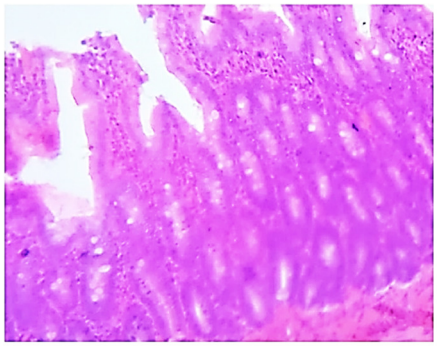

Figure 2: The intestinal histological appearance is devoid of lesion. Stain: Haematoxylin-eosin; Magnification: x400.

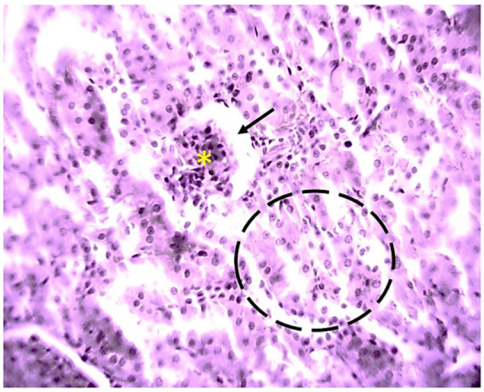

Figure 3: Photomicrograph of the kidneys of rat exposed to Methanolic and Aqueous extracts of *Carica papaya* Leaf and Seed.

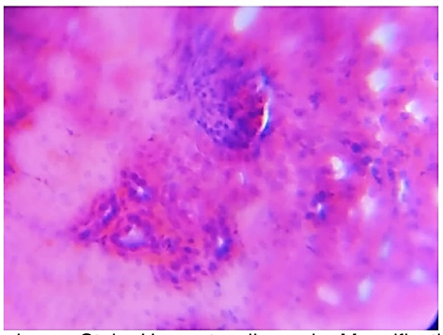

Figure 4: There is no visible lesion in the renal parenchyma. Stain: Haematoxylin-eosin; Magnification: x400

and 9). There was no visible lesion in splenic parenchyma as evidenced by the organization of parenchyma into red (black) and white pulps (white). Magnification: x400; Stain: Haematoxylin-eosin (Figures 9 and 10).

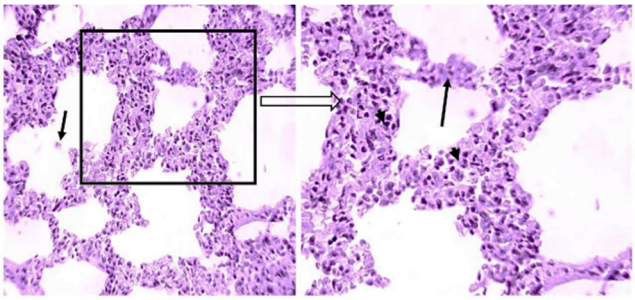

Figure 5: Photomicrograph of the lungs of rat exposed to Methanolic and Aqueous extracts of *Carica papaya* Leaf and Seed.

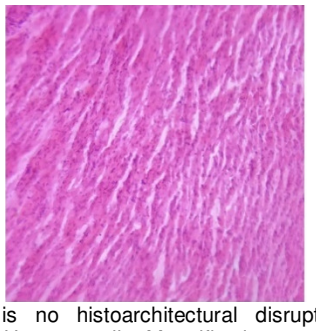

Figure 6: There is no histoarchitectural disruption in the heart parenchyma. Stain: Haematoxylin; Magnification: x400

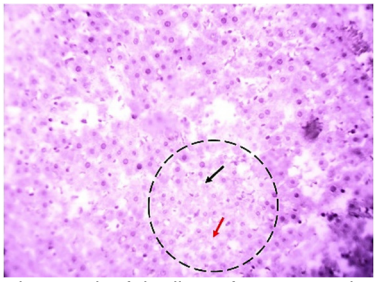

Figure 7: Photomicrograph of the liver of rat exposed to Methanolic and Aqueous extracts of *Carica papaya* Leaf and Seed.

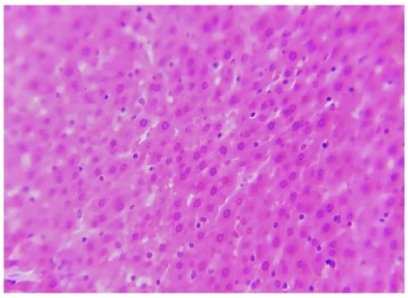

Figure 8: The hepatic parenchyma bears no visible lesion. Stain: Haematoxylin; Magnification: x400

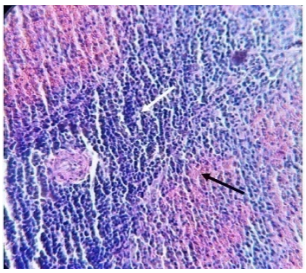

Figure 9: Photomicrograph of the spleen of rat exposed to Methanolic and Aqueous extracts of *Carica papaya* Leaf and Seed.

