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Original Research Article

Evaluation of *Spondias mombin*, *Senna occidentalis* and *Musa sapientum* against *Vibrio cholerae* O1 in experimental mice

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A B S T R A C T

The rapid emergence of multiple antibiotic resistant strains of *Vibrio cholerae* O1 in cholera management has necessitated the development of alternative therapeutic agents. This study investigated aqueous and ethanolic extracts of leaves of *Spondias mombin* and *Senna occidentalis* and stem sap of *Musa sapientum* against two epidemic strains of *V. cholerae* O1 (BA O1 and CVC O1) in experimental mice. Acute toxicity and *in vivo* studies were carried out using Swiss albino adult mice (15- 25 g) at varying dosages on the haematological and biochemical markers, and histopathology. Studies of acute toxicity on all the effective extracts revealed no lethality. The LD_{50} of the extracts were found to exceed 5000 mg/kg body weight. Haematological and biochemical parameters showed that there was significant effect ($p<0.05$) of the extracts at various concentrations on the parameters compared to the control group of mice. *In vivo* studies on the intestinal samples of mice showed mild loss of villi at lower dosage regime, at higher dosages, no lesion was observed compared to the control groups. Therefore, aqueous extracts of *S. mombin* and *S. occidentalis* are recommended as good alternatives in the treatment of epidemic cholera.

Keywords

Histopathology; Antimicrobial; *Spondias mombin*; *Senna occidentalis; Musa sapientum* ; cholera; Nigerian Herbs

Introduction

Vibrio cholerae is the regular cause of cholera that most frequently necessitates hospitalisation (Sack *et al.,* 2003). Historically, a large number of cholera epidemics have been associated with multiple-antibiotic resistant (MAR) of *V. cholerae* (Fakruddin *et al.,* 2011). The antibiotics resistance pattern of epidemic strains has also changed frequently, and the emergence of *V. cholerae* O1 and O139 with different antibiograms have been documented (Faruque *et al.,* 1998). In many instances, the emergence of strains with altered antibiotic resistance has been associated with large epidemic of cholera (Faruque *et al.,* 1998). The development of MAR organisms has constituted a global problem as far as

treatment of some infectious diseases is concerned (Greenwood *et al.,* 2009, Anthonia and Olumide, 2010). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from various sources such as medicinal plants.

Plants derived medicines have been part of traditional health care in most part of the world for thousands of years, and there is increasing use of them as sources of agent to fight microbial disease (Ajayi and Akintola, 2010; Ghaleb *et al.,* 2009; Mohana *et al.,* 2008). In recent years, there has been gradual revival of interest in the use of medicinal plants in developed as well as developing countries, because medicinal plants constitute an effective source of antimicrobial natural product with proven potential of treating infectious diseases comparable to synthetic drug agents (Valarmathy *et al.,* 2010; Akinyemi *et al.,* 2006). Members of the plants, *Spondias mombin, Senna occidentalis,* and *Musa sapientum* commonly called "*Iyeye*", "*Ewe ori-esi*" and "*Ogede wewe*" in the Yoruba land belonging to the family Anacardiaceae, Fabaceae and Musaceae respectively, have been reported to possess antimicrobial properties and have been used in treating numerous microbial problems (Taylor, 2006). The leaves of these plants are a common remedy to various digestive problems such as stomach aches, dysentery, diarrhoea, constipation, heart diseases, and liver detoxifier and as antiviral, antibacterial, antihelminthic, antifungal, anticarcinogenic, and antispasmodic agents.

Previous evaluation of the antimicrobial property of *Spondias mombin, Senna occidentalis* and *Musa sapientum,* justify their use as antimicrobial agent against epidemic strain of *Vibrio cholerae* O1. This research was designed to evaluate the toxicity and effectiveness of these plants in experimental animals.

Materials and Methods

Plants

The plants used for this study are *Spondias mombin, Senna occidentalis* and *Musa sapientum* (leaves and stem) were harvested fresh from the field.

Collection of *Vibrio cholerae* strains

Bauchi isolate (BA O1) of *Vibrio cholerae* serogroup O1, biotype El tor, serotype Ogawa and controlled strain of *Vibrio cholerae* (CVC O1) serogroups O1, biotype El tor, serotype Inaba from Centre of Disease Control (CDC) Kenya were obtained from the department of Molecular Biology and Biotechnology, Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State, Nigeria.

Acute Toxicity Study (LD_{50})

Six weeks to 8weeks female adult mice weighing between 16 g to 25 g and were physically examined. The animals were housed in plastic cages of 53 cm x 38 cm x 20 cm. They were fed and allowed to stabilize for 1week before use. Prior to use, the mice were grouped into four of five mice per group and allowed to fast for 18 h. Different extract concentrations were prepared (1000 mg/kg, 3000 mg/kg, and 5000 mg/kg) and administered orally. Three groups were fed with each concentration of extracts and the fourth group were fed with distilled water. The mice were then monitored for exhibition of symptoms for 72 hr period. The symptoms were scored in order of mortality rates (Akhila *et al.,* 2007).

Test for Biochemical Markers

The biochemical parameters were determined using the RANDOX kit (Randox Laboratories Ltd., Crumlin, United Kingdom) and analyzed photometrically using the Rx Monza Analyser.

Urea

Urea test was carried out by the Urease-Berthelot principle (Weatherburn, 1967). Urea in the serum was hydrolysed to ammonia in the presence of urease, to give a blue green coloured complex, which was determined by photometry at 546nm. Normal values in the serum of mice ranges from 17 - 71 (U/I).

Bilirubin (BIL)

Bilirubin was determined using the method described by Jendrassik and Grof, (1938). Direct (conjugated) bilirubin reacted with diazotised Sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin was determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotised Sulphanilic acid. These were determined by photometry at 578nm. Normal values in the serum of mice ranges from 0 - 1.99 (mg/dl).

Creatinine (CREA)

This was done by a method described by Bartels and Bohmer, (1972). Creatinine in alkaline solution reacted with picric acid, which form a deep yellow complex. The amount of the complex formed is directly proportional to the creatinine concentration in the serum, which was measured photometrically at 492nm. Normal values in the serum of mice ranges from 0.12 – 0.9 (mg/dl).

Alkaline Phosphate (ALP)

This was carried out using the optimized standard method according to the recommendations of the Deutsche Gesellschaft fur Klinische Chemie, (1972). P-nitrophenylphosphate was hydrolysed in the presence of alkaline phosphatase. This was the measured photometrically at 405nm. Normal values in the serum of mice ranges from 35 - 96 (U/I).

Aspartate Aminotransferase (AST)

This was carried out by a colorimetric method described by Reitman and Frankel, (1957). AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-Dinitrophenylhydrazine to form a corresponding hydrazone, a brownish red coloured complex in an alkaline medium. The colour intensity is directly proportional to the AST concentration in the serum and was measured by photometry at 546nm. Normal values in the serum of mice ranges from 54-298 (U/I).

Alanine Aminotransferase (ALT)

This was carried out by a colorimetric method described by Reitman and Frankel, (1957). ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-Dinitrophenylhydrazine to form a corresponding hydrazone, a brownish red coloured complex in an alkaline medium. The colour intensity is directly proportional to the ALT concentration in the serum and was measured photometrically at 505nm. Normal values in the serum of mice ranges from 15-84 (U/I).

Packed Cell Volume (PCV)

Blood samples were collected through puncture of the eye and introduced into heparinized tubes up to about three-quarter capacity. With one end of the tube sealed up with a sealant, the tubes were then placed into the microhematocrit centrifuge and preset to a speed of 10,000 rpm for 5mins. The PCV were then read on a microhematocrit reader (Brian *et al.*, 2000). Normal values in mice range from 39 – 49 (%).

Effective Study (ED_{50}): *In Vivo* Evaluation of Plant Extracts in Adult Mouse Model

Adult mice (6weeks to 8weeks) were obtained, weighed and physically examined. The animals were housed in plastic cages of 53cm x 38cm x 20cm. They were fed and allowed to stabilize for 1week before use. The mice were tested in four groups of five mice each. Each mouse were orally inoculated with 100µl (10^6cells) of *V. cholerae* via a 20guage vial needle. The mice were then dosed with individual plant extract (83mg/ml, 166mg/ml and 332.5mg/ml for SMWE, SMEE and COWE respectively) and at 4hr after administration of toxigenic strains of *V. cholerae*. Each group of animals was monitored for 24hr.

Routine Histopathology

Mice intestinal samples were harvested following infection. These were immediately washed briefly in PBS and then fixed in 10% formalin in PBS overnight at 4^oC and imbedded in paraffin prior to sections being cut for staining with hematoxylin and eosin (HE).

Slides prepared from paraffin sections of colonized intestine were treated with xylene to remove wax and the rehydrated in graded ethanol (absolute, 90%, 70%, 50%, and 0%) stained with xylene and counterstained with hemoxylene. The stained specimen were further dehydrated in graded ethanol and then mounted for photomicrographs. The photomicrographs were taken in bright field under a leitz DIALUX research microscope.

