Available online at www.elixirpublishers.com (Elixir International Journal)

Biosciences

Elixir Biosciences 79 (2015) 30315-30318

ISSN: 2229-712X

Anti-tumor/anti-cancer activities and toxicity assessment of leaf methanol extract of *Annona senegalensis* pers. (annonaceae)

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ARTICLE INFO

Article history:

Received: 6 December 2014;

Received in revised form:

20 January 2015;

Accepted: 5 February 2015;

Keywords

Anti-tumor,

Annona senegalensis,

Toxicity,

Mean survival time,

Hematological parameters.

ABSTRACT

This present study was aimed determining the potency of methanol extract of leaves of *Annona senegalensis*. on tumor-bearing Swiss albino mice (20 in number)and to evaluate the safety of the leaves in ethnomedicinal prescription. The animals were divided into 5 groups of 4 animals each. Mean survival time (MST) and percentage increase in life span were highest in group IV with values 3.0 ± 0 .03 and 5±0.05 respectively at 1000 mg / kg body weight (b.w). Packed cell volume (PCV) showed progressive decrease as the dosage increased from 100 mg/kg to 1000 mg/kg in all the groups when compared with doxorubicin control groups with value 34.5. Viable tumor cell counts were 22.2 (group III 100 mg/kg), 20.1 (group IV 1000 mg/kg) and 18.0 group V (doxorubicin 10 mg / kg standard drug) and values are statistically different from the MEAS control (group II) with value 28.2. Non-viable tumors cell counts were on the increase as the doses increased; 5.10 (group III 100 mg/kg), 6.47(group IV 1000 mg/kg) and 7.24 (group V 0.8 mg/kg doxorubicin standard drug) while the MEAS control (group II) was 5.00 and values were compared. All hematological parameters showed increase at the doses (intraperitoneal) investigated except total WBC white blood cells with slight decrease in values among the groups. Toxicity studies (LD_{50}) showed that the leaf methanol extract was safe even at 5000 mg/kg b.w. The study therefore showed that leaves methanol extract of *A. senegalensis* Pers. had anti-tumor properties on the experimental animals and can therefore serve as a medication for tumor problems.

Introduction

Annona senegalensis tends to grow in semi arid to sub humid regions it is also found in savannah, part of tropical rain forest island of Sudan and South Africa, often but not exclusively on coastal based rocks with mostly sandy, loamy soils from sea level up to 2400 m at mean temperature between 17 ^{0}C and 30 ^{0}C and mean rainfall solitary plant within woodland savannah under storey also frequently in swamp forest or riverbanks or on a former crop land left fallow for an extended period [1]. Preliminary phytochemicals analysis of leaves showed the presence of steroids, triterpenes Anthocyanins, coumarins, flavonoids and alkaloids [2].

Leaves are also part of the diet of west African giraffe [3]. The stem bark can be processed to produce yellow- brown dye insecticide, or medicine for treating a wide array of ailments, including worms parasitic on the intestine or flush(notably guinea worms) diarrhea, lung infections, tooth aches and even snake bites [3].

Cancer; also know as malignant tumor, is a group of involving abnormal cell growth with the potential to invade or spread to other parts of the body [4]. Not all tumors are cancerous. Benign tumor do not spread to other parts of the body. Possible signs and symptoms include, a lump, abnormal bleeding, a prolonged cough, unexplained weight loss, and a change in bowel movements, among others. While these symptoms may indicate cancer, they may also occur due to other issues [4]. There are over 100 different known cancer that affect humans and each is named according to the part of body affected.

Many cancers can be prevented by not smoking, eating more vegetables fruits and whole grains, eating less meat and minimizing sunlight exposure, and being vaccinated against certain infectious disease [5]. Early detection through screening is useful for cervical and colorectal cancer [5]. Cancer is often treated with some combination of radiation therapy, surgery, chemotherapy, and targeted therapy [6]. The most common types of cancer in males are lungs cancer, prostate cancer, colorectal cancer.

In female the most common type are breast cancer, colorectal cancer, lung cancer, cervical cancer [6]. Rate are increasing as more people live to an old age and as lifestyle changes occur in the developing world . Tumor also sometimes referred to as neoplasm is an abnormal cell as result of abnormal growth or division of cells [7]. Prior to abnormal growth (known as neoplasia), cell often undergo an abnormal of growth, such as metaplasia or dysplasia. The most common types of primary tumor in adults are; meningiomas and astrocytomas such as globlastomas [7]. In children the most common type is medulloblastomas Based on finding the tumor are divided into different grade or severity. A tumor develops when a lesion or lump is formed in your body due to abnormal cellular growth while in the case of cancer, the cellular growth is uncontrollable and it spreads in the body [7].

