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A BRIEF REVIEW ON RECENT ADVANCES IN CLINICAL RESEARCH OF *ANNONA MURICATA*

Trupti P. Sawant and Dayanand P. Gogle*
Post Graduate Teaching Department of Botany, RTM Nagpur University, Nagpur- 440033.

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For Correspondence:

Dayanand P. Gogle*
Address:
Post Graduate Teaching Department of Botany, RTM Nagpur University, Nagpur- 440033.

ABSTRACT

Annona muricata (Graviola) has been used as a traditional or folk medicine as it has been reported to have broad range of therapeutic effects apart from its nutritious value. In order to reveal its medicinal properties for which "Acetogenins" plays vital role, many researches and phytochemical screenings have been done to isolate various chemical components from acetogenins of Graviola. Along with it, different researches have been done on its anticancer (breast cancer, ovarian cancer), antimicrobial, wound healing capacity and many more. While the Soursop / Graviola / *Annona muricata* is gaining popularity as a medicinal plant, it is necessary to know the clinical research and development work carried out in India and elsewhere on this subject, so an attempt is being made to review the available literature on such work and is being presented here in this article.

INTRODUCTION:

Medicinal plants are considered to be the main sources of biologically active compounds that can be used for the treatment of various ailments including cancer. Out of the 250,000-500,000 plant species on the earth, only 1-10% have been studied chemically and pharmacologically for their potential medicinal value especially for chemotherapeutic effect [1]. The era of chemotherapy began in 1940s with the first use of nitrogen mustards and antifolate drugs [2]. Thereafter, cancer drug discovery and development have been the major research endeavour around the globe as evidenced by several peer-reviewed papers in the scientific literature [3]. Excessive free radical formation is one of the hallmarks of cancer cells. Several studies have shown that plant-derived antioxidant neutraceuticals scavenge free radicals and modulate oxidative stress-related effects [4]. Various compounds isolated from plants are known to be effective against proliferating cells. They exhibit cytotoxic effects either by damaging DNA or by locking the formation of mitotic spindle during stages of cell division [5]. However, most of the cytotoxic chemopreventive drugs exhibit side effects [6] at some point of time during therapy, and hence, there is a need to isolate compounds that are potent and selective with minimal side effects on normal cells [7].

The fruit-pulp is soft with an agreeably sour flavour. It is usually eaten raw but unfortunately contains a quantity of fibre. It may be more acceptable after some preparation that is either as juice, ice-cream, jellies but not jams. Commonly called Soursop, *Annona muricata* is a plant, which belongs to the family Annonaceae. H. M. Burkill in the Useful Plants of West Tropical Africa described the Soursop plant as "a small tree attaining a height of about eight metre. A native of tropical America, but now widespread in the tropics, it is thought to have reached Africa (Angola) by 1686. The trunk and timber do not appear to have any particular uses." Soursop is a medicinal plant that has been used as a natural remedy for a variety of illnesses. Several studies by different researchers demonstrated that the bark as well as the leaves has anti-hypertensive, vasodilator, anti-spasmodic (smooth muscle relaxant) and cardio depressant (slowing of heart rate) activities in animals. Researchers had re-verified Soursop leaf's hypotensive (reduce blood pressure) properties in rats. Other properties and actions of Soursop documented by traditional uses include its use as anti-cancerous, antidiabetes, anti-bacterial, anti-fungal, anti-malarial, anti-mutagenic (cellular protector), emetic (induce vomiting), anti-convulsant, sedative (induces sleep), insecticidal and uterine stimulant (helps in childbirth). It is also believed to be a digestive stimulant, antiviral, cardio tonic (tones, balances and strengthens the heart), febrifuge (cures fever), nerviness (balances/calms the nerves), vermifuge (expels worms), pediculocide (kills lice) and as an analgesic (pain-reliever) [8]. Graviola (Soursop) belongs to the genus Annonaceae and comprises about 150 species. It is a small tree of tropical South America, no more than 20 feet tall; the oblong to oval leaves are leathery, very

dark and shiny green. They have a pungent odour when crushed. The tree has larger individual yellow flowers on woody stalks (pedicels). Graviola fruit is prickly and oblong or somewhat curved; with a length of 13 inch and a weight of up to 8 pound. The tree may bear fruits anywhere on its trunk or branches. The fruit has 40 - to 100 black seeds. The creamy, aromatic pulp is used in ice cream and as a juice: it is rich in vitamin B and C. It has a musky, sub - to acid flavour.

Common names - Nangka blanda, soursop, guanabana, graviola, prickly custard apple, durian benggala, zuurzak, Brazilian pawpaw, Sirsak, Sauersack [9].

RECENT ADVANCES IN CLINICAL RESEARCH OF *ANNONA MURICATA*

Clinical research is a branch of healthcare science that determines the safety and effectiveness of medications, devices, diagnostic products and treatment regimens intended for human use. These may be used for prevention, treatment, diagnosis or for relieving symptoms of a disease. Clinical Research is different from clinical practice. In clinical practice one uses established treatments, while in clinical research evidence is collected to establish a treatment. The term clinical research refers to the entire bibliography of a drug/device/biologic, in fact any test article from its inception in the lab to its introduction to the consumer market and beyond. Once the promising candidate or the molecule is identified in the lab, it is subjected to pre-clinical studies or animal studies where different aspects of the test article (including its safety toxicity if applicable and efficacy, if possible at this early stage) are studied.