Statistical Analysis

The data are expressed as mean. The significance of differences among all steps of the groups was analyzed through analysis of variance (ANOVA); also, the interaction between the concentration of plant extracts and the plant extract types were analyzed using general linear model (GLM); if the F value was found to be significant, differences between means were then analyzed with the post-hoc (Duncan) test. Differences were considered statistically significant when the p value was < 0.05 (Duncan, 1977).

Results and Discussion

Acute Toxicity Study (LD_{50}) of Effective Crude Plant Extract

For all the extracts used (*S. mombin* water extract, *S. mombin* ethanol extract, *S. occidentalis* water extract), no mortality was recorded after 72 h of observation.

Haematology and Biochemical Parameters

Table 1, 2, 3 shows the analysis of variance (ANOVA) of the effect of the plant extracts at 1000mg/kg, 3000mg/kg and 5000mg/kg body weight. There was significant difference ($p<0.05$) in the

haematological and biochemical parameters at each concentration.

Table 4 compares the effects of the plant extracts at varying dosage on the haematological and biochemical parameters compared to the control groups. Table 5 shows the interactive effect of concentration of extract and plant extract type on haematology and biochemical parameters. The interaction gave a better understanding of the how the concentration and extract affected all the blood parameters. The interaction shows whether the effect of the extracts on the blood parameters depended on the concentration of such extracts.

This study investigated aqueous and ethanolic extracts of leaves of *Spondias mombin* and *Senna occidentalis* and stem sap of *Musa sapientum* against two epidemic strains of *V. cholerae* O1 (BA O1 and CVC O1 *in vivo* on female Swiss albino mice (18 – 22g) according to the guideline set by Organization for Economic Co-operation and Development (OECD) for testing chemicals and Akhila *et al.* (2007). For this study, doses were fixed at 1000mg/kg b.w, 3000mg/kg b.w and 5000mg/kg b.w.

In acute toxicity test, no adverse effect or mortality was detected in the mice up to 5g/kg body weight per oral during the 72 h observation. Thus, according to the guideline set by Organization for Economic Co-operation and Development (OECD) for testing chemicals, these plants are not toxic and safe for use (OECD, 2001; Akhila *et al.,* 2007). This result is in agreement with the earlier works reported by Ayoka *et al.* (2005); Uchendu and Isek (2008); Sini *et al.* (2010). Also, water extract of *S. occidentalis* did not show any adverse effect or mortality detected in mice up to 5000mg/kg. This is also in agreement with research work by Sini *et al.* (2010).

During the study it was noted that at various doses of *S. mombin* extract, newly born mice were found. *Spondia mombin* is reported to have uterine stimulant action as well as abortive effects in three studies with laboratory animals (mice and guinea pig) (Offiah and Anyanwu, 1989; Barros *et al.,* 1970; Akubue *et al.,* 1983). According to the database file for *Spondias mombin*, extracts of *S. mombin* are a common midwife's remedy to help induce labour, reduce bleeding and pains during and after childbirth, to bring on the flow of breast milk, and as a vaginal wash to prevent or treat uterine or vaginal infection after childbirth.

Further studies conducted in assessing the toxic effect on the haematology and biochemical marker include the packed cell volume (PCV), the liver function tests (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin (BIL)) and the kidney function test (creatinine (CRE) and urea (URE)).

At 1000mg/kg, there was significant effect of the various extracts on the haematological and biochemical parameters. Water extract of *S. occidentalis* and ethanolic extract of *S. mombin* displayed erythropoesis as indicated by significant increase in the PCV compared to *S. mombin* water extract. Erythropoesis is an increase in the level of red blood cells than normal compared to the whole blood. This finding corresponds with reports by Taylor

Table.1 Effect of plant extract on the Haematology and Biochemical Parameters Examined during Acute Toxicity Study at 1000 mg/kg body weight

Plant Extracts	PCV	ALP	ALT	AST	D/BIL	T/BIL	CREA	URE
SMWE	26.67^{a}	3.63^{a}	17.91^{a}	38.33^{a}	0.37^{a}	0.52^{a}	0.15^{a}	24.64^{a}
SMEE	49.33^{b}	34.96^{ab}	40.70^{b}	67.69^{b}	0.58^{b}	0.61^{b}	0.21^{b}	30.32^{b}
SOWE	54.00^{b}	63.15^{b}	84.50^{c}	77.01^{c}	0.85^{c}	0.95^{c}	0.66^{c}	76.80^{c}
Control groups	42.00^{b}	7.32^{a}	20.00^{a}	61.58^{b}	0.40^{a}	0.62^{b}	0.41^{a}	25.43^{a}
Normal range	39 - 49	35 - 96	15 - 84	54 - 298	0 -1.99		0.12-0.9	17-71

The values are expressed as mean (n=3). Values with the same superscript are not significantly different at $p<0.05$.

SMWE = Spondias mombin *water Extract; SMEE* = Spondias mombin *Ethanol Extract; SOWE* = Senna occidentalis *water Extract; PCV=Packed cell volume; ALP=Alkaline phosphatase; ALT=Alanine Aminotransferase; AST=Aspartate Aminotransferase; D/BIL=Direct bilirubin; T/BIL=Total bilirubin; CREA=Creatinine; URE=Urea.*

Table.2 Effect of Plant Extract on the Haematology and Biochemical Parameters Examined during Acute Toxicity Study at 3000 mg/kg Body Weight

Plant Extracts	PCV	ALP	ALT	AST	D/BIL	T/BIL	CREA	UREA
SMWE	35.60^{a}	12.63^{a}	82.50^{c}	106.68^{b}	1.23^{c}	2.00^{c}	0.21^{a}	53.42^{c}
SMEE	36.33^{a}	47.19^{b}	60.31^{b}	75.00^{a}	0.36^{b}	0.62^{b}	0.25^{a}	23.46^{a}
SOWE	47.00^{b}	3.73^{a}	15.73^{a}	140.37^{c}	0.06^{a}	0.32^{a}	0.74^{b}	24.38^{b}
Control groups	42.00^{b}	7.32^{a}	20.00^{a}	61.58^{b}	0.40^{a}	0.62^{b}	0.41^{a}	25.43^{a}
Normal range	39 - 49	35 - 96	15 - 84	54 - 298	0 -1.99		0.12-0.9	17-71

The values are expressed as mean (n=3). Values with the same superscript are not significantly different at $p<0.05$.

KEYS: *SMWE* = Spondias mombin *water Extract; SMEE* = Spondias mombin *Ethanol Extract; SOWE* = Senna occidentalis *water Extract; PCV=Packed cell volume; ALP=Alkaline phosphatase; ALT=Alanine Aminotransferase; AST=Aspartate Aminotransferase; D/BIL=Direct bilirubin; T/BIL=Total bilirubin; CREA=Creatinine; URE=Urea.*

Table.3 Effect of Plant Extract on the Haematology and Biochemical Parameters Examined during Acute Toxicity Study at 5000 mg/kg Body Weight

Plant extracts	PCV	ALP	ALT	AST	D/BIL	T/BIL	CREA	UREA
SMWE	42.00^{b}	60.48^{b}	32.50^{b}	105.07^{c}	0.53^{a}	0.94^{b}	0.17^{a}	45.61^{c}
SMEE	35.37^{a}	83.49^{c}	33.96^{b}	77.02^{a}	0.62^{a}	0.67^{a}	0.42^{b}	23.47^{a}
SOWE	52.00^{c}	5.01^{a}	23.00^{a}	103.11^{b}	1.20^{b}	1.83^{c}	0.58^{c}	29.58^{b}
Control groups	42.00^{b}	7.32^{a}	20.00^{a}	61.58^{b}	0.40^{a}	0.62^{b}	0.41^{a}	25.43^{a}
Normal range	39 - 49	35 - 96	15 - 84	54 - 298	0 -1.99		0.12-0.9	17-71

The values are expressed as mean (n=3). Values with the same superscript are not significantly different at $p<0.05$.

KEYS: SMWE = *Spondias mombin* Water Extract; SMEE = *Spondias mombin* Ethanol Extract; SOWE = *Senna occidentalis* water Extract; PCV=Packed cell volume; ALP=Alkaline phosphatase; ALT=Alanine Aminotransferase; AST=Aspartate Aminotransferase; D/BIL=Direct bilirubin; T/BIL=Total bilirubin; CREA=Creatinine; URE=Urea.

