Niger. J. Physiol. Sci. 37 (June 2022): 127 – 135
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Full-Length Research Article

Biochemical Studies on the Colorectal Tissues of Wistar Rats Treated with Methanol Leaf and Stem Bark Extracts of *Annona muricata* (Soursop)

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Summary: *Annona muricata* (AMC), is a tropical plant species of the Annonaceae family known for its medicinal uses in ameliorating and/or protecting several organs and tissues in the body. In this study, we elucidated the influence of the methanol extracts of AMC leaf and stem bark on the biochemical and histological integrity of the colorectum. Different doses (100, 200, 400, 600, and 800mg of extract/kg body weight) of methanol extracts of the leaves and stembark were orally administered to adult male Wistar rats of average weight (100 - 150 g) for 28 days. At the end of the experimental period, the rats were sacrificed and colorectal tissues harvested for analyses. Adenosine triphosphatase (ATPases), glucose-6-phosphate dehydrogenase (G6PD) lactate dehydrogenase (LDH) activities, and tissue protein (TP) concentration, were evaluated by colorimetric method using appropriate test kits. The results in the colorectal tissue analysed showed that total ATPase, LDH and G6PD (energy markers) activities increased significantly in the groups which received methanol leaf and stem bark extracts of AMC when compared with control. However, a general decrease was detected in TP and Na^+/K^+-ATPase activity but there was a twist in Na^+/K^+-ATPase activity in stem bark doses of 200mg/kg and 400mg/kg which showed significant increases in dose-dependent manner when compared with the control. Even though G6PD activity showed fluctuating patterns for both extracts, the highest dose (800mg/kg) reflected the most significant increase when compared with the control. The histology confirmed the toxicological results by the biochemical parameters. Under the condition of this study, we inferred that the methanol leaf and stem-bark extracts of AMC may significantly influence the enzymes by reducing and increasing total ATPase and Na^+/K^+-ATPase activities depending on the doses and administration should therefore depend on the desired outcome.

Keywords*: Adenosine triphosphatase, Annona muricata, Colorectum, Glucose-6-phosphate dehydrogenase, Lactate dehydrogenase*

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Manuscript received- January 2022; Accepted- April 2022

DOI: https://doi.org/10.54548/njps.v37i1.16

INTRODUCTION

Over the history of humans, natural products derived from plants and other by-products have been utilized in the treatment of different types of disease conditions. Numerous chemotherapeutic agents of foremost importance have derived either directly from plants natural products of plants or its derivatives. Outside these vital drugs, there are many other plant preparations including crude herbal concoctions which have been established to have promising results for the prevention, management, amelioration, and treatments of various disease conditions. One of such plant preparations is the one that was prepared from a plant widely seen and grown in subtropic and tropic regions of the world, and from the Annonaceae family (Qazi *et al*., 2018; Handayani and Yuzammi, 2021; Hernández Fuentes *et al*, 2022). One of the most popular species of this family is the Annona muricata, that is locally known and called Soursop. The plant's fruit-pulp, seeds, root-bark stem-bark and leaves extracts and many other species of Annonaceae (Handayani and Yuzammi, 2021; Hernández Fuentes *et al*, 2022), have been studied broadly for its antioxidant properties as well as the anticancer and anti-inflammatory effects (Agu *et al*., 2017a; Agu and Okolie, 2017a; Agu and Okolie, 2017b). Phytochemical analyses have shown that a bioactive member of polyketide constituents known as acetogenins have been isolated from Annonaceous plant species, and lots of these compounds (including flavonones) are found in diverse parts of *Annona muricata* (Moghadamtousi *et al*., 2013), and these compounds apart from reported medicinal properties against pathologic conditions, could also help to enhance (or even negatively affect) the integrity of cellular and tissue components, viz, membrane proteins, enzymes, as well as, intracellular biomolecules. Thus, this study was designed to examined the effects of the crude extracts of *Annona muricata* leaf and stem bark on colorectal tissues of Wistar rats, by investigating the colorectal tissue adenosine triphosphatase (ATPase; sodium-potassium, Na^+ K^+-ATPase and calcium, Ca^{2+}-ATPase) and energy markers, glucose 6 phosphate dehydrogenase (G6PD) as well as lactate dehydrogenase (LDH) activities as well as the Na^+/K^+-ATPase and Ca^{2+}-ATPase activities influenced by identified acetogenin and flavonone.

MATERIALS AND METHODS

Experimental rats: One hundred and forty-four (144) adult male Wistar rats comprising between 100 g – 150 g body weight were utilized in this experimental study. These rats were purchased in the Department of Anatomy, University of Benin, Benin City and were acclimatized for two weeks at the animal house of the Department of Biochemistry, prior to the study. The animals were fed with water and standard rat chow ad libitum. The Research Ethics Committee Guideline Principles on Animal Handling of the College of Medicine, University of Benin (CMR/REC/2014/57) provided written approval for the study, which was strictly followed.

***Annona muricata* crude extract preparation:** A large quantity of the plant's fresh leaves and stem bark were collected from trees in house-hold gardens in Benin City and within the vicinity of the University of Benin in Edo State, Nigeria, and its identification was done at the Plant Biology and Biotechnology department of same university with the voucher authentication specimen number of UBHa 0205, deposited at the Department's Herbarium. The samples (leaves and stem bark) were exposed to room temperature (about 25oC) for 4 weeks, to dry and were separately pulverized. After macerating each pulverized plant sample in methanol for 48 hours, it was filtered through cheese cloth. The extracted materials were then concentrated in vacuo using a rotary evaporator to produce viscous gels that were air-dried to gel-like solids. Using distilled-deionized water as a solvent, the gel-like crude methanol extracts from both plant parts were reconstituted to produce a stock solution. Each reconstituted crude extract was kept in small-capped plastic containers in a refrigerator at -4oC prior to its used.

Extract administration: The extracts were administered via gavage, which served as an oro-gastric tube. The rats were given special care to avoid any oral or esophageal injuries.

