Activities of aqueous extracts of soursop (*Annona muricata*) leaves on immune response, haematology and serum biochemistry of broiler chickens

¹Oluwayinka, E. B., ¹Okunuga, F. S., ¹Oni, O. O., ²Olukunle, J. O., ³Akinkuotu, O. A. and ⁴Oluwayinka, O. G.

¹Department of Veterinary Medicine and Surgery, ²Department of Veterinary Physiology and Pharmacology, ³Department of Veterinary Microbiology and Parasitology, Federal University of Agriculture Abeokuta,

⁴Department of Veterinary Microbiology and Parasitology, University of Ibadan, Nigeria

Abstract Corresponding author: jacobseb@funaab.edu.ng +2348035761336

Poultry production worldwide is still challenged by diseases and researchers are continually searching for different means of combating the causative agents. *Annona muricata*'s antiherpes and anticancer activities may be helpful in tackling Marek's disease of poultry birds. This study was carried out to investigate the effects of aqueous extract of *Annona muricata* leaves on the haematology, some liver enzymes, body weight gain and immune response to Newcastle disease vaccination in broilers at dosages of 50mg/kg, 100mg/kg and 200mg/kg. Twenty (20) Marshall Broilers which were 35 days old at the start of the experiment were used; the chicks were divided into 4 groups. The aqueous extract of *Annona muricata* leaves was administered orally for 14 days; Blood and serum samples were collected at the beginning and end of experiment. Haematology, some liver enzymes test and haemagglutination inhibition (HI) test against Newcastle disease vaccination was carried out. A significant ($p < 0.05$) decrease in antibody titre against Newcastle disease vaccine was observed in only the group of birds fed with 200mg/kg of the extract. Birds fed with 200mg/kg of the extract had the highest values of packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC) and white blood cell (WBC) count but there was no significant difference ($p < 0.05$) in these values in comparison to the control group. There was no significant difference ($p < 0.05$) in the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) between treated and control groups. Therefore, aqueous extract of *Annona muricata* reduces immune response to Newcastle disease vaccine at 200mg/kg dosage but does not have any deleterious effect against the haematology and liver functions of birds fed with doses as high as 200mg/kg.

Keywords: *Annona muricata*, haematology, serum biochemistry, immune response, chicken

Introduction

The consumption of poultry and their products in Nigeria has increased rapidly like in other parts of the world. Increase in the rate of intensification of poultry production and intensification of poultry production has brought with it some management defects with a consequent high rate of disease incidence and spread (Frank, 2001, David, 2009). Among many other ways of treating disease is herbal medicine.

Annona muricata is a small evergreen tree herb found in many regions of the tropical world. It is also called "Soursop" or graviola soursop, 'guabana', and known as 'Abo' in Nigeria. It is a small, upright evergreen tree that grows between 5 to 6 meters in height. Young branchlets are rusty-hairy and the malodorous leaves are about 6 - 20 cm long and 2 - 6 cm wide (Vasquez, 1990, de Feo, 1992). The plant is said to contain annaceous acetogenins including quinolones, isoquinolines,

annopentocin, anomuricins, coreximine and reticuline (Kim *et al.*, 1998). There has been a long history of its anti-viral activity against Herpes simplex virus-1 (Padmaa, 1998; Rajeswari *et al.*, 2012), antileishmanial activities, cytotoxic activities against the neoplastic cells (Alvarez-Gonzalez, *et al.*, 2008), antibacterial activities (Pathak, *et al.*, 2010), antiparasitic and pesticidal properties and wound healing activities (Padmaa *et al.*, 2009) of the aqueous, methanolic or alcoholic extracts of any part of the plant in Natural medicine. Its antitumor activities were first discovered in the 1970s and since then, several researches have been carried out on it. Its antitumor activity has been demonstrated in human breast cancer cells (Dai, 2011; George, *et al.*, 2012), cervical, ovarian, bladder, skin cancer cell line (Yuan, 2003; Tormo *et al.*, 2003), lungs cancer cell (Wang *et al.*, 2002) and pancreatic carcinoma cell (Zeng *et al.*, 1996) and its use in cancer treatment in human.

The anticancer and antiherpes activities of this plant have drawn researchers' attention to its potentials in solving Marek's disease challenges in poultry production.

Materials and methods

Fresh leaves of *Annona muricata* were collected and dried at normal room temperature for two weeks before it was ground. The aqueous extract was prepared at a concentration of 36.8mg/ml and stored in the refrigerator after use each day.

