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Comparative analysis of *Annona muricata* ethanol leave and stem extracts on testosterone levels of N-Methyl- N- Nitrosourea (NMU) induced prostate cancer in albino rats

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Abstract

The aim of study is to determine the effect of *A. muricata* leave and stem bark extract on testosterone level of N-Methyl-N- Nitrosourea (NMU) induced prostate cancer in albino rats. The rats were divided into 7 groups of 5 rats; group 1 served as normal control, group 2 was the prostate cancer (PC) negative control group, group 3 was administered NMU + finasteride, group 4 = PC + 250 mg/kg of ethanol leaf extract of *A. muricata*, group 5 = PC + 500 mg/kg of ethanol leaf extract of *A. muricata*, group 6 = PC + 250 mg/kg of ethanol stem bark extract of *A. muricata* and group 7 = PC + 500 mg/kg of ethanol stem bark extract of *A. muricata*. Induction of prostate cancer using cyproterone acetate, testosterone propionate and N-methyl-nitroso urea lasted for 21 days after which treatment with extracts of *A. muricata* commenced and lasted for 28 days. The result from this study showed a significant increase ($p<0.05$) in testosterone concentration especially for group 2 when compared to the control. Also, treatment of prostate cancer induced rats administered with ethanol leave and stem extract of *A. muricata* significantly decreased ($p<0.05$) testosterone level in a dose dependent manner. On the basis of our findings, it is concluded that the *A. muricata* leaves and stem can be used as an anticancer agent for the management of prostate cancer.

Keywords: *Annona Muricata*; Finasteride; Prostate Cancer; Testosterone

1. Introduction

Prostate cancer is the most frequent cancer in men and the second highest cause of mortality by cancer for the male population. Approximately 29% and 9% of leading new cancer cases and deaths, respectively, in the United States were attributed to the prostate in 2012 (American Cancer Society, 2012). A 1.33-fold increasing trend of incidence rate between 1999 and 2002 was reported in Korean men (Won et al, 2019). Furthermore, prostate cancer may become problematic if a less than 15-year survival is predicted (Johansson, et al 2014). In Nigeria, 17.35% of male cancer death is attributed to the prostate (Wiredu and Armah, 2016). Treatment, on the other hand, has adverse effects, and in some cases unneeded, as some men do not die from their cancer and may harbor tumors that are indolent even in the absence of therapy (Johansson et al., 2014). Prostate cancer affects more than 50% of men in their 60s and as much as 90% in their 70s and 80s. In the United States in 2000, there were 4.5 million visits to physicians with issues relating to Prostate cancer. Globally and nationally, more and more people are turning to complementary and alternative medicine for various ailments of which the use of medicinal plants is foremost.

Annona muricata, commonly called soursop, is a small erect evergreen tropical fruit tree plant belonging to the family *Annonaceae*, growing 5 to 6 meters in height. The leaves of *A. muricata* have been reported to contain several groups of substances collectively called annonaceous acetogenins. Monotetrahydrofuran annonaceous acetogenins, *cis*-

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corossolone, annocatalin, annonacin, annonacinone, solamin, and corossolone have been isolated from the leaves of *A. muricata* (Onuoha et al., 2023). The first 2 isolates have significant cytotoxic activity *in vitro* against human hepatoma cell lines, Hep G (2) and Hep 2,2,15. Annocatalin showed a high selectivity toward the Hep 2,2,15 cell line (Liaw et al., 2012). Additionally, acetogenins (annoreticuin-9-one) and *cis*-annoreticuin isolated from *A reticulata* and *A montana*, respectively, have been reported to have cytotoxicity against certain cancer cell lines. Acetogenin targets the human pancreatic tumor cell line (PACA-2), human prostate adenocarcinoma (PC-3), and human lung carcinoma (A-549), while *cis*-annoreticuin, targets human hepatoma carcinoma cell line (Hep G2) (Liaw et al., 2012). The dichloromethane extract of the seeds of *A muricata* yielded annoreticuin-9-one, while the flesh of the fruit yielded *cis*-annoreticuin (Onuoha et al, 2023). The presence of Annonaceous acetogenins, muricoreacin and murihexocin C (mono-tetrahydrofuran acetogenins) in the leaves of *A muricata* (*Annonaceae*) with significant cytotoxic activities targeting human prostate adenocarcinoma (PC-3) and pancreatic carcinoma (PACA-2) cell lines has been demonstrated. Leaves of *A muricata* in ethyl acetate showed a higher death rate to HeLa cells than the ethanol distilled water extract. Similarly, chloroform extract application to HeLa cells showed a higher death rate than ethyl acetate extract. The chloroform extracts appear to be a better option for cancer causing viruses (Astirin et al., 2013). The aqueous extract is said to contain general glycosides, saponins, and flavonoids (Onuoha et al., 2023). In an acute toxicity study (LD_{50} < 5000 mg/kg body wt), the aqueous extract did not show any toxicity on systemic organs (Arthur et al., 2011). The ethanolic extract of the leaves of *A muricata* is said to have hypoglycemic and antidiabetic effects (Gupta et al., 2015). Furthermore, its protective effect on lipid profile has been documented

2. Materials and Methods

2.1. Plant sample collection

Annona muricata leaves and stem used for this research work was gotten from a Compound at Ihiagwa, Owerri, Imo state and was air-dried and weighed every two days till a constant weight is gotten indicating that the leaves and stems have completely dried. The dried *Annona muricata* leaves and stem were then ground to fine powder and then stored in an air tight container for further analysis.