Sub-chronic toxicity assessment: During period of usage, extracts were administered to the rats based on calculated doses per weight of rat (i.e. equivalent volume). This dose calculations were done weekly per weight of rats for the sub-chronic studies, as the weekly weights of the rats per group were recorded i.e. at day 0, day 7, day 14, day 21 and day 28. Untreated rats (group one) served as the control and was administered 2 ml of distilled water (Agu, 2016; Agu *et al*, 2017a; Pongri and Igbe, 2017).

Observations in sub-chronic assessment (clinical signs and mortality): The rats were observed for signs of weakness, increased or decreased appetite, weight loss and other physiological changes including mortality. Clinical signs to be assessed before dosing, immediately and 4hrs after dosing, include level of sedation, restlessness, change in nature of stool, urine and eye colour, excretion of worms, diarrhoea, haematuria, uncoordinated muscle movements etc. The animals were observed for toxic symptoms such as weakness or aggressiveness, anorexia, weight loss, diarrhoea, discharge from the eyes and ears, stridor and mortality (Fielding and Matheron, 1991; Vijayalakshmi *et al*, 2000; Suanarunsawat *et al* 2009).

Experimental protocol for sub-chronic toxicity studies: The methanol extracts of the leaves and stem-bark were administered at increasing doses from 100 mg/kg (group 2), 200 mg/kg (group 3), 400 mg/kg (group 4), 600 mg/kg (group 5) and 800 mg/kg (group 6). The group 1 rats which served as control, were given 2 ml of distilled water (0 mg/kg). Each group had six (6) rats each.

Animal Sacrifice: After the 28 days treatment prior to sacrifice, the rats were fasted overnight. Their weights were taken and then sacrificed after being anaesthetized with chloroform. Their abdomen was opened, and colorectal part of the intestine, i.e. about 1.5cm from the anus, was harvested. Part of the colorectal tissue was preserved in a plain sample container containing normal saline prepared with pH=7.0 phosphate buffer solution, while the other part of the colorectal tissue was preserved in formal-saline prior to tissue slide preparation for histological studies. The tissues stored in the normal saline-phosphate buffer solution were homogenized in phosphate buffer (pH=7.0) using mortar and pestle (0.5g of tissue per 5ml), centrifuged at 10,000g for five minutes and supernatant collected and preserved in a freezer for biochemical analyses.

Biochemical analysis: Colorectal ATPases assays (Total ATPase, Mg^{2+}, Ca^{2+}, and Na^{+}/K^{+}-ATPases) were done using the procedure described by Adam-Vizi and Seregi (1982); colorectal tissue lactate dehydrogenase (LDH) activity, glucose-6-phosphate dehydrogenase (G6PD) activity, and tissue protein (TP) concentration, were investigated using ready-to-use Randox® kit method.

Isolation and elucidation of compounds: The compounds which served as the ligands that were docked against the ATPases had been isolated and elucidated, as well as, previously reported by Agu *et al*. (2017a).

Molecular Docking Experiments

Preparation of Protein: The Protein data bank (PDB) ID of 4GL7, (the starting crystallography structure) for calcium ATPase and 1MO8 for rat alpha1 Na, K-ATPase nucleotide-binding domain) which was the requirement for docking was obtained from http: /www.rcsb.org a protein data bank repository. Water and ligand coordinates were removed prior to docking, and the appropriate charges were added. The proteins were viewed in Discovery Studio to study the interactions of their 2-D amino acid residues.

Preparation of the Ligand: ChemDraw was used to create the 3D structures of the elucidated compounds, which were then converted to PDB format for docking studies using GaussView. The ligand molecules (compounds) were optimized appropriately.

Molecular docking: Molecular Docking calculations were performed using Autodock Vina. The modeled structures of calcium ATPase and nucleotide-binding domain of rat alpha1 Na^{+}/K^{+}-ATPase, and elucidated compounds were loaded the Autodock/Vina and all the water molecules were removed prior to the upload. Protein snapshots were taken using PYMOL.