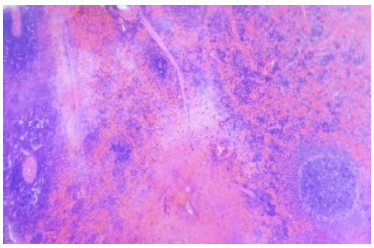

Figure 10. The splenic parenchyma is intact and devoid of lesion. Stain: Haematoxylin; Magnification: x400

DISCUSSION

The current study demonstrated that both methanolic and aqueous extracts of *Carica papaya* leaf and seed have anthelminthic activity against larval stages, as they completely stopped the motility of *Ascaris suum* similar to the report by Dikibo et al, (2012) in West African dwarf goats, *Carica papaya* seed was used for therapy against natural helminthic infection in goats. Other research publications on non-ruminants has suggested that the latex and seeds of the *Carica papaya* may have potential anthelmintic effects on helminths in mice, rats, pigs, and poultry (Dikibo et al., 2012, Baies et al., 2022).

According to Timothy et al. (2022), the anthelmintic properties of *C. papaya* seed extracts are related to the presence of benzyl isothiocyanate. The current study revealed that the efficacy of the plant extract are similar to the available patented drugs sold in the market. Methanolic leaf extracts at 200mg/kg and aqueous seed extracts at 12.5mg/kg and beyond, could be of value in the treatment of pigs ascariosis irrespective of solvent used to extract the active ingredients, which encourages further investigation of their use as therapeutic against *Ascaris suum* infections in endemic regions. Again, anthelminthic property may be attributed to the presence of papain in the seeds and leaf of of *C. papaya* and this is possible because papain is capable of digesting bacteria and parasitic cells, hence its use as an anthelmintic and antibiotics as reviewed by (Andrew et al., 2016).It is therefore, probable that a compound with similar mode of action to albendazole (benzimidazole) may be present in the plant and may be more soluble in water than ethanol and which can be used as an acaricide.

The seed of *Carica papaya* were similar to thiabendazole in terms of their activity against gastrointestinal helminths in SokotoRed goats, according to a prior study by (Ameen *et al.,* 2018). According to the results of another study, the seeds, stembark and leaves were both safe and effective at getting rid of intestinal

helminthes (Goku *et al.,* 2020). As was previously argued, this may be due to their unusual chemical makeup, which includes alkaloids, saponins, glycosides, and fixed oils. Since some of these phytoconstituents have been documented to possess anthelminthic activities (Garcia *et al.,* 2019), their presence thus may have contributed to the observed activity in this study. This finding might also support the conventional wisdom that the stem bark, flowers, roots, and seeds have anthelminthic properties in them and have been used in management of several conditions, chiefly among them are their roles in managing helminth infections (Agyare *et al.,*2014). The findings of this work may be of great advantage to a poor society since the plant is readily available year-round. Phytochemical screening of *C. papaya* leaves and seed extracts (Figure 2) revealed abundant secondary chemicals such as tannins, flavonoids, terpenes, and these have been linked to the plant's purported anti-microbial and ethno-medical effects Aziz *et al*., (2018).The presence of alkaloid, carbohydrate, glycoside, phenols, tannin, saponin, and oxalate shows the greater intensity of their presence in methanolic, ethanolic, hexane, and ethyl acetate extract as reported by Cyuzuzo *et al.,(*2020) who demonstrated that extracts exhibit significant inhibitory activity against *Candida albicans*. Another study by Siddiqui *et al.,* 2018, revealed that peels of banana and papaya fruits are potentially good source of antioxidant and antibacterial agents. The antimicrobial activity of the plant, which was visible in the antimicrobial activity against the tested organisms used for their study, was caused by these phytoconstituents, according to Alorkpa*et al*., 2016. Ethanol extract showed a significant broad-spectrum antimicrobial activity against both gram-positive and gram-negative bacteria.

Toxicology studies on the plant has shown that it contains less oxalate and alkaloids than other regularly consumed food products, and that the seeds and leaves are safe for ingestion (Fajimi and Taiwo, 2005).Acute toxicity testing helps to identify any negative effects that are anticipated to appear soon after receiving a single dosage of an experimental chemical (Loha *et al*., 2019). It has previously been established that any substance having LD_{50} of 5 g/kg ingested orally is safe or essentially non-toxic (OECD, 2001).