2.2. Sample Extraction

The extraction method used in the study was maceration extraction method which involves simple soaking, filtering and then evaporations. 800g of the ground plant material (*Annona muricata*) was weighed into a conical flask and 2000 ml of 70% ethanol was added, ensuring that the solvent properly cover the plant material. The mixture was allowed to stand for 48 hours with constant stirring. After 48 hours the mixture was filtered using filter paper and the filtrate evaporated in a water bath to obtain an extract.

2.3. Calculation of Percentage Yield

The extract was weighed using an electronic weighing balance then the percentage yield was calculated as follows:

$$\text{The percentage yield} = \frac{\text{weight of extract}}{\text{Weight of ground plant leave)}} \text{ x } 100$$

2.4. Animal Environment, Handling and Ethics

Thirty-five (35) male albino rats of body weights mean of 96g were purchased from the Animal Breeding Unit, Zoology Department, University of Nigeria Nsukka, Enugu state, Nigeria. The animals were kept in stainless–steel cages in a well–ventilated room of temperature 28 ± 2°C and relative humidity of 55–65% with a diurnal 12 h light cycle. The rats had access to water and pelletized standard finishers mesh (Vital finisher) (United Africa Company Nigeria Plc., Jos, Nigeria) *ad libitum*. A period of 2 weeks was allowed for acclimatization of the rats to environmental conditions.

The Thirty-five (35) male albino rats were divided into seven (7) groups of five (5) rats each.

Table 1 Experimental design

Groups	Description	Treatments	No. of rats
One	Normal control	Feed and water only	5
Two	Negative control	50 mg/kg of cyprosterone acetate (CA) + 100 mg/kg testosterone propionate (TP) + 50 mg/kg of NMU	5
Three	Standard control	50 mg/kg of CA + 100 mg/kg TP + 50 mg/kg of NMU + 50 mg/kg of finasteride	5
Four	Low dose leave	50 mg/kg of CA + 100 mg/kg TP + 50 mg/kg of NMU + 250 mg/kg of leave extract	5
Five	High dose leave	50 mg/kg of CA + 100 mg/kg TP + 50 mg/kg of NMU + 500 mg/kg of leave extract	5
Six	Low dose stem	50 mg/kg of CA + 100 mg/kg TP + 50 mg/kg of NMU + 250 mg/kg of stem extract.	5
Seven	High dose stem	50 mg/kg of CA + 100 mg/kg TP + 50 mg/kg of NMU + 500 mg/kg of stem extract	5

2.5. Induction of prostate cancer and extract administration

Induction of prostate cancer was carried out using the method of Bosland and Prinsen (1975) with little modification. After 2 weeks of acclimatization, the rats were given 50 mg/kg of cyprosterone acetate in normal saline via intraperitonial route for 18 days, after which the rats received subcutaneous injection of testosterone propionate (100 mg/kg) in olive oil for another 3 days, this was followed by a single shot (dose) of NMU (50 mg/kg) in normal saline via intraperitonial injection.The different doses of extracts and finasteride were administered orally following induction of prostate cancer for 28 days.

2.6. Collection of animal Sample for analysis

At the end of 28 days, the animals were anaesthetized with chloroform and then sacrificed. Whole blood was collected in plain sample bottles through cardiac puncture using sterile needles and syringes. The clotted blood was centrifuged at 3000 rpm for 10 mins to obtain serum. Liver, kidney and prostate were also harvested for histopathological examination.

2.7. Determination of testosterone level

Serum testosterone (T) was assayed from blood obtained from left ventricular puncture. The samples were assayed in batches from a standardized curve using the enzyme linked immunosorbent assay (ELISA) method (Tietz, 1995). The microwell kits used were from Syntro Bioresearch Inc., California USA. Using 10 µl of the standard, the samples and control were dispensed into coated wells. 100 µl T conjugate reagent was added followed by 50 µl of anti-T reagent. The contents of the microwell were thoroughly mixed and then incubated for 90 min at room temperature. The mixture was washed in distilled water and further incubated for 20 min. Absorbance was measured with an automatic spectrophotometer at 450 nm. A standard curve was obtained by plotting the concentration of the standard versus the absorbance and T concentration was determined from the standard curve.

2.8. Statistical Analysis

The data generated in this study were processed and analyzed using ANOVA, comparing the average mean and standard deviation of the different groups at $P \leq 0.05$.