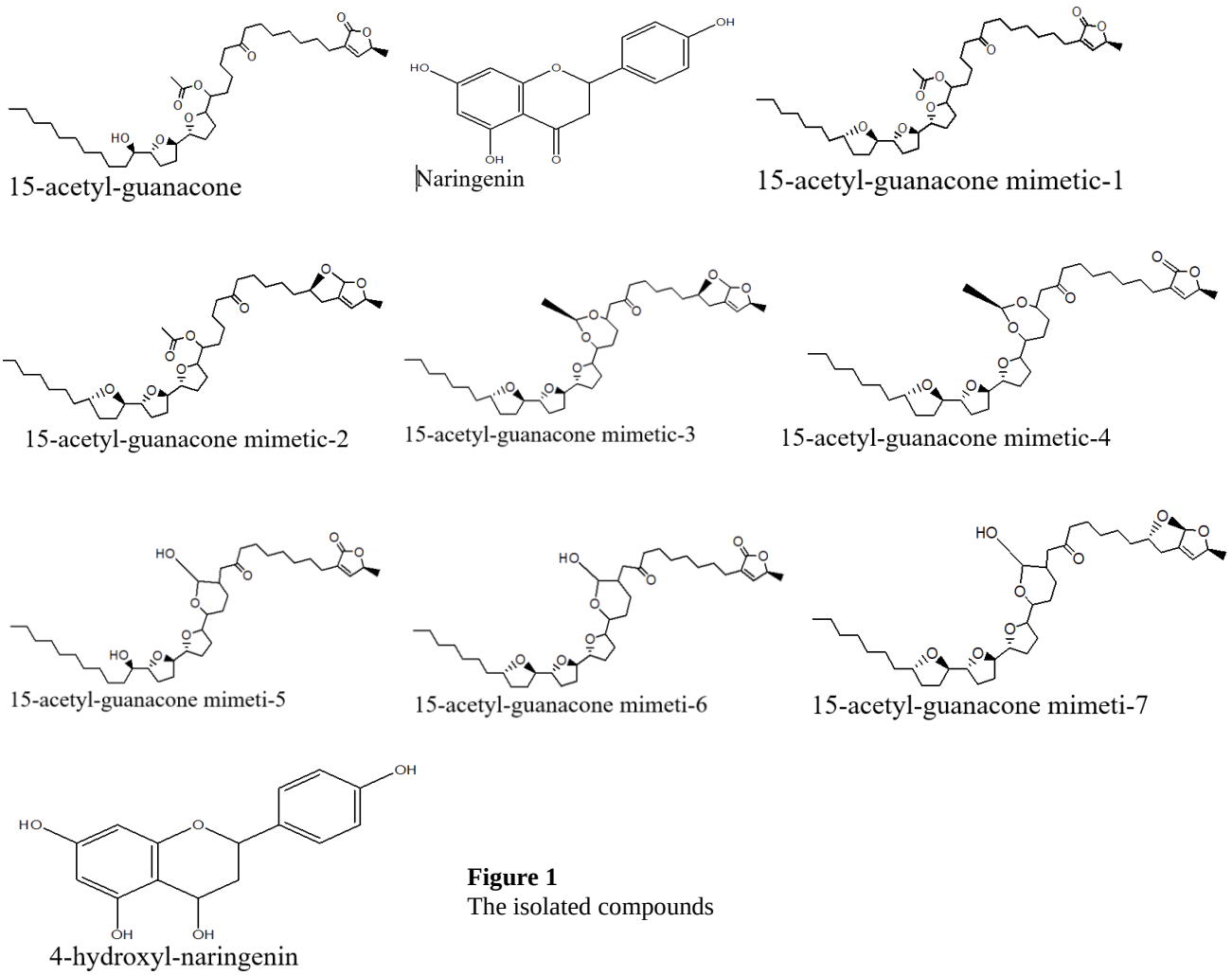

Figure 1
The isolated compounds

Statistical analysis: Prior to descriptive analysis, data were entered into a Microsoft Excel spreadsheet (v.10). The data is presented as mean SEM and was analyzed with Duncan's multiple range analysis of variance, ANOVA. Pearson's correlation (p=0.05) was used for correlation analyses in the Statistical Package for Social Sciences, SPSS®, Version 21.0, IBM Corp., Armonk, NY, USA. Graph Pad software®, Prism 5, Version 5.01, La Jolla, CA, USA, was used to create histograms and line plots. Significant values were defined as $p < 0.05$

RESULTS

Table 1 shows the results of the groups administered the leaf extracts of A. muricata. Total ATPase in group 3 and 6 increased significantly. While Na+, K+ ATPase of group 4 increased significantly, there was significant decrease in group 6 when compared with the control groups. Table 2 shows the results for the groups administered the stem-bark extracts of A. muricata. The total ATPase in groups 4 recorded a significant increase but a significant decrease was observed in group 6 ($p < 0.05$). Also, while Na+, K+ ATPase showed an increase in group 3, group 6 recorded a decrease that was significant. The results recorded a generalized decrease in Na+, K+ ATPase activities at higher doses of the extracts administered. Thus, from the observation, it could be inferred that the groups administered both extracts demonstrated a significant reduction in both total and Na+, K+ ATPase activities with subsequent significant increases close to the control (for stem-bark), at the highest dose of 800mg/kg ($p < 0.05$); a response that may be termed bi-phasic to the extracts.

Table 1:
Membrane-bound colorectal ATPase activities of rats administered methanol leaf extracts of *Annona muricata* ($x10^{-1}$ U/mg wet weight of colorectal tissue)

Assay/ Groups	Total ATPase	Mg^{2+}ATPase	Ca^{2+} ATPase	Na^{+}/K^{+}- ATPase
1	10.66 ± 0.00^{ab}	3.00 ± 0.00^{abd}	1.23 ± 0.07^{a}	4.91 ± 0.00^{a}
2	8.10 ± 0.00^{b}	3.91 ± 0.00^{bd}	1.81 ± 0.00^{b}	3.00 ± 1.00^{bh}
3	14.67 ± 0.30^{c}	6.92 ± 0.31^{cf}	1.56 ± 0.00^{c}	4.10 ± 2.60^{cg}
4	11.98 ± 0.10^{de}	3.27 ± 0.30^{d}	1.78 ± 0.09$^{d\ g}$	5.84 ± 0.00^{dg}
5	10.32 ± 0.20^{e}	1.10 ± 0.50^{g}	1.95 ± 0.13^{e}	2.98 ± 1.20^{eh}
6	18.47 ± 3.10^{f}	8.82 ± 0.60^{f}	1.70 ± 0.10$^{f\ g}$	1.00 ± 1.60^{f}

The values are shown as mean ± SEM (n = 6). By one way Duncan's multiple range ANOVA, means with different superscripts are significantly different ($p < 0.05$) down the column, with the control (group 1) taking the superscript 'a' alphabet.

Table 2:
Membrane-bound colorectal ATPase activities of rats administered methanol stem-bark extracts of *Annona muricata* ($x10^{-1}$ U/mg wet weight of colorectal tissue).

Assay/ Groups	Total ATPase	Mg^{2+}ATPase	Ca^{2+} ATPase	Na^{+}/K^{+}-ATPase
Group 1	15.88 ± 0.02[a]	2.30 ± 0.00[acd]	10.44 ± 0.07[acf]	3.17 ± 0.02[abef]
Group 2	7.85 ± 0.93[b]	4.94 ± 0.13[be]	4.03 ± 0.00[be]	2.17 ± 1.00[bef]
Group 3	26.77 ± 5.10[c]	2.12 ± 0.30[c]	9.15 ± 1.00[cf]	15.51 ± 5.00[c]
Group 4	17.50 ± 0.40[ad]	2.68 ± 0.50[d]	6.30 ± 0.40[d]	8.60 ± 0.20[d]
Group 5	11.00 ± 1.61[e]	5.86 ± 0.00[e]	4.74 ± 1.00[e]	3.05 ± 1.00[ef]
Group 6	19.77 ± 1.60[f]	7.96 ± 1.20[f]	10.10 ± 1.77[f]	3.04 ± 1.10[f]

The values are shown as mean ± SEM (n = 6). By one way Duncan's multiple range ANOVA, means with different superscripts are significantly different (p < 0.05) down the column, with the control (group 1) taking the superscript 'a' alphabet

The colorectal tissue profile of some metabolic enzyme activities of groups treated with the leaf and stem-bark extracts are presented in Tables 3 and 4. There was a significant increase in LDH activity of the rats administered the leaf and stem-bark extracts as the concentrations increased through group 3 compared with the control that was not administered any extract(s). However, there were significant decreases in the observed LDH activity at the group 4 concentration for both the leaf and stem-bark, followed by a significant increase at the highest concentration (group 6).