Timothy *et al.,* (2022) reported that The LD_{50} of papaya aqueous leaf extract which was given above 5000 mg/kg, was tolerated at a single large dose by Albino rats. Additionally, evaluation of the organ/body weight profiles as well as any haematological parameter was unaffected by the repeated administration of the test doses. In this present study, from the time the extracts were administered until 21 days later, all the treated rats were carefully inspected for any symptoms of toxicity for the initial acute toxicity trials. No one showed any overt toxicity or behavioral changes at dosages between 10 and 5000 mg kg-1. Except for those that were euthanized for histological evaluation, all of the treated rats were still alive three weeks after the observation period ended.

Aqueous papaya seed extract has been shown in prior research to be non-toxic, with LD_{50} values above 5000 mg/kg body weight of rats (Siddique et al., 2018) This research also came to similar conclusions, but went further to establish the LD_{50} for methanol, leaf, and seed extracts, which was discovered to be identical to the aqueous extract of Carica papaya seed. This demonstrates that papaya seed and leaf extracts from *Carica papaya* are both suitable for use in therapeutic settings. There were some histological abnormalities in the rats' vital organs after oral administration of the maximum dose (5000 mg kg-1) of the *Carica papaya* leaf and seed extract (Table 1a and 1b), albeit none of them resulted in death (Figure 1a-6b).

Histopathologic examination of internal organs of rats treated with varying doses of leaf and seed extracts showed areas of moderate thickening of alveolar septa with cellular debris in the alveolar space, the kidney had glomerular shrinkages and renal tubular distortion, the liver showed diffused hepatocellular degeneration typified by cytoplasmic vacuolation and nuclear degeneration (Figure 5a). Dikibo *et al*., (2012) discovered comparable lesions in the livers of Sprague-Dawley rats given varied doses of *Carica papaya* seed. The findings of that study demonstrated that Carica papaya can be hepatotoxic, especially when consumed in excess. Additionally, according to Fernandez-Checa and Kaplowitz (2005), hepatocytes are particularly vulnerable to damage because of their role in metabolite uptake and handling. They also deal with medicines and other harmful compounds. Kidney of Wistar rats when aqueous and methanolic leaf and seed extract of *C.papaya* was administered at a dose rate of 5000 mg kg^{-1}. There was moderate glomerular shrinkage, mild widening of urinary visible lesion and focal renal tubular distortion, these observations were made by Igbinovia *et al.,*(2015) who observed the effect of *Carica papaya* seed extract on the histology of the kidney in Wistar rats. Most organs exposed to lower doses and distilled water were relatively normal. Three weeks post-treatment, some organs especially the intestine, heart, liver and kidneys showed marked signs of villi regeneration, renal parenchyma was good, and other organs too showed a normal architecture as shown in the histopathological section of the intestine, liver, kidney, heart and spleen. It can be inferred from these results that the extract may be toxic to rats exposed to multiple doses of over 5000 mg kg^{-1} for a prolonged period.

Analysis of blood parameters is important in the evaluation of risks associated with test compounds under investigation as changes in the haematological system have a greater indicative value for animal toxicity (Olson *et al.,* 2000) In the present study, treatment with *Carica papaya* extracts on hematological parameter such as; WBC, HGB and RBC were found to be significantly increased in their values as compared to the control rats. Tarkang *et al*., (2012), reported similar findings in a study were *Carica papaya* aqueous extracts was administered for 28 and 90 days up to a dose of 1gKg-1BW in wistar rats

and an induced dose-dependent significant increase in all hematological parameters was observed. This is as a result of the stimulation of the hematopoietic system, leading to the production of WBC (leukopoiesis), RBC (erythropoiesis) and platelets (Guyton and Hall, 2006).The increase in HGB value in the treated rats may also be attributed to boosting up of the immune system (Adedapo *et al.,* 2007), or could suggests a strong immuno-modulatory, antioxidant and endothelial protection and repair activity of *Caraica papaya* extracts. Earlier studies had reported the membrane-protective activity, protection against hemolysis of the RBC (Imaga, 2009). An increase in HCT, as observed in this study is said to indicate an intracellular accumulation of water which sometimes appear in fasting rat, as also reported by Goku et al, (2020), Vismarra et al., (2023). However, an increase in the following parameters (HGB, HCT and RBC) could be an indication of dehydration (Halim *et al.,* 2011). To gain insight into pathological alterations and the nature of disease, assays of biochemical parameters were carried out to compare the lipid, glycemic, liver, and renal profiles of experimental and control animals and parameters such as TP, AST, ALT and ALP were determined although, earlier researchers describe that possible liver dysfunction when with herbal product/plant. (Everds, 2004) reported that an increase in albumin level maybe associated to abnormal liver function or dehydration state. As discussed by Ramaiah, (2007) abnormality in AST, ALT, and ALP levels is more specific for liver cell injury. In the present study there was no significant difference in the values of liver enzymes as compared to the control rats.

Conclusion

The findings of this study support the use of *Carica papaya,* particularly the leaf and seeds as an anthelmintic agent in the management of helminthosis or worm infestation by traditional or herbal practitioners and indigenous people.

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