3. Results

Table 2-4 showed that groups of prostate cancer induced rats treated with ethanol leave and stem extract of *A. muricata* significantly decreased ($p<0.05$) testosterone level in a dose dependent manner with the most effective result seen in highest leave concentration.

Table 2 Testosterone level of NMU-induced rats treated with *A. muricata leaf*

parameter	**NC**	**PC**	**PC + F**	**PC + AL_L**	**PC+AL_H**
Testoterone (ng/ml)	0.824 ± 0.52^{a}	2.105 ± 0.73^{c}	1.327 ± 1.01^{ab}	1.583 ± 0.37^{b}	1.023 ± 0.06^{a}

Values represent mean ± standard deviation of triplicate determinations and values with different alphabets indicate significant difference at $p < 0.05$.; NC = normal control, PC = prostate cancer, F = finasteride, AL_L = *A. muricata* leave (low dose), AL_H = *A. muricata* leave (high dose)

Table 3 Testosterone level of NMU-induced rats treated with *A. muricata stem extract*

parameter	**NC**	**PC**	**PC + F**	**PC + AS_L**	**PC+AS_H**
Testoterone (ng/ml)	0.824 ± 0.52^{a}	2.105 ± 0.73^{c}	1.327 ± 1.01^{ab}	1.672 ± 0.41^{b}	1.371 ± 0.32^{ab}

Values represent mean ± standard deviation of triplicate determinations and values with different alphabets indicate significant difference at $p < 0.05$. ; NC = normal control, PC = prostate cancer, F = finasteride, AL_L = *A. muricata* stem (low dose), AL_H = *A. muricata* stem (high dose)

Table 4 Testosterone level of NMU-induced rats treated with *A. muricata* leave and stem

				Leave extracts		**Stem extracts**	
	NC	PC	PC + F	PC + AL_L	PC+AL_H	PC + AS_L	PC + AS_H
Testoteron e (ng/ml)	0.824 ± 0.52^{a}	2.105 ± 0.73^{c}	1.327 ± 1.01^{a}	1.583 ± 0.37^{b}	1.023 ± 0.06^{a}	1.672 ± 0.41^{b}	1.371 ± 0.32^{a}

Values represent mean ± standard deviation of triplicate determinations and values with different alphabets indicate significant difference at $p < 0.05$. dose), AL_H = *A. muricata* leave (high dose), PC + AS_L = *A. muricata* stem (low dose), PC + AS_H = *A. muricata* stem (high dose).

4. Discussion

The result from this study showed a significant increase ($p<0.05$) in testosterone concentration in group 2 (following administration of cyprosterone acetate, testosterone propionate and NMU) compared to the normal control group. The result also showed that treatment of prostate cancer induced rats with ethanol leave and stem extract of *A. muricata* significantly decreased ($p<0.05$) testosterone level in a dose dependent manner compared to group 2 (PC) with the leave extract showing greater Ameliorative effect when compared to the stem bark extract.

Decreased testosterone level seen following treatment with leave and stem bark extract of *A. muricata* implies decreased dihydrotestosterone (DHT) and vascular endothelial growth factor (VEGF) levels, this indicate that they can restrain the development of prostate cancer via decreasing the androgen level, similar to the mechanism of finasteride as seen in result of analysis done according to Glass et al. (2010). The level of testosterone in the finasteride treat group was also significantly reduced when compared to the untreated group (group 2). Finasteride, a type II 5α-reductase inhibitor, suppresses both the plasma and intraprostatic testosterone concentrations, leading to reductions in glandular size, epithelial cell height and the synthetic and proliferative activities of testosterone as studies done by Huynh (2012) which states that finasteride also enhances the apoptotic index of epithelial cells and is thus employed as a positive control. The result of this study also showed that testosterone level was significantly reduced in *A. muricata* leave extract treated group compared to stem bark extract treated group. This could be as a result of major phyto-therapeutics resident in the leaves of *A. muricate* (Onuoha et al 2023). Studies have demonstrated high level of bioactive components in the leaves of *A. muricata* such as flavonoids, annonaceous acetogenins, saponins, alkaloids and other phenolic compounds (Onuoha et al 2023).

5. Conclusion

From the above findings it can be concluded that treatment with ethanol leave and stem bark extract of *A. muricata* showed ameliorative effect on prostate cancer induced rats, the leave extract showed more phyto-therapeutic effect when compared to the stem bark extract. The study thus substantiates the traditional use of *A. muricata* for the management/treatment of prostate cancer. Hence, A. *muricata* might be used an anticancer agent for the management of cancer.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict-of-interest to be disclosed.

Ethics approval and consent to participate

The study was approved by the Ethical Committee on Human Research of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria. With approval NO. FUT/SOBS/BCH/COM.2/013/2022 and done in accordance with the highest International Criteria of Animal Experimentation of Helsinki.

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