Table.4 Comparison between the Effects of the Plant Extracts With Varying Dosage on Haematological and Biochemical Parameters

Plant Extract	Dosage (mg/kg)	PCV	ALP	ALT	AST	D/BIL	T/BIL	CREA	URE
SMWE	1000	26.67^{a}	34.96^{b}	84.50^{c}	38.33^{a}	0.58^{a}	0.61^{a}	0.21^{a}	76.80^{c}
	3000	35.67^{ab}	12.63^{ab}	82.50^{c}	106.68^{d}	1.23^{b}	2.00^{c}	0.21^{a}	53.42^{b}
	5000	42.00^{b}	60.48^{c}	32.50^{b}	105.07^{c}	0.53^{a}	0.94^{b}	0.17^{a}	45.61^{a}
SMEE	1000	49.33^{b}	63.15^{ab}	40.70^{b}	67.69^{a}	0.85^{c}	0.95^{c}	0.15^{a}	24.64^{b}
	3000	36.33^{a}	47.18^{a}	60.31^{c}	75.00^{b}	0.36^{a}	0.62^{a}	0.25^{b}	23.46^{a}
	5000	35.37^{a}	83.49^{b}	33.96^{a}	77.02^{c}	0.62^{b}	0.67^{b}	0.42^{c}	23.47^{a}
SOWE	1000	54.00^{b}	3.63^{a}	17.92^{b}	77.01^{a}	0.37^{b}	0.52^{b}	0.66^{ab}	30.32^{a}
	3000	47.67^{a}	3.73^{a}	15.73^{a}	140.37^{c}	0.057^{a}	0.32^{a}	0.74^{b}	24.38^{a}
	5000	52.00^{b}	5.01^{a}	23.00^{c}	103.11^{b}	1.203^{c}	1.83^{c}	0.58^{a}	29.58^{a}
Control groups		42.00^{b}	7.32^{a}	20.00^{a}	61.58^{b}	0.40^{a}	0.62^{b}	0.41^{a}	25.43^{a}
Normal range		39 - 49	35-96	15- 84	54 - 298		0-1.99	0.12-0.9	17-71

The values are expressed as mean. Values with the same superscript are not significantly different at $p<0.05$.

KEYS: SMWE = Spondias mombin water Extract; SMEE = Spondias mombin Ethanol Extract; SOWE = Senna occidentalis water Extract; PCV=Packed cell volume; ALP=Alkaline phosphatase; ALT=Alanine Aminotransferase; AST=Aspartate Aminotransferase; D/BIL=Direct bilirubin; T/BIL=Total bilirubin; CREA=Creatinine; URE=Urea.

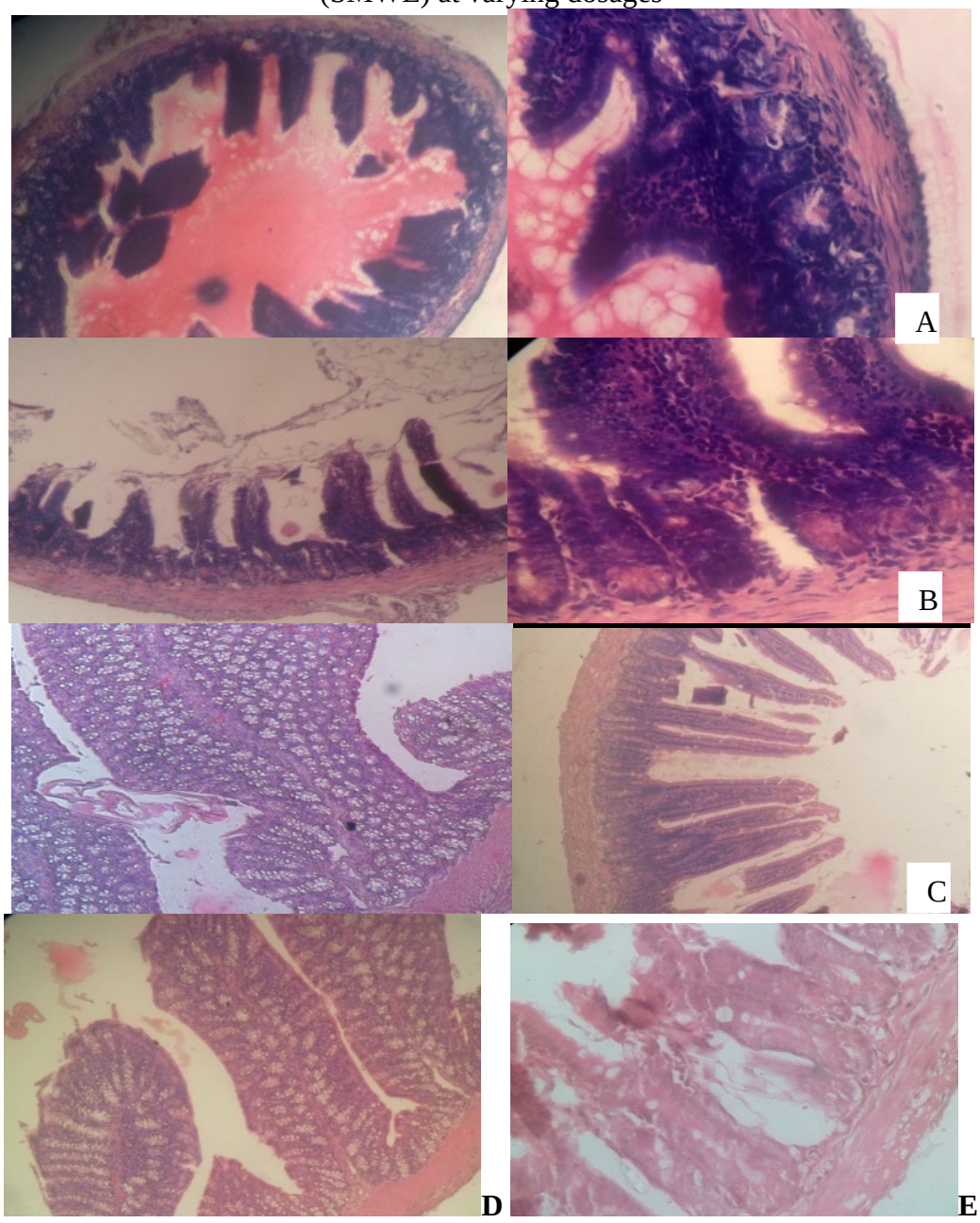
Table.5 Interactive Effect of Concentration of Extracts and Plant Extract Types on Haematology and Biochemical Parameters

Plant Extracts	PCV	ALP	ALT	AST	D/BIL	T/BIL	CREA	UREA
SMWE	37.00^{a}	35.01^{b}	66.50^{c}	83.48^{b}	0.79^{c}	1.16^{c}	0.20^{a}	58.81^{c}
SMEE	39.00^{a}	69.68^{c}	45.06^{b}	73.21^{a}	0.61^{b}	0.74^{a}	0.27^{b}	23.85^{a}
SOWE	50.83^{b}	4.11^{a}	18.91^{a}	106.48^{c}	0.56^{a}	0.88^{b}	0.67^{c}	26.96^{b}
Control groups	42.00^{b}	7.32^{a}	20.00^{a}	61.58^{b}	0.40^{a}	0.62^{b}	0.41^{a}	25.43^{a}
Normal range	39-49	35- 96	15 - 84	54- 298		0 -1.99	0.12-0.9	17-71

The values are expressed as mean. Values with the same superscript are not significantly different at $p<0.05$

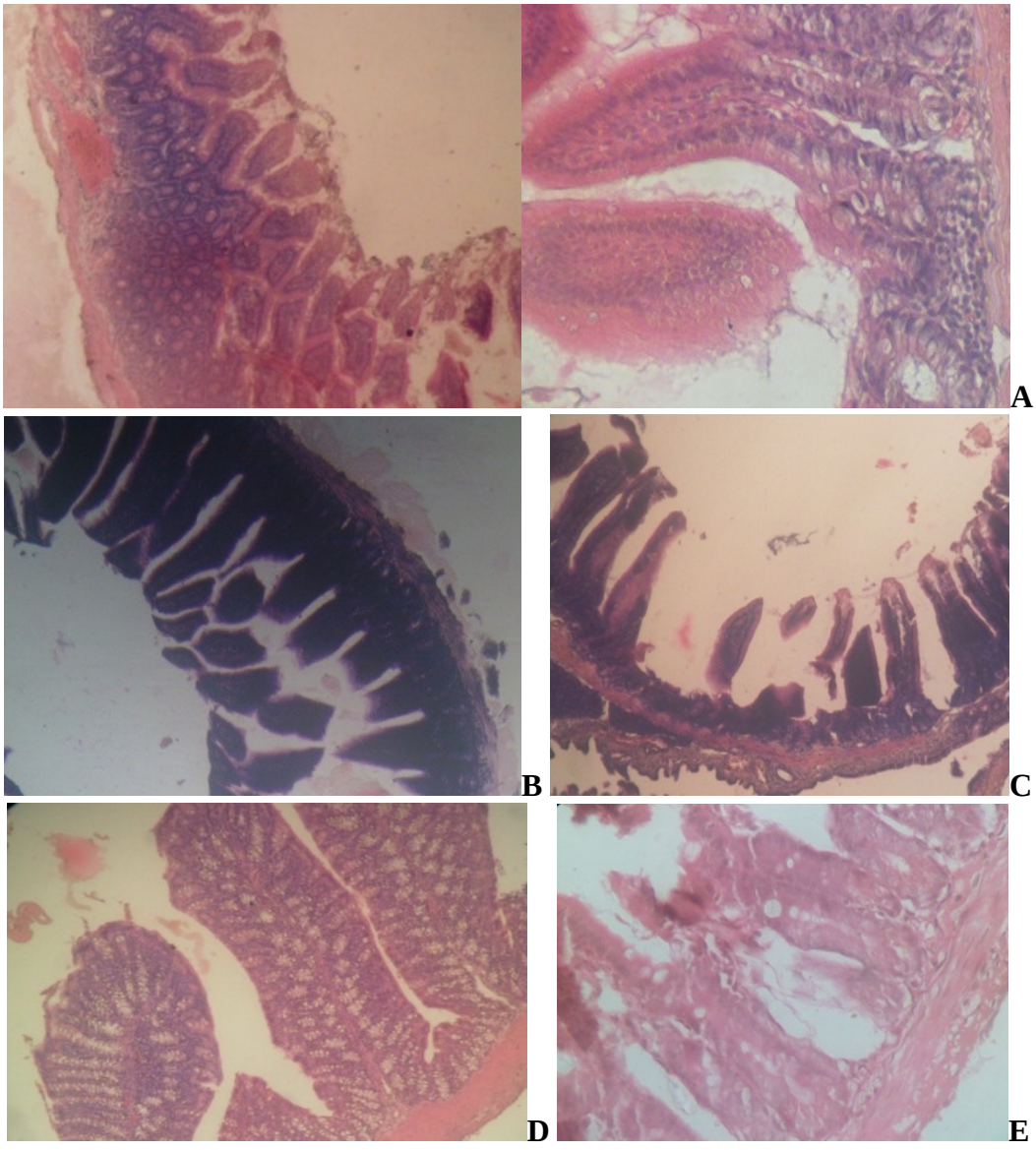
KEYS: SMWE = *Spondias mombin* water Extract; SMEE = *Spondias mombin* Ethanol Extract; COWE = *Senna occidentalis* water Extract; PCV=Packed cell volume; ALP=Alkaline phosphatase; ALT=Alanine Aminotransferase; AST=Aspartate Aminotransferase; D/BIL=Direct bilirubin; T/BIL=Total bilirubin; CREA=Creatinine; URE=Urea.