There are many claims that the fruit of the Graviola tree can kill cancer far better than chemotherapy, or can seek out and attack malignant cells. The most popular claim about Graviola is that the fruit is 10,000 times more effective than chemotherapy Soursop has many medicinal properties. And various clinical researches are done on *Annona muricata* to prove its different medicinal activities. Following are some clinical research done upon *Annona muricata* – Antibacterial activities, Antinociceptive and Anti-ulcerogenic Activities, Anti Hyperglycemic , Anti Tumor Activities, Antineoplastic Activities , Anti Ovarian Activities and Anticancer activities on different cell lines like T47D cell lines. And Wound healing capacity, Chemo preventive potential, Antioxidant activity.

1. **Antibacterial Activities:**

Antibacterial effects of aqueous and ethanolic extracts of pods of soursop were examined against Staphylococcus aureus, Vibrio cholerae and Escherichia coli. The bioactivity of water-based soursop extracts against S. aureus and V. cholerae may be related to the chemical structure of the active substances. In an investigation of the bactericidal properties of eight species of annonaceae, it was confirmed the ability of trachylobanoic acid to inhibit S. Aureus. Annonaceae contain other bioactive substances, including a range of acetogenins with a wide spectrum of action, including

antibiotic effects. Structurally, Annonaceous acetogenins are series of C-35/C-37 natural products derived from C-32/C-34 fatty acids and combined with a 2-propanol unit [10]. *Annona muricata* extract of leaves exhibited a broad spectrum of activity against a panel of bacteria (B. subtilis, Staph aureus, K. pneumonia, P. vulgaris, etc.) responsible for common bacterial diseases like pneumonia, diarrhoea, UTIs and skin infections [11].

Antibacterial effect (*in vitro*) of *Annona muricata* against gram positive and gram negative bacteria

Gustavo Hitzschky Fernandes Vieira & co-workers in 2010 investigated the antibacterial effects of aqueous and ethanolic extracts of pods of soursop were examined against Gram positive and Gram negative bacteria. The objective of this study was to evaluate the bactericidal effect soursop extracts upon four bacterial species commonly associated with food intoxication: Staphylococcus aureus, Vibrio cholerae, Escherichia coli and Salmonella spp.

Evaluation of bactericidal effects: The bactericidal effect of the extracts was evaluated with the modified Kirby-Bauer disk diffusion method. The strains were inoculated in TSA and incubated at 35 ºC for 24 hours. Cultures were then adjusted to a concentration of 10.CFU/mL by making a suspension in 0.85% saline solution match the 0.5 McFarland turbidity standards. Using a sterilized swab, aliquots from each tube were spread on dishes with Muller-Hinton agar (Difco), extract was added and incubated at 35 ºC for 24 hours. Disks soaked with sterile distilled water and ethanol p.a. were used as negative control (Fig 1). Extracts producing halos of bacterial growth inhibition greater than 13 mm were considered effective.

Table 1: Bactericidal effect of water- and ethanol-based soursop extracts (*Annona muricata*)

Extract	Volume µL/dish	Diameter of inhibitory halo (mm)						
		SA	VC	SAL	EC1	EC2	EC3	EC4
Water-based	50	14	17	-	-	-	8	-
	100	14	18	-	-	-	8	-
	150	15	19	-	-	-	9	-
	200	16	23	-	-	-	10	-
Ethanol-based	50	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-
	150	-	-	-	-	-	-	-
	200	-	-	-	-	-	-	-

** - no activity; SA (Staphylococcus aureus ATCC25923); VC (Vibrio cholerae classic 569B); SAL (Salmonella Enteritidis); EC1 (Escherichia coli - fish); EC2 (E. coli - shrimp); EC3 (E. coli - river); EC4 (E. coli - lake).*

The all volumes of soursop extract were bactericidal to *S. aureus* and *V. cholerae*. The greatest halos (16 and 23 mm) were observed at 200 µL/dish.

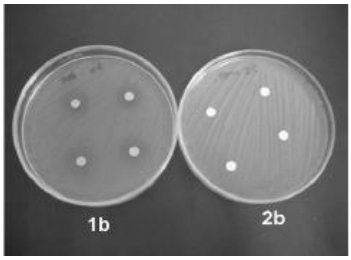

Fig 1: Antibacterial activity of water soursop extracts against *Escherichia coli* (1b) and disks soaked with sterile distilled (negative control) (2b).

The fact that the only strains resistant to all our extracts (*Salmonella* and *E. coli*, sampled at the lake "Lagoa da Fazenda") were Gram-negative, may be related to cell wall structure. According to TORTORA *et al*., the cell wall of Gram-negative bacteria acts as a barrier to a number of substances, including antibiotics. This would also explain why medicinal plants tend to be more effective against Gram-positive than Gram-negative cultures The results of the present study confirm the importance of laboratory-testing medicinal plants used in indigenous medicine in search of new substances capable of inhibiting *S. aureus*, classic *V. cholerae* and *E. coli* [12].

Phytochemical Screening and Antimicrobial activities of *Annona muricata* (L) leaf extract.

Solomon-Wisdom and his co-workers from Abuja in their study of phytochemical screening and microbial activities of *Annona muricata* leaf extract. The aim and objective of this research work is to screen the aqueous and alcoholic extract of *Annona muricata* for their biologically active chemicals, with a view to provide a scientific basis for use of the leaves for prevention and treatment of diseases. And to determine the antimicrobial activities on some selected micro organisms. The antibacterial activity was done using agar cup method.