Healthy broiler chicks were collected at day old and raised to 35 days old before the start of the experiment. They were weighed and randomly selected into four groups. They were fed with broiler starter feed for 6 weeks and broiler finisher feed for the remaining part of the experiment. Water

was served *ad libitum* and birds were kept in a standard poultry house. Routine vaccinations and medications were done for all the chicks except vaccination against Newcastle disease. Aqueous extract was administered orally using a feeding canula. Blood samples were collected at the beginning of the experiment and at the end jugular vein of the chickens into an **Ethylene Diamine Tetra-Acetic Acid (EDTA)** and plain bottle (for serum). Serum was separated from the blood into ependoff tubes and stored at -20°C while blood collected in the EDTA bottles were preserved in the refrigerator until use.

Hematological parameters like red blood cell count (TEC) was done using Hayem's solution (Lamberg and Rothstein 1978), packed cell volume (PCV) using microhaematocrit method (Hutchison 1960), hemoglobin concentration as described by Franco (1984), Total White Blood Cell Count (WBC) was determined as described by Lillie (1977), differential white blood cell count was done using a thin blood smear that was prepared based on the diagnostic method of Garcia (2001), alanine transferase (ALT) and aspartate aminotransferase (AST) were determined spectrophotometrically according to the method of Rietman and Frankel, 1957, using a commercial Randox kit, Alkaline Phosphatase (ALP) was determined spectrophotometrically by the method of Babson and Read, 1959, as described in Randox diagnostic kit manual. **Haemagglutination Inhibition (HI) test** was performed according to (Allan and Gough, 1974)

The antigen used for HI was a reconstituted commercial NDV La Sota vaccine. For this purpose, a total of 5 ml of chicken blood was collected aseptically into an EDTA bottle. The blood was centrifuged at 1500 rpm for 15 min and the plasma and buffy

coat was pipetted off. After washing thrice with Phosphate Buffer Saline (PBS), 0.5% suspension of RBC in PBS was used in HI test.

Data was presented as mean $\pm$ standard deviation (SD). Statistical analyses were subjected to one-way ANOVA and Student's t-test using SPSS version16 and Graphpad prism 6. Significant differences between groups were determined at $p < 0.05$.

Results

In Figure 1, the antibody titre against Newcastle disease vaccine in birds fed with 50mg/kg was the highest ($\text{Log}_2 8$) with that of the group fed with 200mg/kg ($\text{Log}_2 5$) of the extract being the lowest when compared to the control group ($\text{Log}_2 6.5$) but the difference is not significant ($p < 0.05$).

Figure 1: Effect of aqueous extracts of *Annona muricata* leaves on chickens' response to Newcastle disease vaccination

Table 1 showed that there was no significant difference in the haematological parameters between the treated and the control group. The packed cell volume (PCV), Haemoglobin (Hb) value and red blood cell (RBC) counts for the control group was lower than that of the treated group. White blood cell count on the other hand, was highest in the group fed with 200mg/kg (14.68 ± 0.63) of the aqueous

extract with the lowest values in groups fed with 50mg/kg (10.80 ± 0.56) when compared with the control group (12.38 ± 1.20). Basophils, monocytes and eosinophils were highest in chickens fed with 200mg/kg of the extract while heterophils was highest in chickens fed with 50mg/kg. Lymphocytes were highest in chickens fed with 100mg/kg respectively.

Table 1: Effect of aqueous extract of *Annona muricata* leaves on haematology of chickens

	Control	200mg/kg	100mg/kg	50mg/kg
PCV (%)	27.2 ± 1.50	31.75 ± 3.94	31.00 ± 0.70	29.40 ± 1.03
HB (g/dl)	8.82 ± 0.31	10.53 ± 1.18	10.16 ± 0.33	9.52 ± 0.33
RBC($\times 10,000\text{m}^3$)	2.34 ± 0.16	2.47 ± 0.34	2.54 ± 0.68	2.46 ± 0.15
WBC ($\times 50\text{m}^3$)	12.38 ± 1.20	14.68 ± 0.63	12.14 ± 1.03	10.80 ± 0.56
HET ($\times 1000\mu\text{l}$)	30.80 ± 2.31	29.75 ± 3.28	28.60 ± 3.75	31.20 ± 2.27
LYM ($\times 1000\mu\text{l}$)	67.80 ± 1.74	67.25 ± 2.29	68.60 ± 2.80	67.20 ± 2.48
Eos ($\times 1000\mu\text{l}$)	0.20 ± 0.20	0.50 ± 0.50	0.80 ± 0.37	0.60 ± 0.40
Baso ($\times 1000\mu\text{l}$)	0.60 ± 0.40	0.75 ± 0.25	0.60 ± 0.24	0.20 ± 0.20
Mono($\times 1000\mu\text{l}$)	0.60 ± 0.60	1.75 ± 0.63	1.40 ± 0.60	0.80 ± 0.37

*Packed Cell Volume (PCV) , Haemoglobin (Hb) , Red Blood Cell (RBC) , White Blood Cell (WBC) Heterophils (Het), Lymphocytes (LYM) Eosinophils (Eos), Basophils (Baso), Monocytes (Mono),

Annona muricata leaves on immune response, haematology and serum biochemistry

Figure 2 showed that the level of AST decreased in the treated group with increased dosages of the extract but the difference was not significant. There was also no significant difference in the levels of ALT and ALP between treated and the

control group although the groups fed with 200mg/kg had the highest values. The ratio of ALT to AST for the chickens fed with 50mg/kg, 100mg/kg and 200mg/kg and control were 0.56, 0.46, 0.53 and 0.45 respectively.