Plate.1 Histopathology of CVC O1 infected mice treated with *S. mombin* Water Extract (SMWE) at varying dosages



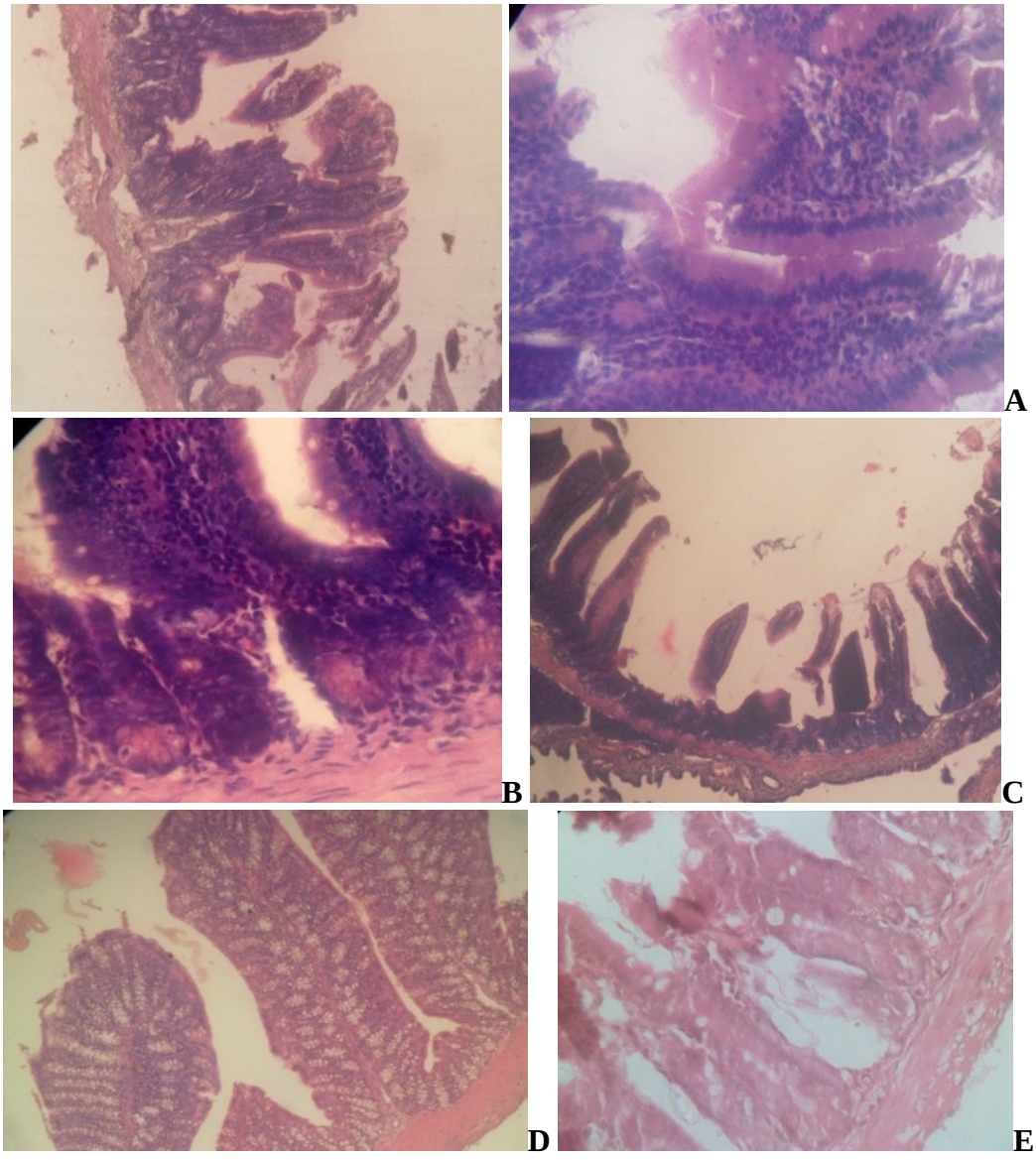
A(1000mg/kg): moderate loss of villi, which are stumpy, clubbed and fused. Necrosis is seen at the crypt of lieberkuhn and mononuclear cell infiltration in the lamina propria; **B(3000mg/kg):** sparse, isolated clubbed shaped villi with vascular degeneration and necrosis of the cells of crypt of lieberkuhn; **C(5000mg/kg):** Goblet hyperplasia seen; **D(positive control):** Normal except goblet hyperplasia; **E(untreated):** Necrosis of the mucosal epithelial cells. The villi were at different sizes and height, stumpy and showed exfoliating mucosa cells. Mononuclear cell infiltration in the lamina propria.

Plate.2 Histopathology of CVC O1 infected mice treated with *S. mombin* Ethanol Extract (SMEE) at varying dosages



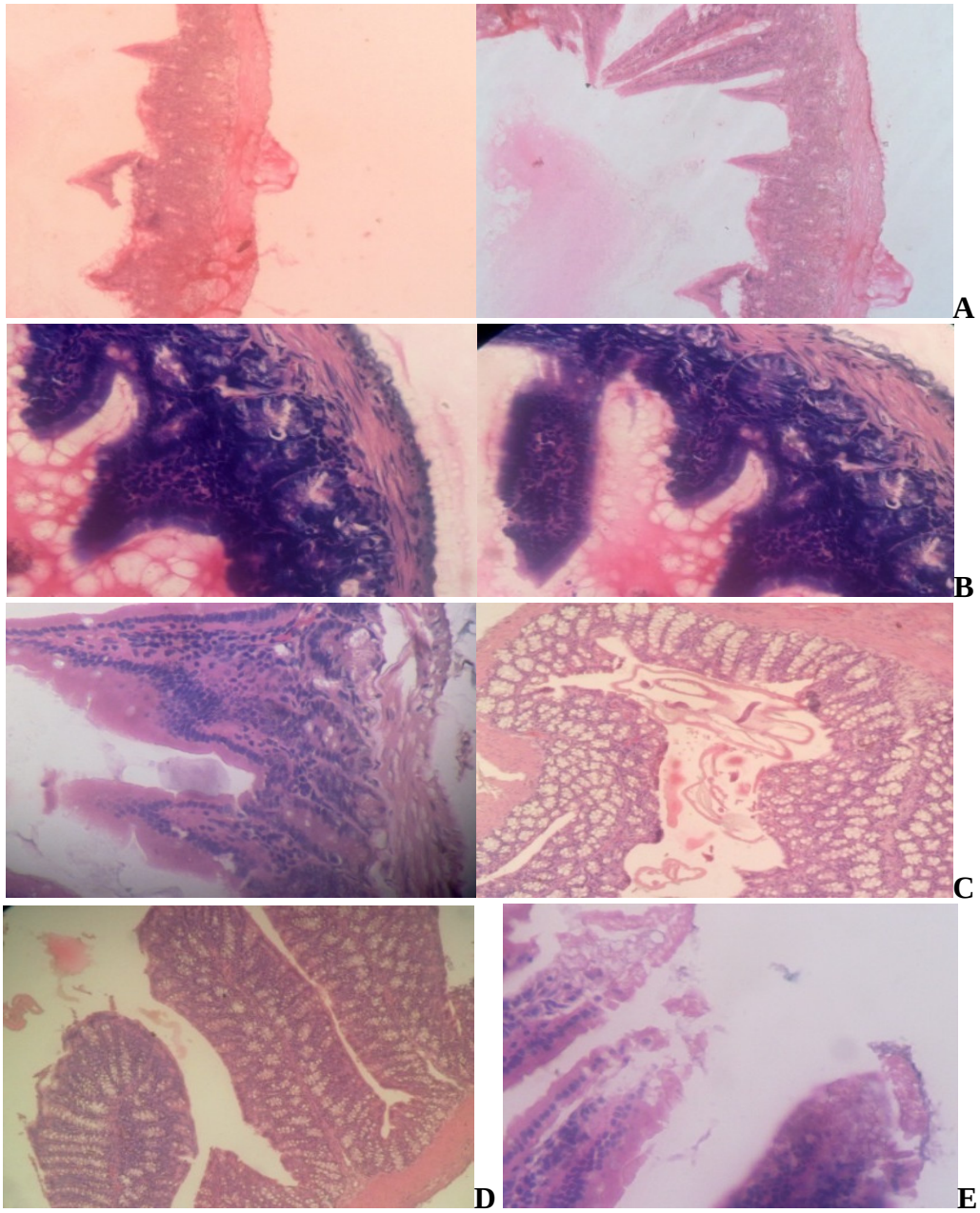
A(1000mg/kg): Stumpy and club-like villi and goblet hyperplasia seen; **B(3000mg/kg):** Mineralization of the villi; **C(5000mg/kg):** Loss of villi and mineralization of the necrotic area; **D(positive control):** Normal except goblet hyperplasia; **E(untreated):** Necrosis of the mucosal epithelial cells. The villi were at different sizes and height, stumpy and showed exfoliating mucosa cells. Mononuclear cell infiltration in the lamina propria