Table 2: Zone diameter of inhibition of methanolic leaf extract on *Annona muricata*

Test Organisms	Concentration of Extract (mg/ml)				Positive control	Negative control
	400	200	100	50	(streptomycin)mg/ml	(water)ml
	Diameter of inhibition (m)					
Bacillus subtilis	19.5 ± 0.5	18.5 ± 0.5	17.5 ± 0.5	14.5 ± 0.5	23.10 ± 0.5	0.00
Staphylococcus aureus	20.5 ± 0.5	17.5 ± 0.5	15.5 ± 0.5	14.5 ± 0.5	23.40 ± 0.5	0.00
Klebsiella pneumonia	18 ± 1.0	16.5 ± 0.5	15.5 ± 0.5	14.5 ± 0.5	21.80 ± 0.5	0.00
Salmonella cyphimurium	16.5 ± 0.5	15.5 ± 0.5	14.5 ± 0.5	14.5 ± 0.5	23.50 ± 0.5	0.00
Escherichia coli	16.5 ± 0.5	13.5 ± 0.5	-	-	26.80 ± 0.5	0.00
Streptococcus pyogenes	17.25 ± 1.15	15 ± 1.0	14.5 ± 0.5	13.75 ± 0.15	22.40 ± 0.5	0.00

Table 3: Zone diameter of inhibition of aqueous leaf extract of *Annona muricata*

Test Organisms	Concentration of Extract (mg/ml)				Positive control	Negative control
	400	200	100	50	(streptomycin)mg /ml	(water)ml
	Diameter of inhibition (m)					
Bacillus subtilis	18.5 ± 0.5	16.75 ± 1.12	-	-	23.10 ± 0.5	0.00
Staphylococcus aureus	17.75 ± 0.13	20.5 ± 4.5	-	-	23.40 ± 0.5	0.00
Klebsiella pneumonia	16 ± 1.0	22.5 ± 0.5	15.25 ± 1.15	14.5 ± 0.5	21.80 ± 0.5	0.00
Salmonella typhimurium	16.5 ± 0.5	17.5 ± 0.5	-	-	23.50 ± 0.5	0.00
Escherichia coli	17.5 ± 0.5	24.5 ± 0.5	-	-	26.80 ± 0.5	0.00
Streptococcus pyogenes	-	-	-	-	22.40 ± 0.5	0.00

Table 4: Results of Phytochemical Screening of the Leaf of *Annona muricata*

Secondary metabolites	Aqueous Extract	Methanol Extract
Steroids	+	+
Cardiac glycoside	+	+
Alkaloid	+	+
Saponin	+	+
Anthraquinone	-	-
Tannin	+	+
Flavonoids	+	+

Key: + = Presence of Secondary Metabolite, - = Absence of Secondary Metabolite

The phytochemical analysis revealed the presence of secondary metabolites like Tannins, Steroids, Cardiac glycoside; Alkaloids, saponins and Flavonoids were present in trace amount in the leaves except Anthraquinone (Table 4). It is not surprising that there are difference in the antimicrobial effects of plant species, due to the phytochemical properties and differences among species. It was found that, Klebsiella pneumonia was inhibited in all the concentrations of both extract (Tables 2 and 3), Streptococcus pyogenes did not show any inhibition with the aqueous extract (Table 3). Staphylococcus aureus had the highest zone of diameter of inhibition 20.5+ 0.5 at 400mg/ml of the methanolic extract, while Bacillus subtilis showed the highest zone diameter of inhibition 18.5±0.5 at 400mg/ml of aqueous extract. The comparative antibacterial activity between methanolic and aqueous extracts of *Annona muricata* and the standard antibiotic streptomycin revealed that the methanolic extract showed significant ($P<0.05$) antibacterial efficacy and could compete with the standard antibiotic, streptomycin [13].

2. Antinociceptive and Anti-ulcerogenic Activities:

The antinociceptive activity of orally administered ethanol extract of *Annona muricata* leaf (AML) was demonstrated in mice and rats. Acetic acid injected into peritoneal cavity is believed to be able lead to an increase of cyclooxygenase (COX) and lipooxygenase (LOX) products in peritoneal fluids as well as promoting the release of other inflammatory mediators such as bradykinin, substance P, TNF-α, IL-1β, IL-8, which finally stimulate the primary afferent nociceptors entering dorsal horn of the central nervous system. The administration of the AML significantly reduced the number of abdominal writhing induced by acetic acid in a dose-dependent manner. The result may suggest that the mechanism of AML may be partly mediated by the inhibition of COX and/or LOX and other inflammatory mediators in peripheral tissues. Also, the antinociceptive activity of AML could also be suggested by the interruption of signal transduction in primary afferent nociceptors. Therefore it is suggested that AML may contain active ingredients which may act both centrally (via inhibition of central pain receptors) and peripherally (through inhibition of COX and/or LOX). Besides that, it is well described that endogenous opioid system is largely involved in the central regulation of pain, as well as in the action of opioid-derived analgesic drugs. The present results exerted that the antinociception elicited by AML seems to be dependent of the activation of opioid system. The antinociceptive effect may due to the activation of opioid or central receptors or the modulation of the effect of endogenous opioid peptides which may participate in the antinociceptive activity at both peripheral and central levels. In addition, AML has been observed to exhibit its antiulcerogenic effect in dose-dependent manner which relates to cytoprotective and antioxidant properties. Gastric mucus acts as an important protective factor for the gastric mucosa. It is not only capable of acting as an antioxidant agent but also reducing mucosal damage mediated by oxygen free radicals. However, the protective properties of the mucus barrier depend on the gel structure and also on the amount or thickness of the layer covering the mucosal surface. Therefore, antiulcer agents should provoke mucosa-strengthening effect and cicatrisation action with low occurrence of side effects. This effect is known as cytoprotection. AML have antioxidant effect and this could be one of the contributory factors in producing its antiulcerogenic effect. In many studies, the control group treated orally with ethanol clearly produced the expected characteristic zone of necrotizing mucosal lesions. On the other hand, oral administration of AML significantly decreased the total lesion area and the percentage of lesion. The preliminary phytochemical screening showed that the presence of tannins, flavonoids and triterpenes in AML may exert its effect in preventing gastric damage development on gastric mucosal [10].