The morbidity and mortality of cancer the second leading cause of death worldwide next to cardiovascular disease, is

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characterized by uncontrolled cellular growth, local tissue invasion, and distant metastases [7]. Free radicals, one of the major causes for the conversion of normal cell to cancerous cells, are generated as a consequences of a number of endogenous metabolic processes involving redox enzymes and bioenergetics electron transfer and exposure to a plethora of exogenous chemicals [8]. In normal metabolic condition, oxidant and antioxidant levels are maintained in balance within humans for sustaining optimal physiological conditions [8]. Moreover, the increase of cancer incidence and lack of appropriate anticancer drugs have forced scientists to pharmacological and chemical investigation of anticancer anti-cancer/anti-tumor agents from medicinal plants [8]. The world wide upsurge in the use of herbal preparation and medicinal plant along with their isolated active compound has provided one of the most important sources for pharmaceutical industry for lead compounds.

This study was designed in order to inhibit or kill the growth of tumor cells which will result in cancer cells with a view to finding an anticancer drugs of plant origin from *Annona senegalensis.*

Materials And Methods

Plant collection and identification

The leaves of *Annona senegalensis* Pers. were collected from a forest around Janniri District Bali local, Taraba State and identified at the Science Laboratory Department of Federal Polytechnic Bali, where a voucher number of **FEDPOBAL2013ANNOO2** was deposited for the plant.

Preparation and extraction of plant material

The plant material was air dried at room temperature for two weeks and was pulverized into coarse powder using electronic blender(Model 5000, China). The powder was then sieved using sieve number 20 mesh (British standard) to obtain a fine powder and weighed. The plant material was then kept in a clean and dried closed jar for further use. 100g of leaf powdered plant material was poured into a 1000 ml capacity separating funnel and soaked with 300 ml of methanol in 150 ml of distilled water at room temperature for 24 hr using cold maceration technique. The extract was filtered through Buchner funnel using whatman filter paper number one. The filtrate were evaporated using rotary evaporator.

Anti tumor/Anti-cancer Studies of Leaf Methanol Extract

Grouping of animals

About 20 Swiss albino mice were divided into five group of four each. All the animals were allowed to acclimatized in the cage for 24hrs. After this, the mice were weighed using electronic balance and their weights recorded.

***In vivo* induction of Tumor**

The animals were given 300 ml formalin (Zayo-Sigma Abuja, Nigeria) in a bottle containing 100 ml distilled water orally (*ad libitum*), and allowed to stay for four weeks for the development of either nasal, pharyngeal or stomach tumors [9]. After tumor development, the animals were then administered the methanol leaf extracts of *Annona senegalensis* (MEAS) intraperitoneal. Group1 received 5ml/kg normal saline, group II, III and IV received 10mg/kg, 100mg/kg and 1000mg/kg respectively while group V received Doxorubicin (Adriamycin® USP) 10mg/kg i.p. The animals were then observed for 4 weeks. Tumor parameters were then taken and compared with standard values. Body weight gained were also taken as a sign of recovery for tumor invasion on vital organs of the mice.

Hematological Parameter Studies in Mice

Hematological parameters such as WBC(counting chambers), HB, and RBC were analyzed using standard procedures [10]. Mean survival time(MST) and increase in life span (%ILS) were calculated thus:

MST= Day of first death + day of last death / 2

%ILS = MST of treated group/MST of control group x 100

Acute toxicity (LD_{50}) Studies

Acute toxicity study of the leave and stem bark of *Annona senegalensis* was carried out according to the method of Lorke (1983). 26 Swill Albino mice of weights between 27g and 43 g were allowed to acclimatized for 24hour. In the first phase, mice were divided into three groups of three animals each and were treated with the extract at doses of 10, 100 and 1000 mg/kg body weight I.P (intra-Peritoneal). They were observed for 24 hour for behavioral changes and mortality for one week.

In the second phase, mice were further divided into three groups of one mouse per group and were administered with the extract doses of 1600, 2900 and 5000 mg/kg b.w i.p and observed for another one week for mortality and general behavioral changes. The LD_{50} was calculated as:.

$$LD50 = GM\sqrt{Minimum\ toxic\ dose\ x\ Maximum\ tolerated\ dose}$$

Results

Table 1: Mean survival time and % increase in life span After tumor induction/ treatment

Experimental group	MST	% ILS
Normal control group1	-	-
10 mg/kg group 2	2.2	35.10
100mg/kg group3	2.5	37.50
1000mg/kg group4	3.0	50.50
Grp5doxo.10mg/kg	3.2	53.62

*Mean survival time (MST), Increase in life span(%ILS), doxo (doxorubicin control drug).

Table 2: Effects of methanol extract of *Annona senegalensis* on tumor cell volume (TCV), packed cell volume(PCV), viable and non viable cell count on MEAS tumor- bearing mice

Parameters	Normal Group1	10 mg/kg group2	100mg/kg group3	1000mg/kg group4	Doxorubicin group5
Body weight	29.5	19.6	20.4	30.2	17.8
TCV	0	36.5	35.4	33.5	32.13
PCV	0	34.5	33.1	32.2	31.0
VCC	0	28.2	22.2	20.1	18.0
NVC	0	5.00	5.10	6.47	7.24

Weight of mice tend not to be stable at the doses administered whereas tumor cell volume (TCV) packed cell volume, viable tumor count values decreased as the dosage increased in groups[table3]. However, non viable tumor cell count was on the increase dosage and significantly different from the MEAS control group

Table 3: Effect methanol extract of *Annona senegalensis* (MEAS) leaves on hematological parameters of MEAS treated albino mice.