Plate.3 Histopathology of CVC O1 infected mice treated with *S. occidentalis* Water Extract (SOWE) at varying dosages



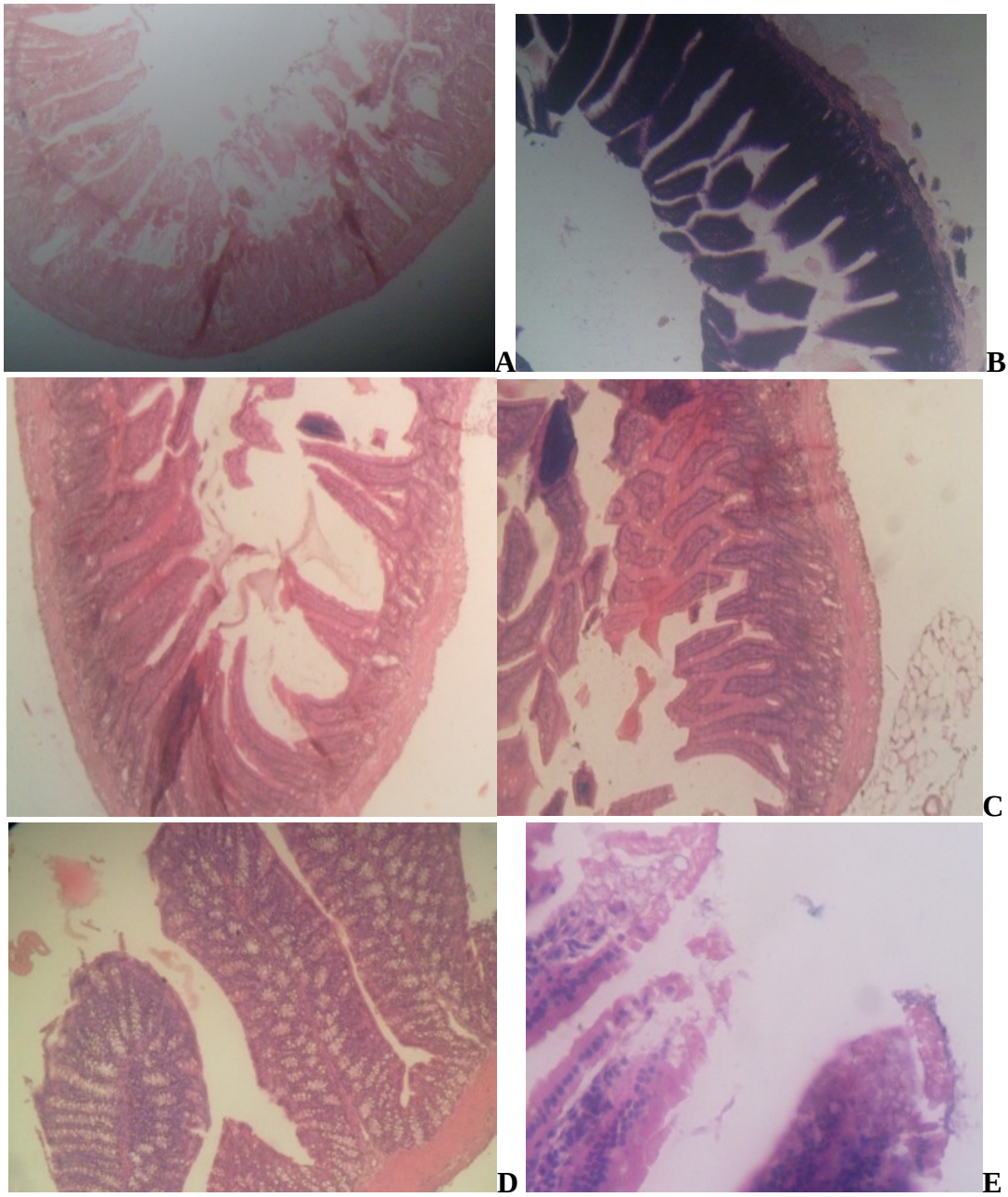
A(2000mg/kg): Mild diffuse loss of villi with proliferating mucosal epithelial cells, some were stumpy isolated and cling together. Necrosis of the crypt of lieberkuhn; **B(3000mg/kg):** sparse, isolated clubbed shaped villi with vascular degeneration and necrosis of the cells of crypt of lieberkuhn; **C(5000mg/kg):** Loss of villi and mineralization of the necrotic area; **D(positive control):** Normal except goblet hyperplasia; **E(untreated):** Necrosis of the mucosal epithelial cells. The villi were at different sizes and height, stumpy and showed exfoliating mucosa cells with mononuclear cell infiltration in the lamina propria.

Plate.4 Histopathology of BA O1 infected mice treated with *S. mombin* Water Extract (SMWE) at varying dosages



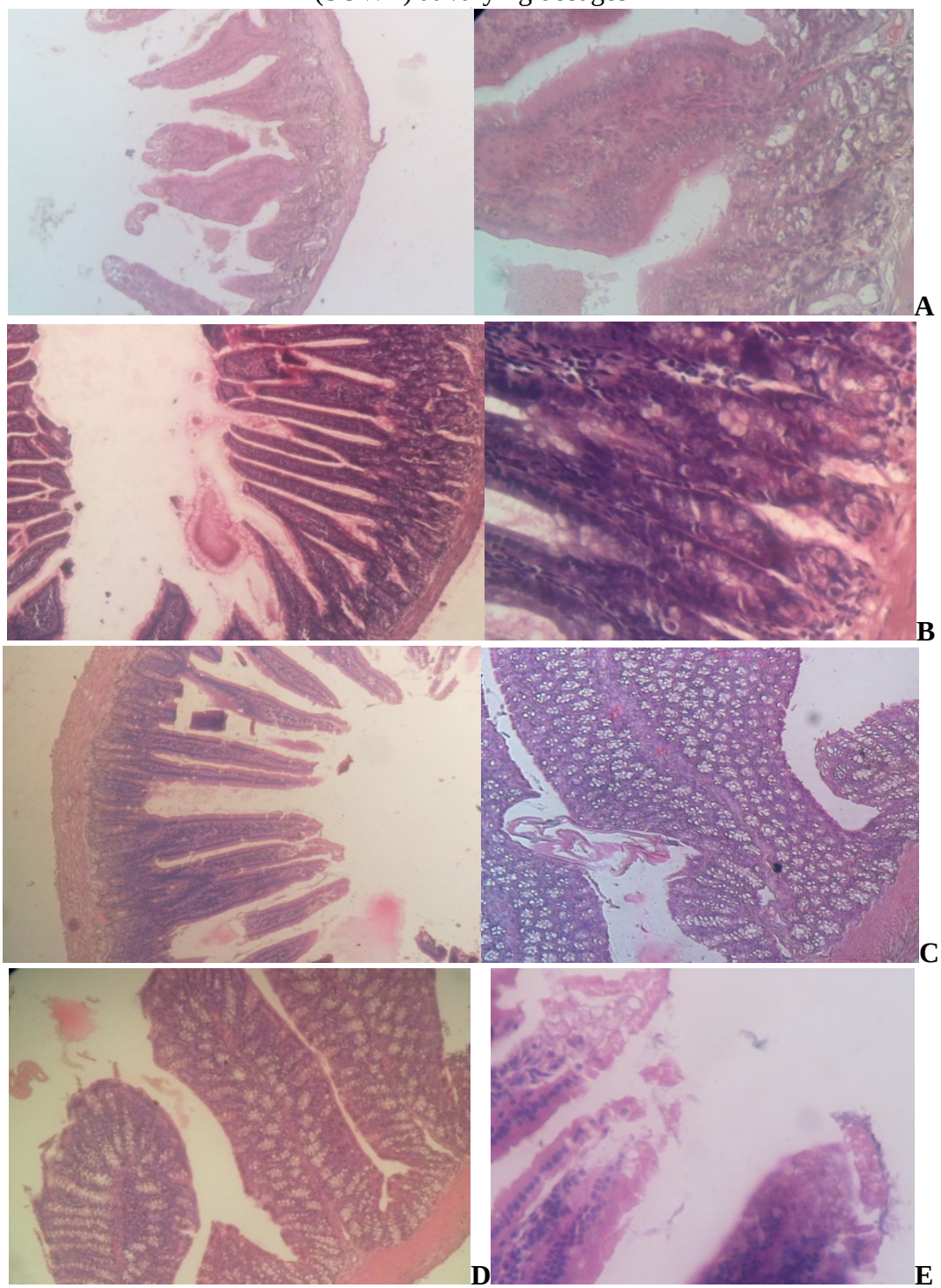
A(2000mg/kg): Slight loss of the villi, with the tips of the villi showing necrosis appearing stumpy and blunt with proliferation of the mucosa epithelial cells; **B(3000mg/kg):** Loss of villi with stunted vacuolar degeneration and necrosis of the epithelial cells in the crypt of lieberkuhn with slightly intestinal mononuclear cell infiltration in the lamina propria; **C(5000mg/kg):** Regeneration of the epithelial cells with some villi appearing stumpy, having necrosis of the epithelial cells at the tip and proliferation of mucosa epithelial at the base of the villi. Severe diffuse proliferation of the goblet cells; **D(positive control):** Normal except goblet hyperplasia; **E(untreated):** Necrosis of the tip of the villi, which are stumpy club-like and fused. Mononuclear cells infiltration of the lamina propria and the lamina muscularis. Proliferation of the mucosa epithelial cells.