Antinociceptive and Anti-Inflammatory Activities of the Ethanol Extract of *Annona muricata* L. Leaves in Animal Models:

Effects on Carrageenan-induced Edema in Rats: The A. muricata ethanol extract anti-inflammatory effect evaluated by the paw edema method induced by carrageenan is shown in Table 4. Edema inhibition was observed 3 h after carrageenan application of doses (p.o.) of 200 (0.73 ± 0.06; 23.16 %; $p < 0.05$) and 400 mg/kg (0.67 ± 0.04; 29.47 %; $p < 0.01$). 4 h after carrageenan injections, the doses of 200 (0.53 ± 0.03; $p < 0.01$) and 400 mg/kg (0.47 ± 0.02; $p < 0.001$) reduced the respective paw edema (29.33 and 37.33%). In this time, indomethacin also reduced the paw edema (42.67%).

Table 5: Effects of the ethanol extract from *A. muricata* leaves on carrageenan-induced paw edema in rats.

Group	Dose	Volume of hind paw (mL)			
	(mg/kg)	1 h	2 h	3 h	4 h
Control	Saline	0.53 ± 0.06	0.72 ± 0.05	0.95 ± 0.06	0.75 ± 0.06
	100	0.52 ± 0.09	0.68 ± 0.06	0.80 ± 0.06	0.63 ± 0.04
Ethanol Extract	200	0.50 ± 0.10	0.65 ± 0.09	0.73 ± 0.06*	0.53 ± 0.03**
	400	0.48 ± 0.07	0.60 ± 0.04	0.67 ± 0.04**	0.47 ± 0.02***
Indomethacin	10	0.47 ± 0.10	0.58 ± 0.05	0.62 ± 0.06**	0.43 ± 0.02***

*Data are mean ± s.e.m. of six rats. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control group.*

The anti-inflammatory activity was confirmed by the paw edema induced by carrageenan in rats, a model widely used to study anti-inflammatory substances. Carrageenan induces paw edema resulting in the release of mediators such as histamine, serotonin, bradykinin, substance P and a platelet activating factor and prostaglandins. In this study, oral treatment with the *A. muricata* extract significantly inhibited the paw edema. This evidence suggests that the anti-inflammatory actions of the ethanol extract are related to inhibition of one or more signalling intracellular pathways involved with these mediators effects [14].

3. Anti Hyperglycemic Activity:

Diabetes Mellitus (DM) is one of the most common metabolic disorders. It is estimated that in the year 2000, 171 million people had diabetes, and this is expected to double by year 2030. Conventionally, insulin-dependent diabetes mellitus is treated with exogenous insulin and non insulin dependent diabetes mellitus with synthetic oral hypoglycaemic agents. However the hormone fails as a curative agent for complications of diabetes and synthetic oral drugs produce adverse health effects. The bark, roots and leaves of *Annona muricata* has been reported to be used as anti-diabetes in the Peruvian Amazon. Many studies have confirmed the effects of methanolic extracts of A. muricata on glycemic control in streptozotocin -induced diabetic Wistar rat [10].

Anti Hyperglycemic effect of *Annona muricata* Bark:

Exploration of Anti-Hyperglycemic and Hypolipidemic Activities of Ethanolic Extract of *Annona muricata* Bark in Alloxan Induced Diabetic Rats:

B. Ahalya and co-workers from A.P, India, very recently in March 2014 investigated the anti-hyperglycaemic and anti-hyperlipidemic effects of Ethanolic extract of *Annona muricata (Annonaceae)* stem in male albino rats. Anti-Hyperglycemic and Hypolipidemic Activities of Ethanolic Extract of *Annona muricata* Bark in Alloxan Induced Diabetic Rats. Diabetes was induced in the albino rats by administration of a single dose of alloxan monohydrate (150 mg/kg, bwt, i.p) and the Ethanolic bark extract of *A. muricata* was administered daily at single doses of 150 and 300 mg/kg, p. o to diabetes induced rats for a period of 14 days. The effect of Ethanolic extract of *A. muricata* bark on blood glucose level was measured in the diabetic rats. Diabetes mellitus is a metabolic syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting in impaired glucose metabolism and other energy-yielding fuels such as lipids and protein. 1 Dyslipidemia is a frequent complication of DM and is characterized by low levels of high density lipoproteincholesterol (HDL-C) and high levels of low density lipoprotein-cholesterol (LDLC) and triglyceride (TG). Several groups of hypoglycemic drugs are currently available to treat DM2. Different types of oral hypoglycemic agents such as biguanides and sulphonylureas are available along with insulin for the treatment of diabetes mellitus, but have side effects associated with their uses3. There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low costs.

Experiment – This study was carried out in healthy, male young adult, albino rats (150-220gms). The animals were housed under standard laboratory conditions of light, temperature and humidity. Before and during the experiment, rats were fed with standard diet. After randomization into various groups and before initiation of experiment the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 hours ad libitum.

Acute study in normal rats: Animals were divided into 4 groups of 3 rats each.

- **Group I:** Rats served as normal-control and received the vehicle (0. 5 ml distilled water/day/rat)
- **GroupII**: Rats (normal) were administered Ethanolic extract of *Annona muricata* bark (150 mg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.
- **GroupIII**: Rats (normal) were administered Ethanolic extract of *Annona muricata* bark *(*300 mg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.
- **GroupIV**: Rats (normal) were administered *Glibenclamide* (500µg/kg b. wt. /day) in distilled

water as a fine aqueous suspension orally.

Acute study in diabetic rats: Animals were divided into 4 groups of 3 rats each.

- **Group I**: Rats served as diabetic-control and received the vehicle (0. 5 ml distilled water/day/rat)
- **Group II**: Rats (diabetic) were administered Ethanolic extract of *Annona muricata* bark (150 mg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.
- **Group III**: Rats (diabetic) were administered Ethanolic extract of *Annona muricata* bark (300 mg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.
- **Group IV**: Rats (diabetic) were administered *Glibenclamide* (500µg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.