*Alanine transferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP)

Discussion

Annona muricata has a long history of safe use as a herbal remedy for several disease conditions for many years (Rieser et al., 1993, Zeng et al., 1996, Wu et al., 2009), and research indicates that the antitumorous acetogenins are selectively toxic to just cancer cells and not healthy cells (Rieser et al., 1993).

The phytochemical screening of methane and aqueous extracts of the leaves of *Annona muricata* revealed the presence of steroids, alkaloids, saponins, tannins, flavonoid, and cardiac glycosides (Fasakin et al., 2008; Larbie et al., 2011; Solomon-Wisdom, 2014). Saponins enhance nutrient absorption, aid proper digestion and help to boost the immunity of birds against diseases through inhibition of urease enzymes (Newton et al., 2002). Flavonoid, which has also been reported to be present

in the extract, have been known to augment the humoral response by stimulating the macrophages and beta-lymphocytes involved in antibody synthesis (Alvarez-Gonzalez et al., 2008, Sharififar et al., 2009). Alkaloids have also been incorporated into poultry diets to reduce amino acid degradation and increase feed intake; they also promotes growth and have immunomodulatory properties (Agarwal et al., 1991, Tschirner et al., 2003). Improvement of protein retention by reducing intestinal decarboxylation of aromatic decarboxylase (Drsata et al., 1996) and enhancement of feed intake by modulating effects on the tryptophan-serotonin pathway have been suggested as part of Alkaloids' mode of action (Mellor, 2001). The presence of saponins and alkaloids in the extract may have reflected greatly in birds fed with 50mg/kg of

aqueous extract of *Annona muricata* and may be the reason for the increase in their antibody titre value against Newcastle disease.

Hematological parameters generally provide information on inflammation, necrosis, various infections of organs, the presence of stress factors *e. t. c.* (Jurcik *et al.*, 2007, Melilo., 2007 and Betarcourt-Alonso *et al.*, 2011). The haematological values of the chickens indicated that all the parameters were within the normal values (Sturki, 1986). The packed cell volume (PCV), Haemoglobin (Hb) and red blood cell (RBC) count of the chickens fed with the different dosages of the extract were slightly higher than the control group. This is in agreement with Fareed *et al.* (2012) who also reported a non significant increase in RBC and Hb levels in rats treated with higher dosages of the same plant. This therefore, may imply that this leaf extract may not induce anaemia in chicken at the tested dosages just as it has been reported in rats Fareed *et al.*, (2012) and in humans Mshana *et al.*, (2000). White blood cells or leukocytes play important roles in immune responses. The non significant increase in WBC count observed in this study correlates with the findings of Larbie *et al.*, (2011) who also observed a non significant increase in WBC in rats fed higher dosages of the extract. This may also imply that the anti inflammatory effect of this plant may not be noticed until the birds are fed with 200mg/kg dosage of the extract. Serum activities of AST, ALT and ALP are the most commonly used biochemical markers of liver injuries. Increased serum levels of AST and ALT have been attributed to the damaged structural integrity of the liver because they are cytoplasmic in their location and are released into circulation after cellular damage (Huang *et al.*, 2007) while hepatobiliary obstruction is one of

the causes of increase in serum ALP levels. The result of this study demonstrated that treatment with different dosages of aqueous leaf extract of *Annona muricata* did not cause any significant difference in serum levels of these enzymes and that the ratios of ALT to AST in the treated groups was not more than one. This finding is in agreement with Larbie *et al.* (2011) that reported that aqueous extract of the leaves of this plant has no deleterious effect on liver function. The aqueous extract of the leaves of *Annona muricata* therefore has little or no effect on the haematology, liver enzymes and immune response to Newcastle disease vaccine.