Table 3:
Colorectal Tissue Lactate Dehydrogenase (LDH), Glucose-6-Phosphate Dehydrogenase (G6PD) Activities and Tissue Protein (TP) Concentrations of AMC Leaf Extracts.

Assay/Groups	LDH (U/mg of tissue)	G6PD (U/mg of tissue)	TP (g/mg tissue)
Group 1	1.03±0.01[a]	8.55±0.22[a]	1.23±0.07[a]
Group 2	1.40±0.10[b g]	9.65±0.20[b g]	1.81±0.00[b]
Group 3	1.50±0.00[c]	5.58±0.00[c h]	1.56±0.00[c]
Group 4	1.10±0.20[d h]	7.03±0.20[d]	1.78±0.09[d g]
Group 5	1.13±0.20[e h]	5.86±0.00[e h]	1.95±0.13[e]
Group 6	1.40±0.19[f g]	9.65±0,20[f g]	1.70±0.10[f g]

The values are shown as mean ± SEM (n = 6). By one way Duncan's multiple range ANOVA, means with different superscripts are significantly different (p < 0.05) down the column, with the control (group 1) taking the superscript 'a' alphabet

Table 4:
Colorectal Tissue Lactate Dehydrogenase (LDH), Glucose-6-Phosphate Dehydrogenase (G6PD) Activities and Tissue Protein (TP) Concentrations of AMC Stem-Bark Extracts.

Assay/Groups	LDH (U/mg of tissue)	G6PD (U/mg of tissue)	TP (g/mg tissue)
Group 1	1.00±0.00[agh]	4.10±0.00[a]	3.40±0.00[ae]
Group 2	1.10±0.01[b i]	2.20±0.00[b]	2.90±0.00[bg]
Group 3	1.20±0.00[c g]	8.21±0.10[c]	2.48±0.10[ch]
Group 4	1.03±0.10[d h]	5.00±0.00[d]	2.08±0.00[d]
Group 5	1.49±0.00[e i]	6.20±0.10[e]	3.20±0.20[eg]
Group 6	1.50±0.00[f]	7.64±0.00[f]	3.40±0.00[ae]

The values are shown as mean ± SEM (n = 6). By one way Duncan's multiple range ANOVA, means with different superscripts are significantly different (p < 0.05) down the column, with the control (group 1) taking the superscript 'a' alphabet.

Table 5:
Binding Affinities of the Ligands against Colorectal Proteins

Receptor	Ligand	Affinity (Kcal/mole)	Dist. from RMSD l.b.	Best mode RMSD u.b.
Ca^{+}-ATPase	15-acetyl-guanacone	-6.60	5.931	11.633
	15-acetyl-guanacone mimetic-1	-5.90	4.337	6.877
	15-acetyl-guanacone mimetic-2	-7.10	4.802	6.593
	15-acetyl-guanacone mimetic-3	-6.80	2.296	5.379
	15-acetyl-guanacone mimetic-4	-5.80	1.834	2.548
	15-acetyl-guanacone mimetic-5	-6.50	3.168	5.146
	15-acetyl-guanacone mimetic-6	-7.70	2.049	3.050
	15-acetyl-guanacone mimetic-7	-7.40	23.821	27.089
	naringenin	-7.50	18.515	19.943
	4-hydroxyl-naringenin	-7.90	20.292	22.758
	Internal ligand	-8.30	1.425	2.067
$Na^{+}K^{+}$ ATPase	15-acetyl-guanacone	-6.20	1.270	2.223
	15-acetyl-guanacone mimetic-1	-6.30	3.939	12.874
	15-acetyl-guanacone mimetic-2	-6.30	2.173	2.826
	15-acetyl-guanacone mimetic-3	-7.60	3.239	15.248
	15-acetyl-guanacone mimetic-4	-7.40	2.679	13.317
	15-acetyl-guanacone mimetic-5	-7.30	3.590	5.738
	15-acetyl-guanacone mimetic-6	-6.70	1.983	3.931
	15-acetyl-guanacone mimetic-7	-7.70	2.540	4.280
	Naringenin	-7.90	2.288	7.363
	4-hydroxyl-naringenin	-7.40	2.432	7.398
	ATP	-7.00	11.177	13.841

Binding affinities greater than 6.00 Kcal/mole is an indication of appropriate bonding of ligand on the protein surface (RMSD = root mean square distance). The results obtained for 15-acetyl-guanacone mimetic-7 and 4-hydroxyl-naringenin suggest that the number of rings and hydroxyl functional groups along the acetogenin and flavonone molecules increased the binding affinity with the colorectal tissue Ca^{+} and Na^{+} K^{+}-ATPases when compared with 15-acetyl-guanacone (though, the binding affinity for the flavonone, naringenin, decreased when 4-1-, was converted to 4-hydroxyl functional group). The flavonone demonstrated to be a better binding compound to Na^{+} K^{+}-ATPase when compared with acetogeni