Induction of Diabetes in Experimental Animals*:* Rats were fasted for 16 hours and were induced with alloxan monohydrate, 150 mg/kg body weights (bwt), intraperitoneally (ip)9. Hyperglycaemia was confirmed when elevated blood glucose level was ≥200 mg. DL–1 after 72 hours of injection10.

Chronic study in diabetic rats

- **Group I:** Rats served as normal-control and received the vehicle (0. 5 ml distilled water/day/rat)
- **Group II**: Rats served as diabetic-control and received the vehicle (0. 5 ml distilled water/day/rat)
- **Group III**: Rats (Diabetic) were administered Ethanolic extract of *Annona muricata* bark (150 mg/kg b. wts. /day) in distilled water as a fine aqueous suspension orally.
- **Group IV**: Rats (Diabetic) were administered Ethanolic extract of *Annona muricata* bark *(*300 mg/kg b. wt. /day) in distilled water as a fine aqueous suspension orally.
- **Group V**: Rats (diabetic) were administered *Glibenclamide* (500µg/kg b. wt. /day) in distilled water as a fine aqueous

Table 6: Variation in blood glucose levels after oral administration of Ethanolic bark extract of *A. muricta* in normal rats acute study.

Treatment (mg/kg)	Changes in Glucose level (mg/dl)				
	0hr	1hr	2hr	4hr	6hr
Group I: Normal (control)	93.9±1.6	92±1.5	90.4±1.2	88±1.2	83±1.26
Group II: AMBE (150 mg/kg b.w)	91±11.0	87±8.5	83.5±10.5*	80±8.2**	87±2.0
Group III: AMBE (300 mg/kg b.w)	90±6.0	76±2.0*	53.6±8.5**	50±7.2***	61.5±6
Group IV: Glibenclamide (500 µg/kg)	93±1.2	86±2.7	72±7.6*	60±7.8**	54±8.2***

*n = 3 rats in each group; Values of BGL are given in mean ± S.E.M; *P < 0.001 when compared with control (no drug) animals.*

Table 7: Variation in blood glucose levels after oral administration of Ethanolic bark extract of *A. muricata* in alloxan induced diabetic rats in acute study.

Treatment (mg/kg)	Changes in Glucose level (mg/dl)				
	0hr	1hr	2hr	4hr	6hr
Group I: Normal (control)	193.33±5.7	192±6.3	191±6.2	189±5.9	185±5
Group II: AMBE (150 mg/kg b.w)	190.1±5.6	170±4.9*	160±5.0**	153±6.2***	165±2.6**
Group III: AMBE (300 mg/kg b.w)	205.1±1.9	175±2.0*	150±2.4**	145±3.2***	151±2.6**
Group IV: Glibenclamide (500 µg/kg)	240.12±1.5	170±2.4*	160±2.1**	148±0.5***	130±0.8***

*n = 3 rats in each group; Values of BGL are given in mean ± S.E.M; *P < 0.001 when compared with control (no drug) animals.*

Table 8: Effects of Ethanolic bark extract of *A. muricata* on Fasting blood glucose (FBG) in alloxan induced diabetic rats in chronic study.

Treatment (mg/kg)	Changes in Glucose level (mg/dl)		
	Initial day	7th day	14th day
Group I: Normal (control)	74.00 ±1.00	83.33±4.16	81.00±1.00
Group II: Diabetic (control)	315.00±5.00	339.00±9.00	362.33±5.69
Group III: AMBE (150 mg/kg b.w)	315.33±3.51	283.33±5.13	210.33±4.51
Group IV: AMBE (300 mg/kg b.w)	312.67±2.52	249.0±7.57	187.33±6.43
Group V: Glibenclamide (500 µg/kg)	312.67±2.52	249.0±7.57	187.33±6.43

*n = 3 rats in each group; Values of BGL are given in mean ± S.E.M; *P < 0.001 when compared with control (no drug) animals.*

Table 9: Effect of Ethanolic bark extract of *A. muricata* on biochemical profiles of the control and treated animals in the chronic study

Treatment (mg/kg)	Changes in Glucose level (mg/dl)				
	TC	TG	HDL	LDL	V LDL
Group I: Normal (control)	76.16 ± 2.39	62.30 ± 2.14	43.50 ±2.52	16.33 ±1.33	14.83 ±1.83
Group II: Diabetic (control)	94.33 ± 2.38	96.16 ± 2.82	30.3 ±1.96*	21.5 ±1.22*	21.1 ±3.42*
Group III: Glibenclamide (500 µg/kg)	80.33 ±2.59**	72 ± 2.59**	50.6 ±1.45**	20 ± 1.56**	14.8 ±2.16*
Group IV: AMBE (150 mg/kg b.w)	95.33±3.79	83.67±3.2	58.0±2.0	20.59±1.43	16.73±3.69
Group V: AMBE (300 mg/kg b.w)	89.00±3.0	77.33±5.03	66.0±4.0	15.46±1.20	14.36 ±2.61

*n = 3 rats in each group; Values of BGL are given in mean ± S.E.M; *P < 0.001 when compared with control (no drug) animals.*

Currently, insulin and synthetic oral hypoglycemic agents like sulfonylureas and biguanides are the major players in management of Diabetes mellitus. Despite the availability of synthetic drugs, there is an ever-Increasing demand of anti-diabetic herbal options. Through present work, Ethanolic bark extract of *A. muricata* seems to be useful in controlling elevated blood glucose levels in diabetes

induced by alloxan in rats. And also lowers hyper triglyceridemia and hypercholesterolemia in alloxan induced diabetic rats. These results indicate that it is worth undertaking further studies on possible usefulness of the Ethanolic extract of the bark of *A. muricata* in diabetes mellitus [15].