Plate.5 Histopathology of BA O1 infected mice treated with *S. mombin* Ethanol Extract (SMEE) at varying dosages



A(2000mg/kg): Severe loss of villi, they appeared stumpy and fused together; **B(3000mg/kg):** Mineralization of the villi; **C(5000mg/kg):** No visible lesion seen; **D(positive control):** Normal except goblet hyperplasia; **E(untreated):** Necrosis of the tip of the villi, which are stumpy club-like and fused. Mononuclear cells infiltration of the lamina propria and the lamina muscularis with proliferation of the mucosa epithelial cells.

Plate.6 Histopathology of BA O1 infected mice treated with *S. occidentalis* Water Extract (SOWE) at varying dosages



A(2000mg/kg): Stumpy and club-shaped villi. Goblet cells hyperplasia with necrosis of the epithelial cells of the crypt of lieberkuhn; **B(3000mg/kg):** Goblet cell hyperplasia in the crypt of lieberkuhn; **C(5000mg/kg):** Goblet cell hyperplasia; **D(positive control):** Normal except goblet hyperplasia; **E(untreated):** Necrosis of the tip of the villi, which are stumpy club-like and fused. Mononuclear cells infiltration of the lamina propria and

the lamina muscularis with proliferation of the mucosa epithelial cells.

(2006); Yadav *et al.* (2009); Hamenoo (2010) stating their hepatoprotective ability and use as blood tonic. Also, *S. occidentalis* water extract brought about significant increase in levels of all the biochemical markers followed by *S. mombin* ethanolic extract and *S. mombin* water extract, though values are within the normal range in strains of Swiss albino mice. These findings are in line with the works reported by Nuhu and Aliyu (2008); Emeka and Funmilayo (2011).

At 3000mg/kg there were also significant effects of the various extracts on the haematological and biochemical parameters. There was significant decrease in the PCV values which fell below normal range in *S. mombin* water extract and *S. mombin* ethanol extract but within normal range in *S. occidentalis* water extract. Decrease in PCV value is an indication in the destruction of red blood cells (i.e. anaemia). This is in agreement with the report by Taylor (2006) that leaves of *S. mombin* have hemostatic action. There was no significant difference in the PCV and CRE values of *S. mombin* water extract and *S. mombin* ethanol extract. This is in consonance with the findings of Igwe *et al.* (2009) when comparing the effect of *S. mombin* on the kidney function profile of rabbits. There was also significant increase in the level of the biochemical markers. Highest values were obtained with *S. mombin* water extract for ALT, BIL and URE, while *S. mombin* ethanol extract gave highest value in ALP and *S. occidentalis* water extract for AST and CRE. Also, these values fall within normal ranges of values.

At 5000mg/kg, the results obtained agree with that of Taylor, (2006); Igwe *et al.* (2009); Yadav *et al.* (2009). There was significant increase in the PCV values of *S. occidentalis* water extract compared to *S. mombin* water extract and *S. mombin* ethanol extract. While PCV values of *S. occidentalis* water extract and *S. mombin* ethanol extract shows normal, it fell below normal in *S. mombin* water extract. There was also significant increase in the level of the biochemical markers. Highest values were obtained with *S. mombin* water extract for AST and URE, while *S. mombin* ethanol extract gave highest value in ALP and ALT while *S. occidentalis* water extract gave highest values for BIL and CRE. There was no significant difference in the values of BIL and ALT of *S. mombin* water extract and *S. mombin* ethanol extract. Also, these values were within normal ranges.

Hyperbilirubinaemia is often the first and sometimes the only manifestation of liver disease (Lee and Ganer, 1983). Impaired hepatic bilirubin clearance due specifically to reduced uptake or possible competition for binding to 2-protein or ligandin, is associated with the administration of drugs such as rifampicin, bunamiodyl, flaraspidic acid and probanecid, but plasma concentration rapidly reverts to normal following discontinuous use of the offending drug (Hauser and Gollan 1988). Another possible explanation for the mild hyperbilirubinaemic effect of the extracts might be as a result of the haemolytic effect of its saponin content. Haemolysis may give rise to mild hyperbilirubinaemia. Although the plasma bilirubin level increases lineally in relation to bilirubin production, the level may still be near normal in subjects with a 50% reduction in red cell survival, provided that hepatic bilirubin clearance is normal (Hauser *et al.*, 1988). Therefore, in correspondence with work done by Hauser *et al.* (1988);

the extracts have no significant negative effect on bilirubin level.

Compared with the control, there is significant effect of the plant extracts with varying concentration on the haematological and biochemical parameters. Results obtained correspond with research works done by Gbolade (2010); Hamenoo (2010); Olaitan *et al.* (2012). *S. mombin* water extract shows no significant effect of the PCV values between dosages of 5000mg/kg compared with the control, but a significant decrease at 1000mg/kg and 3000mg/kg with their values lower than normal. There was no significant effect in the creatinine values among the three doses. This indicates there was no damage on the kidney. However, the increase in urea level in all groups may be due to optimum conditions with regular basis and high nutritional intake. Therefore, no alteration in the creatinine concentrations substantiate this hypothesis, hence renal function was not to be affected by treatment (Taylor, 1989). It also shown that *S. mombin* water extract brought about significant increase in the level of AST, ALT, ALP and BIL. Highest values were obtained with dosage 1000mg/kg for ALT, 3000mg/kg for AST and BIL and 5000mg/kg for ALP. Despite this increase, the values were within normal range.

For *S. mombin* ethanol extract, PCV value at 1000mg/kg shows no significant difference compared to the control group, but a decrease at 3000 mg/kg and 5000mg/kg. There was an observed decrease in PCV values as doses increases (dose-dependent) but remained fairly constant in the treated animals when compared to the control. This is corroborated by earlier findings of Igwe *et al.*, (2011). There is also a significant decrease in the creatinine and urea values when compared with the control values. Ethanol extract of *S. mombin* brought about significant increase in level of AST, ALP, ALT and BIL. Highest values were obtained with 1000mg/kg for BIL, 3000mg/kg for ALT while 5000mg/kg gave highest values for ALP and AST. This result is in agreement with the work reported by Igwe *et al.* (2011).

In *S. occidentalis* water extract, result obtained relates with the works reported by Adedapo *et al.* (2009) and Vitalone *et al.* (2011). Result shows significant increase in the PCV values at all concentration compared with the control group values. This suggests erythropoesis ability of this extract (Yadav *et al.*, 2009). Also, there is significant decrease in the ALP, AST, and BIL values with slight increase in the AST value at all doses compared with the control group values. This suggests the hepatoprotective ability of *S. occidentalis* extract (Yadav *et al.*, 2009). For the creatinine and urea value, there was increase in the CRE values compared to the control group but are not dose dependent. Urea values show no significant difference among the group of doses.

The interactive effect of concentration of extracts and the plant extract types on the haematology and biochemical parameters were significant on all the parameters. The interaction gave a better understanding, showing whether the effect of the extract on the blood parameters depended on the concentration of such extract. There was significant increase in the PCV values of *S. occidentalis* water extract compared to other group of extract with no significant effect between the two extract of *S. mombin*. Also, lowest values of ALP, ALT and BIL were observed in the Swiss albino mice administered with *S. occidentalis*

water extract, which suggests the hepatoprotective ability of *S. occidentalis* in accordance to the work done by Yadav *et al.* (2009). Highest values were obtained with *S. mombin* water extract for ALT while *S. mombin* ethanol extract gave highest increase in ALP. There was also significant decrease in the level of total bilirubin, greatly evident in the indirect (unconjugated) bilirubin levels. This is in agreement with the work of Nusrat (2010), suggesting that the hepatoprotective effect it has on the liver act not on the liver's ability to conjugate bilirubin, but on its ability to assemble bilirubin. The liver however would have shown defectiveness in conjugating bilirubin under intense damage of hepatocytes exhibited by extensive periportal fibrosis, central fibrosis, necrosis, inflammation and cytoplasmic vacuolation/fatty degeneration (Nusrat, 2010); which was not exhibited by the liver injury induced in the present study.