References

- Allan, W. H. and Gough, R. E. 1974. A standard Haemagglutination Inhibition test for Newcastle disease. A comparison of macro and micro methods. *Veterinary Records*, 95: 120-123.
- Alvarez-Gonzalez, I., Garcia-Aguirre, K. K., Martino-Roaro L., Zepeda-Vallejo, G. and Madrigal-Bujaidar, E. 2008. Anticarcinogenic and genotoxic effects produced by acetogenins isolated from *Annona muricata*. *Abstracts/Toxicology Letters*, 180S: S32-S246.
- Babson, A. L., Read, P. A. and Phillips, G. E. 1959. The importance of the substrate in assays of acid phosphatase in serum. *American Journal Clinical Pathology*, 32(1): 83-87.
- George, C. V., Naveen, D. R., Kumar, V., Rajkumar, P. K. Suresh and Kumar, R. A. 2012. Quantitative Assessment of the Relative Antineoplastic Potential of the n-butanol Leaf Extract of *Annona*

- muricata Linn.in Normal and Immortalized Human Cell Lines. Asian Pacific Journal of Cancer Prevention, 13(1) 699–704.
- Dai, Y., Hogan, S., Schmelz, E. M., Ju, Y. H., Canning, C. and Zhou, K. 2011.** Nutrition and cancer, 63 (5): 795-801.
- David, S. 2009.** Mareks Disease and Leukosis. Poultry health and management, 4th edn. Blackwell Publishers, Cowley road Oxford. pp. 235-238.
- De Feo, V. 1992.** Medicinal and magical plants in the Northern Peruvian Andes. Fitoterapia, 63, 417-40.
- Franco, R. S., Yasin, Z., Palascak, M. B., Ciruolo, P., Joiner, C. H. and Rucknagel, D. L. 1984.** The effect of fetal haemoglobin on the survival characteristics of sickle cell. European Journal of Haematology, 108(3):1073.
- Frank, F. 2001.** Marek's disease: History, actual and future Perspectives. Lohmann Inform. No. 25, pp. 1-5.
- Garcia, L. S. 2001.** Diagnostic medical parasitology. 4th Ed. ASM Press, Washington D.C, USA.
- Hutchison, H. E. 1960.** On determining the packed cell volume. Journal of Clinical Pathology, 13: 529.
- Kim, G. H., Zeng, L., Alali, F., Rogers, L. L. and Wu, F. E. 1998.** **Muricoreacin and murihexocin C**, mono-tetrahydrofuran acetogenins from the leaves of *Annona muricata* in honour of Professor G.H. Neil Towers 75th birthday. Phytochemistry, 49: 565-571.
- Lamberg, S. L. and Rothstein, R. 1978.** Laboratory Manual of Hematology and Urinalysis. Avi. Publishing Company, Inc, West Port Connecticut, USSR.
- Lillie, R. D. 1977.** H. J. Conn's Biological Stains. Williams and Wilkins Comp., 9th Ed. Baltimore, USA, NJ. pp 33.
- Padma, P., Pramood, N. P., Thyagarajan, S. P. and Khosa, L. L. 1998.** Effect of the extract of *annona muricata* and *Petunia nyctagiliflora* on herpes simplex virus. Journal of ethnopharmacology, pp 61: 81-83.
- Padmaa, P. M., Chansouria, J. P. N. and Khosa, R. L. 2009.** Wound Healing Activity of *Annona muricata* extract. Journal of Pharmacy Research, 2(3): 404-406.
- Rajeswari, V. D., Gajalakshmi, S. and Vijayalakshmi, S. 2012.** **P h y t o c h e m i c a l** and pharmacological properties of *Annona muricata*: a review. International Journal of Pharmacy and Pharmaceutical Sciences. ISSN-0975-1491
- Rietman and Frankel, S. A. 1957.** Colorimetric method for the determination of SGOT and SGPT. American Journal of Clinical Pathology, 28: 56-58.
- Tormo, J. R., Royo, I., Gallardo, T., Zafra-Polo M. C., Hernández P., Cortes, D. and Pelaez, F. 2003.** In vitro antitumor structure-activity relationships of threo/trans/threo mono-tetrahydrofuranic acetogenins: Correlations with their inhibition of mitochondrial Complex I. Oncology Resources, 14(3):147-154.
- Vasquez, M. R. 1990.** Useful plants of Amazonian Peru. Second Draft. Filed with USDA's National Agricultural Library. USA.
- Wang, L. Q., Min B. S., Li Y., Nakamura,**

- N., Qin , G. W., Li, C. J. and Hattori, M. 2002 .Annonaceous acetogenins from the leaves of *Annona montana*. *Bioorganisation of Medical Chemistry*, 10(3):561-565.
- Yuan, S. S. 2003. Annonacin, a monotetrahydrofuran acetogenin, arrest cancer cells at the G1 phase and causes cytotoxicity in a Bax- and caspase-3-related pathway. *Life Science*, 72(25), 2853-286.
- Zeng, L., Wu , F. E., Oberlies , N. H., McLaughlin , J. L. and Sastrodihadjo, S. 1996. Five new monotetrahydrofuran ring acetogenins from the leaves of *Annona muricata*. *Journal of Natural Production*, 59(11):1035-1042.

Received: 9th February, 2017

Accepted: 21st June, 2017