Anti Hyperglycemic effect of *Annona muricata* Leaves:

The nutrients in soursop leaves are believed to stabilize blood sugar levels in the normal range. Besides, the extracts of soursop leaves can be used as one of the natural diabetes remedies. All this makes these leaves beneficial for diabetics. Nigerian researchers have evaluated the effects of methanolic extract of Soursop leaves on the pancreatic islet cells of streptozotocin induced- diabetic rats. The results of the study indicated that Soursop extract treatment decreased the blood glucose concentration of diabetic rats due to the regeneration/proliferation in the pancreatic Beta cells (Beta-cells). The pancreas is a gland organ in the digestive and endocrine system of vertebrates. It is both an endocrine gland producing several important hormones, including insulin, glucagon and somatostatin, as well as an exocrine gland, secreting pancreatic juice containing digestive enzymes that pass to the small intestine. These enzymes help in the further breakdown of carbohydrates, proteins & fats in the enzyme. Beta cells (beta-cells, Beta-cells) are a type of cell in the pancreas in areas called the islets of Langerhans. They make up 65-80 per cent of the cells in the islets. Beta cells make and release insulin, a hormone that controls the level of glucose in the blood. Streptozotocin (Streptozocin, STZ, Zanosar) is a naturally occurring chemical that is particularly toxic to the insulin-producing beta cells of the pancreas in mammals. It is used in medicine for treating certain cancers of the Islets of Langerhans and used in medical research to produce an animal model for Type 2 diabetes. Streptozotocin-induced hyperglycemia in rats is considered a good model for the preliminary screening of agents active against Type 2 diabetes and is widely used. Generally, destruction of Beta-cells starts three days after STZ administration and reaches its peak at three to four weeks in rats. Streptozotocin induced diabetes in laboratory animals has been widely used for research on diabetes and its long-term complications [16].

Protective effects of *Annona muricata* linn. (Annonaceae) leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of streptozotocin-treated diabetic rats

S Adewole, J Ojewole investigated the protective effect of AML aqueous extract on serum lipid profiles & oxidative stress in hepatocytes of streozotocin treated diabetic rats. Extracts from various morphological parts of *Annona muricata* Linn. (Annonaceae) are widely used medicinally in many parts of the world for the management, control and/or treatment of a plethora of human ailments, including diabetes mellitus (DM). The present study was undertaken to investigate the possible protective effects of A. muricata leaf aqueous extract (AME) in rat experimental paradigms of DM. The animals used were broadly divided into four (A, B, C and D) experimental groups. Group A rats

served as 'control' animals and received distilled water in quantities equivalent to the administered volumes of AME and reference drugs' solutions intraperitoneally. Diabetes mellitus was induced in Groups B and C rats by intraperitoneal injections of streptozotocin (STZ, 70 mg kg-1). Group C rats were additionally treated with AME (100 mg kg-1 day-1, p.o.) as from day 3 post STZ injection, for four consecutive weeks. Group D rats received AME (100 mg kg-1 day-1 p.o.) only for four weeks. Post-euthanization, hepatic tissues were excised and processed biochemically for antioxidant enzymes and lipid profiles, such as catalase (CAT), reactive oxygen species (ROS), glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), thiobarbituric acid reactive substances (TBARS), triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL), respectively. Treatment of Groups B and C rats with STZ (70 mg kg-1 i. p.) resulted in hyperglycaemia, hypoinsulinaemia, and increased TBARS, ROS, TC, TG and LDL levels. STZ treatment also significantly decreased ($p<0.05$) CAT, GSH, SOD, GSH-Px activities, and HDL levels. AME-treated Groups C and D rats showed significant decrease ($p<0.05$) in elevated blood glucose, ROS, TBARS, TC, TG and LDL. Furthermore, AME treatment significantly increased ($p<0.05$) antioxidant enzymes' activities, as well as serum insulin levels. The findings of this laboratory animal study suggest that A. Muricata extract has a protective, beneficial effect on hepatic tissues subjected to STZ-induced oxidative stress, possibly by decreasing lipid peroxidation and indirectly enhancing production of insulin and endogenous antioxidants [17].

Effect of Annona muricata (Linn) on The Morphology of Pancreatic Islet Cell of experimentally- Induced Diabetic Wistar Rats.

D Adeyemi and coworkers in 2007 designed this study to evaluate the effects of methanolic extract of *Annona muricata* leaves on the pancreatic islet cells of streptozotocin induced- diabetic rats. Thirty adult Wistar rats were randomly assigned into three groups (A, B and C) of ten rats each. Group A was the control, Group B was untreated diabetic group and group C was A. muricata-treated group. Diabetes mellitus was experimentally induced in groups B and C by a single intra-peritoneal injection of 80mg/kg streptozotocin dissolved in 0.1M citrate buffer. Group A rats were intraperitoneally injected with equivalent volume of citrate buffer. Daily intra peritoneal injection of 100mg/kg A. muricata was administered to group C rats for two weeks. The rats were sacrificed and the pancreas were removed and fixed in Bouins fluid. The tissues were processed for paraffin embedding and sections of 5µm thickness were produced and stained.

Histo-pathological examination of the pancreas. :

The animals were sacrificed by cervical dislocation and the pancreas of each of the animals was dissected out. The splenic part of the pancreas was fixed in Bouin's fluid by total immersion for 24

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hours after which it was processed via paraffin wax embedding method of Drury and Wallington (1980) [18]. Sections of 5µm thickness were produced from the tissue blocks and stained with hematoxylin and eosin, Gomori aldehyde fuchsin and Gomori chrome alum hematoxylin phloxine for light microscopic examination of the pancreatic islets architecture. The sections were examined under a Carl Zeiss research microscope (Axioskope 40, Germany) with a digital camera attached. Digital photomicrographs of the pancreatic sections were taken at various magnifications.