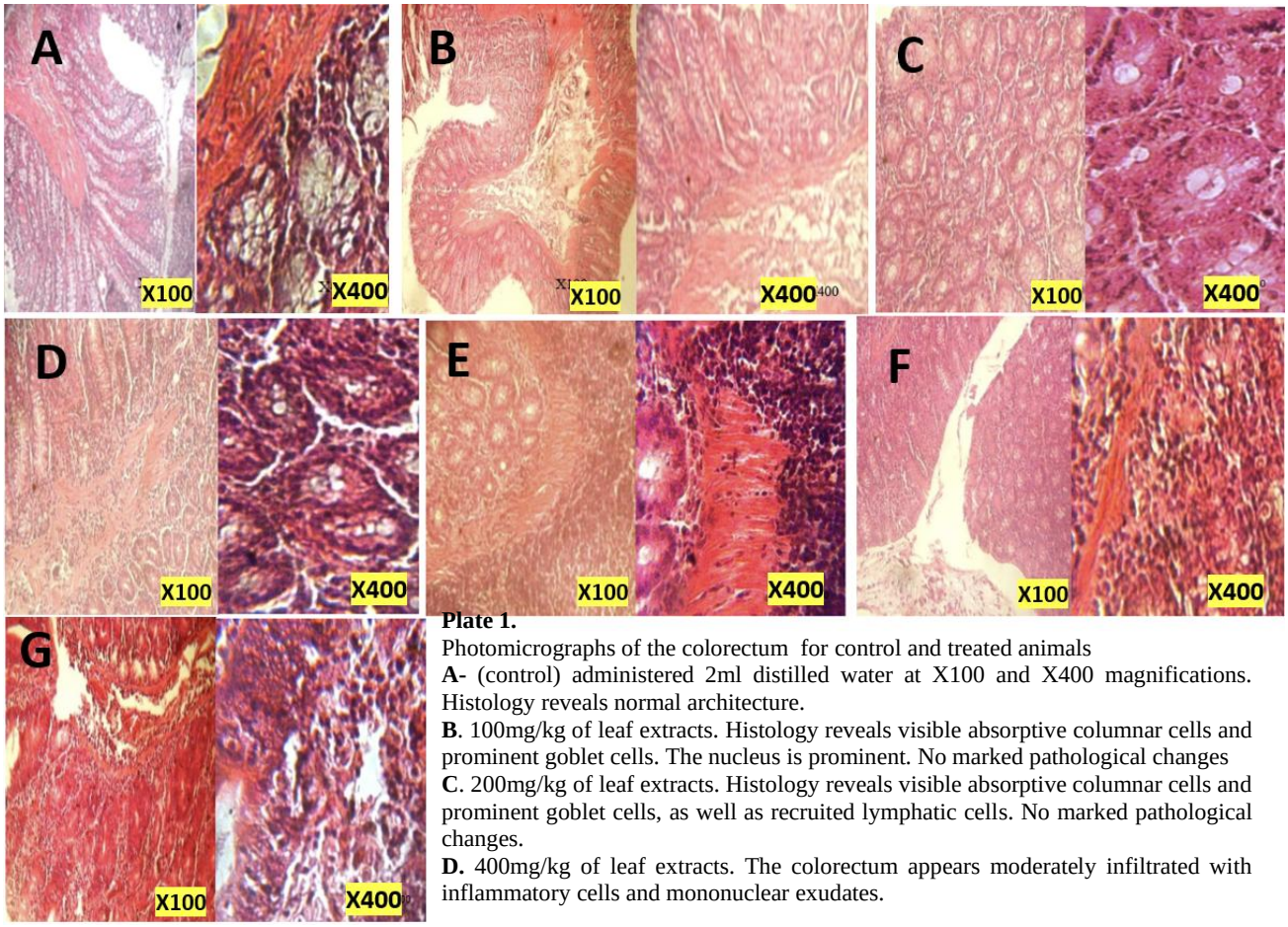

Plate 1.
Photomicrographs of the colorectum for control and treated animals
A- (control) administered 2ml distilled water at X100 and X400 magnifications. Histology reveals normal architecture.
B. 100mg/kg of leaf extracts. Histology reveals visible absorptive columnar cells and prominent goblet cells. The nucleus is prominent. No marked pathological changes
C. 200mg/kg of leaf extracts. Histology reveals visible absorptive columnar cells and prominent goblet cells, as well as recruited lymphatic cells. No marked pathological changes.
D. 400mg/kg of leaf extracts. The colorectum appears moderately infiltrated with inflammatory cells and mononuclear exudates.
E. 600mg/kg of leaf extracts. The colorectum appears moderately infiltrated with inflammatory cells and mononuclear exudates
F. 800mg/kg of leaf extract. Greatly observed histological changes mostly associated with inflammatory exudates and mononuclear cell infiltration.
G. 800mg/kg of stem-bark extract. Varying degrees of histological changes mostly associated with inflammatory exudates and mononuclear cell infiltration

The G6PD activity for the rats administered the leaf extract demonstrated decreases through groups 3 and 5, with increased activity in group 6 rats, compared with the control; whereas the G6PD activity of the rats administered the stem-bark extract showed marked increased activity for the group 3 rats, when compared with the control. The LDH and G_6PD activities for the leaf and stem-bark treated rats did not show any particular observable pattern of interest.

Binding properties of the isolated compounds: Binding properties of the isolated compounds: The binding properties between the isolated compounds of Annona muricata are presented in Table 5, Plate 1 and Figures 2 to 5.

Binding affinities greater than 6.00 Kcal/mole is an indication of appropriate bonding of ligand on the protein surface. The results obtained for 15-acetyl-guanacone mimetic-7 and 4-hydroxyl-naringenin suggest that the number of rings and hydroxyl functional groups along the acetogenin and flavonone molecules increased the binding affinity with the colorectal tissue Ca2+ and Na+ K+-ATPases when compared with 15-acetylguanacone (though, the binding affinity for the flavonone, naringenin, decreased when 4-1-, was converted to 4-hydroxyl functional group). The flavonone demonstrated to be a better binding compound to Na+ K+-ATPase when compared with acetogeni (Table 5, Figures 2 - 5).

Photomicrographs of the colorectum for control and treated animals at X100 and X400 magnifications revealed normal architecture for control. In the 100mg/kg leaf extracts treated rats, histology revealed visible absorptive columnar cells and prominent goblet cells. The nucleus was prominent. However, there was no marked pathological changes. Rats treated with 200mg/kg leaf extracts, revealed visible, absorptive columnar cells and prominent goblet cells, as well as recruited lymphatic cells and no marked pathological changes. In the rats given 400mg/kg and 600mg/kg leaf extracts, the colorectum appeared moderately infiltrated with inflammatory cells and mononuclear exudates. For the rats treated with 800mg/kg leaf extracts and stem-bark extracts, there were varying degrees of histological changes mostly associated with inflammatory exudates and mononuclear cell infiltration (Plate 1, A - G)..