Parameters	Normal	10mg/kg	100mg/kg	1000mg/kg	Doxorubicin
	Gp1	GpII	GpIII	GpIV	GpV
Hb(g)	14.88	9.82	10.60	11.45	11.70
RBC	8.62	3.81	4.75	5.42	5.81
WBC	7.82	20.07	11.92	8.85	9.12

* All values are within normal range of NCI, Gp(group) .

Table 4: Acute toxicity study of leaf of *Annona senegalensis* methanol extract

Group	Dosage (mg/kg)	Animal died/Animal survived
Phase I (n=3)		
Group I	10	0/3
Group II	100	0/3
Group III	1000	0/3
Phase II (n=1)		
Group I	1600	0/1
Group II	2900	0/1
Group III	5000	0/1

*LD_{50} > 5000 mg/kg b.w [biological unimportant; [11]]

Figure 1: An albino mice showing stomach tumor after 4weeks induction.

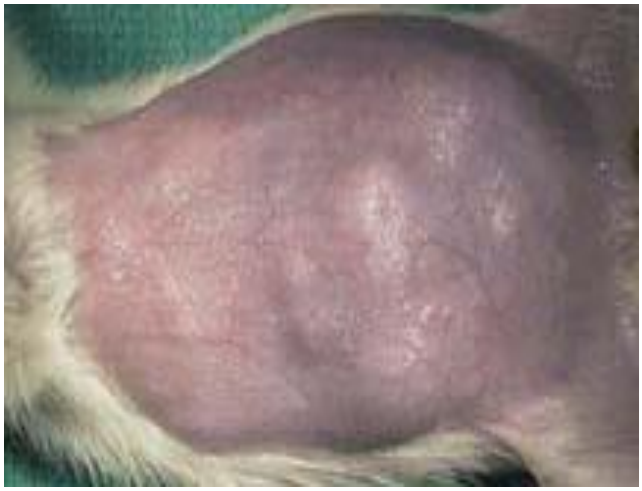

Figure 2: Mice showing benign tumor of the GIT after 2 weeks induction of formalin/water orally.

Discussion

Preliminary phytochemical analysis of *Annona senegalensis* leaves showed the presence of steroids, triterpenes anthocyanins, coumarins, flavonoids and alkaloids. These phytoconstituents exhibit diverse pharmacological and biochemical actions. Therefore, the antitumor properties of methanol extract of leaves was as a result of the presence of the listed phytochemicals. There were no doubts in any case that these constituents in the plant played crucial roles in the inhibition of tumor growth in the mice in all the groups, since the there were decrease in weights in the mice after 4 weeks induction and a significant weight gain in all the groups after extract administration i.p. This weight increase occurred in a dose dependent fashion in all the groups.

The potency of *A. senegalensis* methanol leaf extract on tumor inhibition and induction of apoptosis in the animals were comparable to the control first line drug adriamycin(doxorubicin 10 mg) (Table 1; fig.1). Thus, the MEAS has proving to be a strong antitumor as well as anticancer agents from the study. Higher dose of extract (1000 mg/kg) increases the mean survival time (MST) and percentage increase in life span (%ILS) of albino mice, and the results are significantly different from the control (group) (Table 1, 2, 3; fig. 2) . This implies that at higher dose a better result is achieved in the antitumor properties of the plant. The implication is that, as the dosage increases, body weight did not reduce significantly in all the groups, while the tumor cell volume, packed cell volume, and viable tumor cell count values decreased. Non-viable tumor cell count values increases with increased dosage(Table 3). Despite the slight reduction in the white blood cell in groups 3 and 4, other parameters such as hemoglobin concentration, red blood cell (RBC) had higher values which are significantly different from the extract control (Table 3).

Toxicity test are generally the first test conducted in any toxicity study, a summary of the result of the mortality and gross symptoms of toxicity seen in mice administered i.p (Intra-peritoneal) with the extract of leave and stem bark of *Annona senegalensis.* There was no noticeable deviation in the behavior of mice administered i.p with 10,100, 1000, 1600, 2900 and 5000 mg/kg compared to that of the control all the dosed mice remained healthy during the 2 weeks.(table 4). There was decrease in the body weight of the mice, the animals use in the study were fed with normal mice diet. The acute lethality, LD_{50} of the extracts indicate a relatively high safety profile.

Conclusion

The study showed that methanol extract of *Annona senegalensis Pers.* Leaves possessed antitumor/anti-cancer properties and can be used to treat tumor in traditional medicine and are safe . This plant part thus represents a source towards new drug discovering.

Acknowledgement

The authors are grateful Zayo -Sigma Abuja Nigeria for the assistance.

Conflict of Interest

The authors declares no conflict of interest.

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