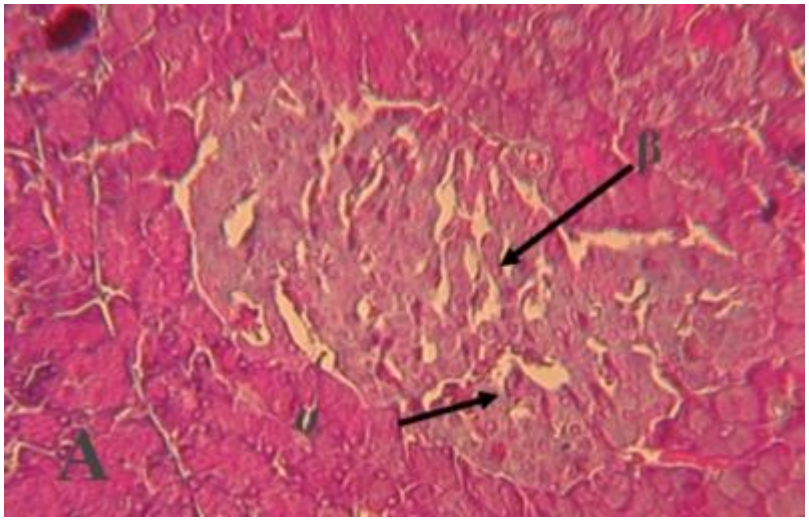

Fig 2: Photomicrograph of a normal (control) pancreatic islet showing cluster of β-cells which are centrally placed and peripherally placed α-cells. (Hematoxylin and Eosin X 2200)

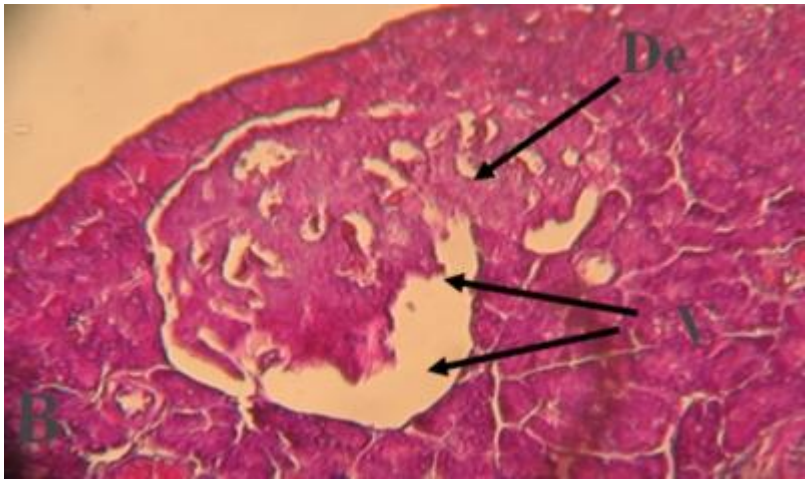

Fig 3: Photomicrograph of pancreatic islet of streptozotocin-induced diabetic rats showing degranulation (De) of β-cells and severe vacuolation (V) of the pancreatic islets. (Hematoxylin and Eosin X 2200)

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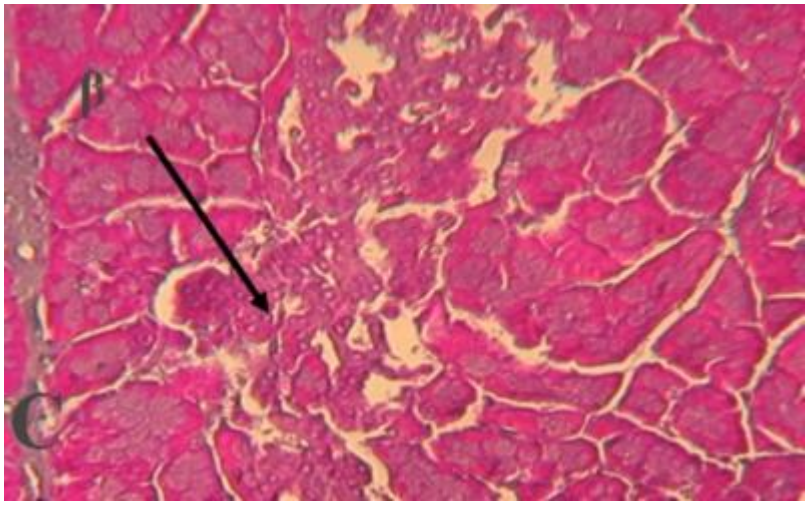

Fig 4: Photomicrograph of pancreatic islets of A.muricata treated diabetic rat showing recovery of the β- cells. As it is evident, the islet cells are regenerated, the inflammatory infiltration has disappeared and there is reduction in the vacuolation cased by administration of STZ (Hematoxylin and Eosin X2200).

The plant extract treated diabetic samples histopathologically approach the corresponding healthy pancreatic samples. The regeneration of the β-cells of the STZ-destructed islets is probably due to the fact that pancreas contains stable (Quiescent) cells which have the capacity of regeneration. Therefore, the surviving cells can proliferate to replace the lost cells. The treatment with extracts of A. Muricata shows a significant antihyperglycemic activity in STZ-induced diabetic rats at the end of the experiment. It has been suggested that bioactive compounds from plants sources having antihyperglycemic activities might act by several mechanisms such as stimulating insulin secretion, increasing repair or proliferation of β-cells and enhancing the effects of insulin and adrenalin. The result of this present study indicated that decreased in the blood glucose concentration of diabetic rats by A. muricata treatment is due to the regeneration/proliferation in the pancreatic β-cells [19].