DISCUSSION

The groups administered the AMC leaf extract showed a steady significant increase in the total ATPase activity and a dose-related significant decrease in Na^+/K^+-ATPase activity at group 4, with subsequent sharp decrease at higher administered doses (groups 5 and 6). The stem-bark AMC extract group showed almost a similar trend.

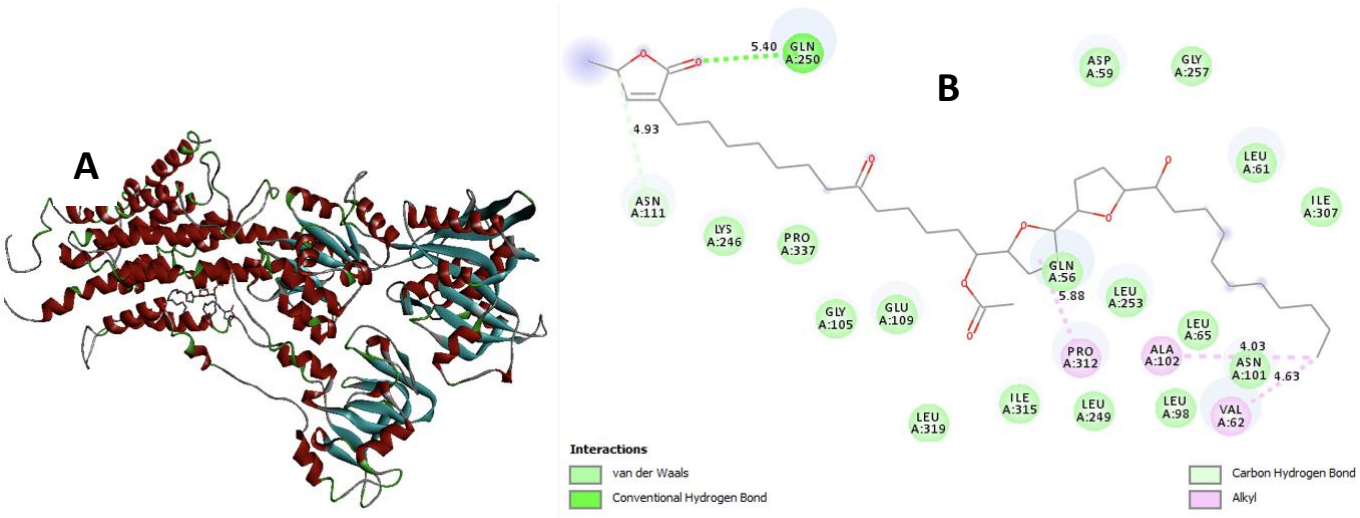

Figure 2
A- Three-D bonding between Ca^{+}- ATPase and 15-acetyl-guanacone
B- Bonding association and active site amino acid residues between Ca+-ATPase and 15-acetyl-guanacone bonding

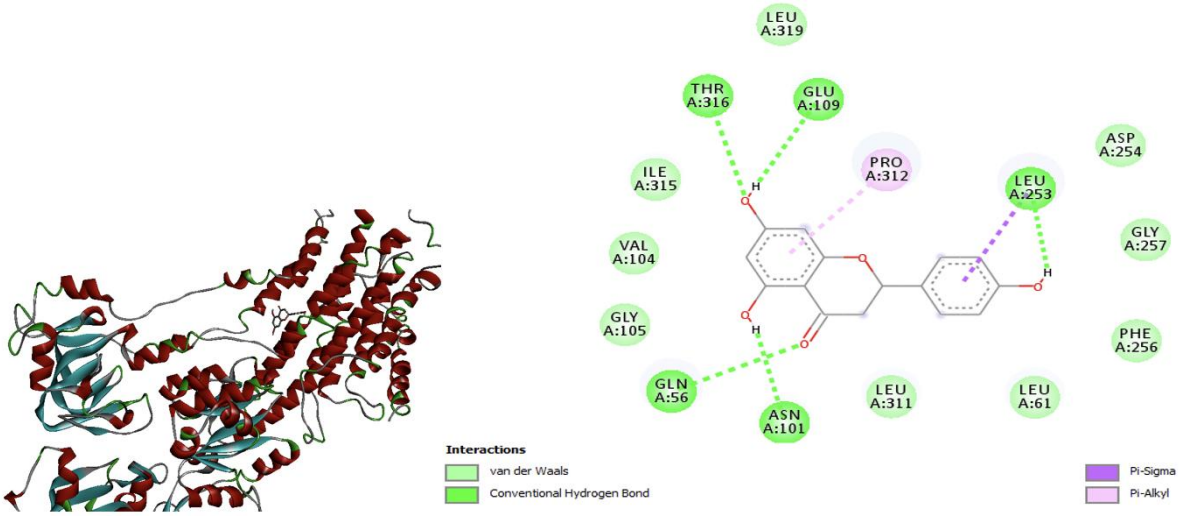

Figure 3
A- Three-D bonding between Ca+-ATPase and Naringenin
B- Bonding association and active site amino acid residues between Ca+-ATPase and Naringenin bonding