AST and ALT assay are important in the diagnosis of liver damage cause by drug toxicity or harmful chemicals (Nelson and Cox, 2005). ALT is a more specific marker of the liver than AST (Nuhu and Aliyu, 2008). High level of this enzyme in the serum may indicate cardiac infracture, muscle injury and hepatic necrosis. Alkaline phosphatase, ALP, is a plasma and endoplasmic reticulum membrane-bound enzyme (Hismiogullari *et al.*, 2011). Transient increase of this enzyme may be noticeable in all types of liver problems. It is important to clarify that ALT and AST levels do not reflect the function of the liver, even though they commonly are referred to as liver function tests or LFTs. They only are used to detect inflammation due to injury or damage to the liver from any source. Even in conditions when AST and ALT are very elevated, the liver may function properly. Bilirubin, a by-product of the routine destruction of red blood cells occurring in the liver is released as bile in the faeces. Elevation of the bilirubin can suggest liver dysfunction.

In vivo evaluation of *S. mombin* and *S. occidentalis* extracts also showed that these antidiarrheal plants prevented intestinal fluid accumulation and inflammation at every dosage regime. However, histology sections of the intestinal samples showed varying degree of infectivity (from no lesion to severe lesions) and inflammatory responses according to the dosage and type of epidemic strain compared to the positive control (treated with tetracycline) and the negative control. Efficacy doses for *S. mombin* water extract, *S. mombin* ethanol extract and *S. occidentalis* water extract were fixed at 2000 mg/kg, 3000 mg/kg and 5000 mg/kg b.w. while the concentrations of the extract were fixed at 83 mg/ml, 116 mg/ml and 332.50 mg/ml respectively.

During efficacy study, *S. mombin* water extract against epidemic strain CVC O1 showed mild loss of villi, infiltration of mononuclear cells, goblet hyperplasia. Ethanolic extract of *S. mombin* showed stumpy and club-like villi and goblet hyperplasia with mineralization of the necrotic area. Water extract of *S. occidentalis* showed mild diffuse loss of villi, which are sparse and club-like with mineralization of the necrotic area. This can be compared to the positive control treated with standard drug (tetracycline) in which goblet hyperplasia was also seen. Negative control shows necrosis of the mucosal epithelial cells with the villi having different sizes and height, which are stumpy and also showing exfoliating

mucosa cells with mononuclear infiltration of the lamina propria.

Efficacy against epidemic strain BA O1, *S. mombin* water extract showed slight loss of villi, vacuolar degeneration and necrosis of the epithelial cells of lieberkuhn, but showed promising regeneration of the epithelial cells at a higher dose regime. Ethanolic extract of *S. mombin* showed severe loss of villi and mineralization of the necrotic area, but at higher dosage regime, there was no lesion seen. Water extract of *S. occidentalis* also showed mild loss of epithelial cells, and as the dosage regime increases, no lesions were observed except for goblet hyperplasia. The positive treated control showed normal except for goblet hyperplasia while the untreated showed necrosis of the tip of villi which are stumpy, club-like and fused. Mononuclear cell infiltrations of the lamina propria and lamina muscularis with proliferation of the mucosa epithelial cells were seen.

The intestinal immune system is exposed to a mixture of foreign antigens from diet, commensal flora and potential pathogens. Understanding how pathogen- specific immunity is elicited while avoiding inappropriate responses to the background of innocuous antigens is essential for understanding and treating intestinal infections and inflammatory disease (McDole *et al.*, 2012). Goblet cells are glandular simple columnar epithelial cells whose sole function is to secrete mucin, which dissolves in water to form mucus. They are found scattered among the epithelial lining of the organ (Donald and James, 2007). They are stimulated by dust, smoke, viruses, bacteria, and other foreign particles. Goblet cells are also essential in oral tolerance – a process by which the immune system is prevented from responding to antigen derived from food products, as peptides from food may pass into the blood stream via the gut, which in theory lead to an immune response. A recent paper published in *Nature*, has shed some light on the process and implicated goblet cell has a role in the process. It was known that CD103 expressing dendritic cells of the lamina propria had a role to play in the induction of oral tolerance (potentially by inducing the differentiation of regulatory T_{cells}) (McDole *et al.,* 2012).

Hyperplasia is considered to be a physiological (normal) response to a specific stimulus and remain subjected to normal regulatory control mechanism. It may be due to increasing demand of basal layer of the epithelial cells (Donald and James, 2007; Ramzi *et al.*, 1999).

The extract of *S. mombin* has shown anti-inflammatory activity in Wistar rats (Nworu *et al.,* 2011). A decoction of the mashed leaves is used by the Ibos (Nigeria) for washing a swollen face. The leaves, ground with sugar, are rubbed on the mouth and gums. A leaf infusion is a common cough remedy or used as a laxative for fever with constipation. A leaf decoction is used for gonorrhea (Abo *et al.,* 1999). The leaves with the leaves of *Vitex quinata* and *Terminalia avicennoides*, are used on the Ivory Coast for fresh wounds preventing inflammation. The infusion of the leaves is used as a treatment of eye inflammation, diarrhoea and venereal diseases (Corthout *et al.*, 1992). Crushed leaves have faint turpentine-like smell, with several reported ethnopharmacological uses (Igwe *et al.*, 2011; Igwe *et al.*, 2008; Ademola *et al.*, 2005; Abo *et al.*, 1999; Corthout *et al.*, 1994). Apart from the leaves, all the other parts of the plant are also important in

traditional medicine. The decoction of the plant is also taken for severe cough with inflammatory symptoms, stomachache, toothache, sore throat, bronchitis, nausea and as poison antidote as well as for treatment of fungal infections.

Also the extract of *Senna occidentalis* has purgative properties; it is also used as a diuretic, liver detoxifier, as a hepato-tonic (balances and strengthens the liver). Further, used in whooping cough and convulsion. In Suriname's traditional medicine, coffee senna is used against throat inflammation, colds, asthma, fever and flu, and also used against poisonous snake bites. It is an excellent remedy for fungus - and bacterial infections.

In conclusion**,** this study has also shown that extracts of *Spondias mombin* and *Senna occidentalis* did not show any toxicity effect and therefore its safe use for human consumption. Also, *In vivo* efficacy studies have also shown that extract of the plant could treat cholera at higher doses. It is therefore recommended that these plants are safe use for human consumption, and their traditional use in the treatment of cholera encouraged. Further study on their potentials in drug development is therefore recommended.

References

Abo, K.A., Ogunleye, V.O. and Ashidi, J.S. 1999. Antimicrobial potential of Spondias mombin, Croton zambesicus and Zygotritonia crocea. Phytotherapy Research 13: 494-497.

Adedapo, A.A., Ayodele, E.A., Ogunshe, A.A.O., Oyeyemi, M.O., Idowu, S.O., Ola-Davies, O.E. and Ademola, I.O. 2009. Haematinic potential of the aqeous crude extracts of Ficus mucoso and Senna occidentalis in rabbits. African Journal of Biomedical Research 12: 47-54.

Ademola, I.O., Fagbemi, B.O. and Idowu, S.O. 2005. Antihelminthic activity of extracts of Spondias mombin against gastrointestinal nematodes of sheep: Studies in vitro and in vivo. Tropical Animal Health Production 37: 223-225.

Ajayi, A.O. and Akintola, T.A. 2010. Evaluation of antibacterial activity of some medicinal plants on common enteric food-borne pathogens. African Journal of Microbiology Research 4(4): 314-316.

Akhila, J.S., Deepa, S. and Alwar, M.C. 2007. Acute toxicity studies and determination of median lethal dose. Current science 7(93): 917-920.

Akinyemi, K.O., Oluwa, O.K. and Omomigbehin, E.O. 2006. Antimicrobial activity of crude extracts of three medicinal plants used in south-west Nigerian folk medicine on some food borne bacterial pathogens. African Journal of Traditional, Complementary and Alternative Medicines 3(4): 13-22.

Akubue, P.I., Mittal, G.C. and Aguwa, C.N. 1983. Preliminary pharmacological study of some Nigerian medicinal plants. Journal of Ethnopharmacology 8: 53-63.

Anthonia, O. and Olumide, O. 2010. In vitro antibacterial potential and synergistic effect of south western Nigerian plant parts used in folklore remedy for *Salmonella typhi* Infection. Nature and Science 8(9): 52-59.

Ayoka, A.O. Akomolafe, R.O., Iwalewa, E.O. and Ukponmwan, O.E. 2005. Studies on the anxiolytic effect of Spondias mombin l (*anacardiaceae*)

extracts. African Journal of Traditional, Complementary and Alternative Medicine 2(2): 153-165.