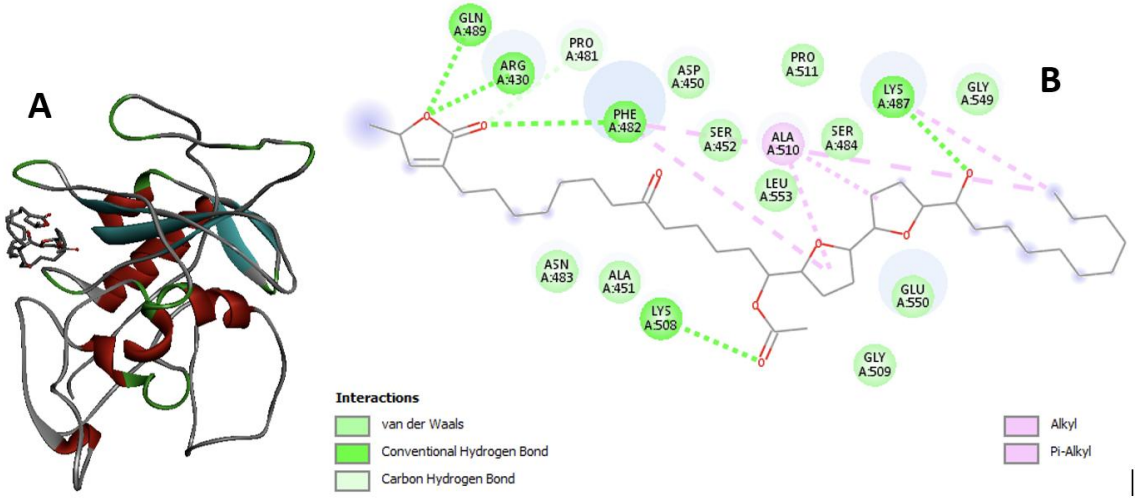

Figure 4
A- Three-D bonding between the nucleotide-binding domain of rat alpha-1 Na^{+} K^{+}-ATPase and 15-acetyl-guanacone
B. Bonding association and active site amino acid residues between the nucleotide-binding domain of rat alpha-1 Na^{+} K^{+}-ATPase and 15-acetyl-guanacone.

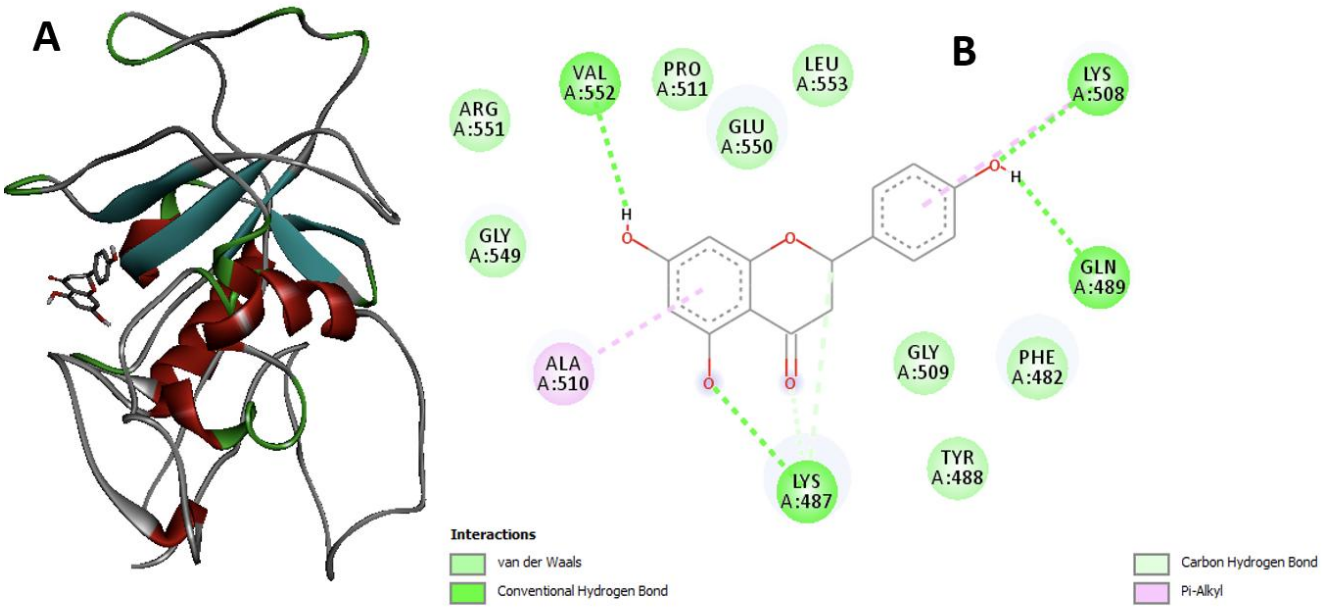

Figure 5
A - Three-D bonding between the nucleotide-binding domain of rat alpha-1 Na^+ K^+-ATPase and Naringenin
B- Bonding association and active site amino acid residues between the nucleotide-binding domain of rat alpha-1 Na^+ K^+-ATPase and Naringenin

The result of this study is in agreement with the findings of Senthil-Nathan *et al*. (2005) who reported significant inhibition of ATPase activity in different animal species exposed to different contaminants. Muzzanti *et al*. in Olubodun and Eriyamremu (2014) stated that a decrease in Na+/K+-ATPase is a reflection of qualitative changes in the phospholipid elements of cells. Rauchova *et al*. (1995) demonstrated that membrane microviscosity changes can affect the kinetic properties of membrane-bound enzymes. There is the possibility that the inhibited ATPase activities may be responsible for the relative inflammatory exudates and mononuclear cell infiltration observed in the histology of this study (Olubodun and Eriyamremu, 2014; Clausen *et al*, 2017).

The observed inhibition of Na^+/K^+-ATPase activities for the leaf (and also, stem-bark) especially above 400mg/kg of the administered extracts could be linked to the diarrhea observed during the acute and sub-chronic toxicity studies, and thus suggests an inhibition of the membrane-bound Na+/K+-ATPase –Osmo-regulatory coupled mechanism of the colorectal cells (colonocytes), where Na^+, Cl^-, and water are lost into luminal content (chyme) of the colon for excretion (Clausen *et al*, 2017; Müller *et al*, 2018).

The Na^+/K^+-ATPase pump is located on the basolateral side of the colonocytes which creates a means of Na+ as well as, Cl- ions transport (electrochemical gradient and secondary active as well as, down-hill drag, respectively), and also passive water transport through the brush border side of the colonocytes by an osmotic diffusion. This Na^+/K^+-ATPase pump is relevant in the whole of this process as the [Na^+] and [K^+] are about 142mEq/L and 4mEq/L in the extracellular fluid, respectively, and 10mEq/L and 140mEq/L in the intracellular fluid, respectively (Guyton and Hall, 2016). The primary active exchange of three Na+ ions out of the colonocytes for two K+ ions into the colonocytes creates a net negative charge inside the colonocytes, which facilitates a subsequent secondary transport of Na^+, and co-transport of Cl^- ions into the colonocytes from the luminal chyme through the brush border into the colonocytes. It is also possible that at a very high dose, the leaf AMC extract may be acting as an irritant to the cells lining the colorectal lumen, or contains osmotically active phyto-molecules, thus initiating a "diluting-effect", where Na^+, Cl^-, and water are passed from the plasma (blood vessels) via the colorectal cells into the colorectal lumen so as to "dilute" the irritating metabolite present in the leaf AMC and facilitate its excretion in a very watery form, resulting in diarrhea (Clausen *et al*, 2017). It is also possible that the leaf (and stem-bark) methanol extracts contain diuretic compound which resulted in the observed increase in urine excreted as well as diarrhea. Along with the excreted watery feaces was observed some worms, especially tape worm, suggesting that *Annona muricata* methanol extracts (leaf and stem-bark) possess anti-helminthic properties.

Studies of the influence of both extracts of AMC on colorectal tissue protein and some energy markers (LDH and G6PD) showed that, there were dose-dependent increases and decreases in colorectal tissue protein for the groups administered the leaf and stem-bark extracts respectively. Colorectal tissue LDH activity increased in a dose-dependent pattern, for the groups administered the leaf and stem-bark extracts of AMC. The colorectal tissue G6PD activity demonstrated fluctuating patterns for all the AMC extracts but showed a significant increase with the highest dose (800mg/kg) when compared with the control groups for both extracts. An increase in LDH activity was reported for plant seedlings exposed to 7 days of anoxia (Chen and Quallis, 2003). Kulkarni and Chavan (2013) however, reported a decrease in LDH activity also in two plant species

subjected to waterlogging stress. The LDH results of the study is consistent with previous reports (Xu *et. al*., 2012; Olubodun and Eriyamremu, 2020). However, it is at variance with the report of Kulkarni and Chavan (2013) and the Davis-Roberts pH-stat hypothesis. The high LDH activity of the colorectal tissues of the groups administered the leaf and stem bark extracts of AMC when compared with the control group may indicate elevated energy demand to overcome the stress induced in the rats by the crude extracts of AMC. The limitation or absence of oxygen as a result of the crude extracts may have induced conversion of aerobic respiration to anaerobic respiration (fermentation) hence increase in the lactate dehydrogenase activity (Xu et. al., 2012; Chikezie *et al*, 2018; Olubodun and Eriyamremu, 2020; Farhana and Lappin, 2022). Conversely, the increase in LDH activity at the highest administered dose (800mg/kg) suggests an increase in the cellular glycolytic-lactic acid pathway, which may have ultimately led to an increase in the colorectal cellular pH level due to lactic acid accumulation. This observation was corroborated by the observation of the histology (plate 1). This may have also had a far-reaching biochemical effect on the colorectal ATPase status. Though, no observable effect could be deduced from the colorectal tissue protein (TP).

The fluctuating pattern observed for G6PD in this study is consistent with Liu *et al*. (2007) also reported fluctuating (increase and decrease) movement after certain amounts of contamination in plants. The increment in G6PD interaction may have increased NADPH production in the colorectal tissues, resulting in tissue tolerance to the administered extracts. The increase in NADPH levels may have stimulated the production of nitrate reductase-dependent Nitric Oxide (NO), which has been shown to regulate tissue growth and development and enhance or boost iron homeostasis (Liu *et al*. 2007). The upsurge in G6PD activity may have exacerbated the increase in antioxidant enzyme activity observed in an earlier report (Agu and Okolie, 2017b, Agu *et al*, 2017b). Increased antioxidant enzyme activities may have aided in the scavenging of reactive oxygen species (ROS) produced by the extracts (Agbafor *et al*, 2017). Glucose-6-phosphate dehydrogenase activity may have played an important role in protecting rat colorectal tissues from oxidative stress caused by both crude extracts and establishing transient colorectal tolerance to the crude extracts (Liu *et al*., 2007; Olubodun and Eriyamremu, 2015; Agbafor *et al*, 2017).

Additionally, the methanol extracts of both plant parts showed histological changes in the colorectal tissues in all animals with marked changes in the highest dose. The control group had a properly aligned cellular morphology whereas, the other groups exhibited presentations of distorted cellular morphologies marked by increased inflammatory bodies. The dysmorphic presentations where significant for the group 8 rats. Several studies have reported histopathological modulations in different organs exposed to various plant species with more effects on the concentrated doses (Ngueguim *et al*, 2016; Samir *et al*, 2017).

In conclusion, the influence of both extracts of AMC on colorectal tissue protein and some energy markers (ATPase, LDH and G6PD) showed that, there were dose-dependent increase in colorectal tissue protein for the groups administered the leaf and stem-bark extracts respectively. However, after a certain dose, the effects were reversed. Colorectal tissue LDH activities increased in a dose-dependent pattern, for the groups administered both extracts. The colorectal tissue G6PD activity demonstrated fluctuating patterns for the extracts but showed a significant increase with the highest dose (800mg/kg) when compared with the control. We inferred that the methanol leaf and stem-bark extracts of AMC may significantly influence the enzymes by reducing and increasing total ATPase and Na^+/K^+-ATPase activities depending on the doses and administration should therefore depend on the desired outcome.

Acknowledgments

''This Research was funded by TETfund Research Fund Grant (2018/2019)''. The Tertiary Education Trust Fund (TETfund) Nigeria (2018/2019 Research Projects Batch 13; Ref: TETFUND/DESS/UNI/BENIN/2018/RP/VOL. 1), is acknowledged for the financial support. The authors are grateful to University of Benin for releasing the funds and the Department of Medical Biochemistry and Biochemistry, University of Benin, for technical support.

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