Ayoka, A.O., Akomolafe, R.O., Akinsomisoye, O.S. and Ukponmwan, O.E. 2008. Medicinal and economic value of Spondias mombin. African Journal of Biomedical Research 11:129–136.

Barros, G., Taylor, L. and Abo, K. 1970. Pharmacological screening of some Brazilian plants. Journal of Pharmaceutics and Pharmacology 22: 116.

Bartels H, Bohmer M, Heierli C. 1972. Serum creatinine determination without protein precipitation. Clin Chim Acta 37:193-197.

Brian, S., Bull, M.D., John, A., Koepke, M.D., Elkin, S.M.B., Onno, W. and VanAssendelft, M.D. 2000. Procedure for determining packed cell volume by microhematocrit method: approved standard Third Edition. Clinical and Laboratory Standard Institute 29(8): 1-18.

Corthout, J., Pieters, L.A., Claeys, M., Vanden-berghe, D.A. and Vlietinck, A.J. 1992. Antiviral caffeoyl esters from Spondias mombin. Phytochemistry 31(6): 1979-2019.

Deutsche Gesellschaft fur Klinische Chemie (DGKC) 1972. Optimised standard colorimetric methods. J Clin Chem Clin Biochem 10: 182-183.

Donald, M. and James, F.Z. 2007. Pathologic Basis of Veterinary Disease. Fourth Edition. Mosby Elsevier. pp. 256-278.

Duncan, R.C., Knapp, R.G. and Miller, M.C. 1977. Test of Hypothesis in Population Means. Introductory Biostatistics for Health Sciences, John Wiley and Son Incorporation, New York. pp 71-76.

Emeka, E.J.I and Funmilayo, D.O. 2011. Hypoglycaemic effect, biochemical and histological changes of Spondias mombin Linn. and Parinari polyandra Benth seeds ethanolic extracts in alloxan-induced diabetic rats. Journal of Pharmacology and Toxicology 6(2): 101-112.

Fakruddin, m., alam, k.m.a., mazumdar, r.m., islam, s., nipa, m.n., iqbal, a. And bhuiyan, h.r. 2011. Antibacterial activity of the extract of terminalia arjuma against multi antibiotic resistant Vibrio cholerae. Journal of Scientific Research 3(1): 129-137.

Faruque, S.M., Albert, M.J. and Mekalanos, J.J. 1998. Microbiology. Molecular Biology Review 62: 1301.

Gbolade, A.A. 2010. Effects of Spondias mombin stem bark and Senna alata leaf extracts on some biochemical parameters in rats. Journal of Pharmacy and Bioresources 7(1): 86-91.

Ghaleb, M.A., Bassam, A.A. and Kamel, M.A. 2009. In vitro activity of certain drugs in combination with plant extracts against *Staphylococcus aureus* Infections. *African Journal of Biotechnology*. 8(17): 4239-4241.

Greenwood, D., Slack, R.C. and Peutherer, J.F. 2009. Salmonella food poisoning and enteric fever. Medical Microbiology 16th edition. Church Hill Livingstone, Edinburgh. Pp. 250-259.

Hamenoo, N.A. 2010. Hepatoprotective and toxicological assessment of Spondias mombin l. (anacardiaceae) in rodents. Department Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Kwaame Nkrumah University of Science and Technology, Kumasi. pp. 1-88.

Hauser, C.S. and Gollan, J. 1988. A membrane transporter mediates access of uridine 5-diphosphoglucuronic acid from cytosol into endoplasmic reticulum of rat hepatocytes: Implications for glucuronidation reactions. Biochim et Biophys Acta. 967: 149-157.

Hismiogullari, A. A., Hismiogullari, S.E., Yavuz, O., Essiz, D. and Rahman, K. 2011. Evaluation of biochemical findings in mice exposed to thiamphenicol treatment. African Journal of Pharmacy and Pharmacology 5(3): 428-431.

Igwe, C.U., Ojiako, O.A., Nwaogu, L.A. and Onyeze, G.O.C. 2008. Lipid lowering effects of aqueous leaf extract of Spondias mombin Linn. The International Journal of Pharmacology 6(1): 1-9.

Igwe, C.U., Ojiako, A.O., Nwaogu, L.A. and Onyeze, G.O.C. 2009. Comparative effects of *Spondias mombin* leaf extracts on kidney function profile on rabbits. Research Journal of Agriculture and Biological Sciences 5(6): 1153.

Igwe, C.U., Onwuliri, V.A., Onyeze, G.O.C. and Osuagwu, C.G. 2011. Spasmogenic activity of ethanolic leaf extract of *Spondias Mombin* Linn on isolated uterine muscle strips of rat: possible hormonal mechanism of action**.** Research Journal of Agriculture and Biological Sciences 7(2): 228-233.

Igwe, C.U., Onwuliri, V.A., Osuagwu, C.G., Onyeze, G.O.C. and Ojiako, O.A. 2011. Biochemical and haematological studies on the ethanol leaf extracts of Spondias mombin Linn. Biochemistry and Analytical Biochemistry 1: 104.

Jendrassik, L. and **Gróf** FS 1938. Bilirubin. Biochem Zeitschrift 297:82-9.

Lee, K.S. and Garner, L.M. 1983. Management of unconjugated hyperbilirubinaemia in the newborn. Seminars in Liver Disease. 3 (1): 52-64.

McDole, J.R., Leroy, W.W., Keely, G.M., Baomel, W., Vjolica, K., Kathryn, A.K., Rodney, D.N. and Mark, J.M. 2012. Goblet cells deliver luminar antigen to CD103+ dendritic cells in the small intestine. Nature 483:345-349.

Mohana, D.C., Satish, S. and Raveesha, K.A. 2008. Antibacterial extracts against some human pathogenic bacteria. Advances in Biological Research 23(3-4): 49-55.

Nelson, D.L. and Cox, M.M. 2005. Lehniger *Principle of Biochemistry*. 4th Edition, Freeman W.H. and Company, New York.

Nuhu, A.A. and Aliyu, R. 2008. Effects of *Cassia occidentalis* aqueous leaf extract on biochemical markers of tissue damage in rats. Tropical Journal of Pharmaceutical Research 7(4): 1137-1142.

Nusrat, A.H. 2010. Hepatoprotective and toxicological assessment of *Spondias Mombin* L. (Anacardiaceae) in rodents. Kwame Nkrumah University of Science & Technology. pp. 70-72.

Nworu, C.S., Akah, P.A., Okoye, F.B., Toukam, D.K., Udeh, J. and Esimone, C.O. 2011. The leaf extract of *Spondias mombin* L. displays an anti-inflammatory effect and suppresses inducible formation of tumor necrosis factor-α and nitric oxide (NO). Journal of Immunotoxicology 8(1):10-6.

Offiah, V.N. and Anyanwu, I.I. 1989. Aborfacient activity of an aqueous extract of Spondias mombin leaves.

Journal of Ethnopharmacology 26: 317-320.

Olaitan, R.A., Theresa, B.E., Mokutima, A.E., Oluwatosin, O.O. and Daniel, E.I. 2012. Evaluation of toxicological effects of *Spondias mombin* in adult male Wistar rats. Journal of Natural Sciences Research 2(7): 144-151.

Organization for Economic Co-operation and Development 2001. Acute oral toxicity – fixed dose procedure. OECD Guideline for Testing of Chemicals 420: 1- 14.

Ramzi, C., Vinay, K. and Tucker, C. 1999. Robbins Pathologic Basis of Disease. Sixth Edition. W.B. Saunders.

Sack, R.B., Siddique, A.K., Longini, I.M., Nizam, A.J., Yunus, M. and Islam, M.S. 2003. A 4-year study of the epidemiology of *Vibrio cholerae* in four rural areas of Bangladesh. Journal of Infectious Diseases 187: 96-101.

Sini, K.R., Karpakavalli, M. and Sangeetha, P.T. 2010. Analgesic and antipyretic activity of *cassia occidentalis* linn. World Applied Sciences Journal 11(10): 1216-1219.

Reitman, S. and Frankel, A.S. 1957. A colorimetric method of determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 28: 53 - 63.

Taylor, E.H. 1989. Creatinine. Clinical Chemistry. New York: John Wiley and Sons. pp. 58-62.

Taylor, L.N.D. 2006. Database Entry for Ubos- *Spondias mombin.* pp. 1-5.

Uchendu, C.N. and Isek, T. 2008. Antifertility activity of aqueous ethanolic leaf extracts of *Spondias mombin* (Anacardiaceae) in rats. African Health Sciences 8: 163-167.

Valarmathy, K., Gokulakrishnan, M., Kausar, M.S. and Paul, K. 2010. A study of antimicrobial activity of ethanolic extracts of various plant leaves against selected microbial species. International Journal of Pharmacological Sciences and Research 1(8): 293-295.

Weatherburn, M.W. (1967). Urea. Analytical Chemistry 39:971-977.

Yadav, J.P., Arya, V., Yadav, S., Panghal, M., Kumar, S. and Dhankhar, S. 2009. *Cassia occidentalis*: A review on its ethnobotany, phytochemical and pharmacological process. Fitoterapia 10: 1016.