Morphological Changes and Hypoglycemic Effects of *Annona muricata* Linn. (Annonaceae) Leaf Aqueous Extract on Pancreatic B-Cells of Streptozotocin-Treated Diabetic Rats

Stephen O.Adewole and coworkers from Nigeria in 2006 investigated the leaf aqueous extract effects of *Annona muricata* Linn. on the morphology of pancreatic β-cells and oxidative stress induced by streptozotocin (STZ)-diabetic rats. Diabetes mellitus was induced in the diabetic animal groups B and C by intraperitoneal injections of STZ (75 mg/kg body weight), while the control group received equal volume of citrate buffer (pH 6.3) solution intraperitoneally. The rats in group C were given A. muricata leaf aqueous extract (AME, 100 mg/kg, p.o.) as from day 5 post STZ injections, and stopped on the 30th day of the study period. The pancreases of the rats were excised and randomly processed for histological staining and biochemical assays for antioxidant enzymes

[such as glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), malondialdehyde (MDA) and serum nitric oxide (NO)]. In diabetic state, pancreatic β-cells of STZ-treated group B rats histologically demonstrated marked alterations in the micro-anatomy and cellular integrities. The morphology of A. muricata-treated rats' pancreases showed viable cellularity with distinct β-cell mass. STZ treatment significantly decreased GSH-Px, SOD, GSH, CAT and pancreatic/serum insulin levels ($p<0.05$). However, STZ treatment increased blood glucose concentrations, MDA, and NO. A. Muricata treated rats showed a significant decrease ($p<0.05$) in elevated blood glucose, MDA and NO. Furthermore, A. muricata treatment significantly increased ($p<0.05$) antioxidant enzymes' activities, as well as pancreatic/serum insulin contents.

Catalase Activity (CAT): The activity of catalase (CAT) was measured using its perioxidatic function according to the method of Johansson and Borg (1988) [20].

Reduced GSH and oxidized glutathione GSSG activities: Pancreatic GSH and GSSG contents were measured as described by Hissin and Hilf (1973) [21].

Superoxide Dismutase Activity (SOD): Pancreatic SOD activity was assayed by the method of Kakkar *et al.* (1984) [22].

Glutathione Peroxidase Activity (GSH-Px): Glutathione peroxidase (GSH-Px) activity was measured by NADPH oxidation, using a coupled reaction system consisting of glutathione, glutathione reductase, and cumene hydroperoxide (Tappel, 1978) [23].

Lipid Peroxidation contents (LPO): The product of the reaction between malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS) were measured by a modified method of Ohkawa *et al*., (1979) [24].

Nitric Oxide (NO): Serum nitrite/nitrate levels were determined by converting the nitrate to nitrite, using enzyme nitrate reductase followed by addition of Griess reagent to colorimetrically quantify the nitrite concentration (Green *et al*; 1982) [25].

Table 10: Pancreatic tissue CAT (µmol/mg protein), GSH (U/g protein) SOD (U/mg protein), GSH-Px (U/mg protein), MDA (nmol/mg protein), Insulin (µU/mg protein) and serum NO (µmol/l) of all groups, A (control), B (STZtreated) and C (STZ- +AME-treated) rats.

Parameters	Control	STZ-treated	STZ + AME-treated
Pancreatic CAT	0.42±0.3	0.21±0.4b	0.43±0.1a
Pancreatic GSH	6.42±1.3	3.53±0.2b	5.6±0.9a
Pancreatic SOD	26.9±1.8	15.7±2.3b	25.4±2.3a
Pancreatic GSH-Px	0.49±0.4	0.28±0.3b	0.51±0.2a
Pancreatic MDA	88±13	138±17b	92±11a
Pancreatic Insulin	15.2±0.4	7.5±0.3b	14.8±0.7a
Serum NO	4.12±0.58	8.38±1.12b	4.57±0.14a

Values are expressed as means (±SEM) of 10 rats for all groups. a Insignificant difference ($p>0.05$) between all groups. b Significant difference ($p<0.05$) in the same row between treatment and control groups.

Table 10 shows the effects of *A. muricata* on biochemical variables suggestive of oxidative stress in STZ-treated animals. There was clear evidence that STZ-induced pancreatic injury was associated with free radical injury and oxidative stress. Oxidative stress was characterized by increased lipid peroxidation and/or altered non-enzymatic and enzymatic antioxidant systems (Zima *et al*; 2001).Also, AME proved significantly better in restoring the altered activity of antioxidant enzymes like CAT, GSH, SOD, GSG-Px and MDA, NO and insulin towards their normal values in the pancreas. In conclusion, the findings of the present study indicate that A. muricata treatment has beneficial effects on pancreatic tissues subjected to STZ-induced oxidative stress by directly quenching lipid peroxides and indirectly enhancing production of endogenous antioxidants. *Annona muricata* protected and preserved pancreatic β-cell integrity [26].

4. Antitumor Activity:

Annonaceous acetogenins are a new class of compounds that have been reported to have potent cell growth inhibitory activities. These components have a wide range of clinical application for cancer chemotherapy. First of all they are very potent inhibitors of the NADH-ubiquinone reductase (Complex I) activity of mammalian mitochondria. The natural substances isolated from the seeds of *Annona muricata* are more potent of than rotenone and also piericidin (piericidin is the most powerful inhibitor of Complex I). These compounds belong to a group of bis-tetrahydrofuran acetogenins, amongst squamocin and otivarin behave qualitatively like rotenone that prevents the transfer of electrons from Complex I to ubiquinone by blocking the ubiquinone-binding site. The inhibition of any step (in this case of the Complex I) in this process will halt the rest of the process. NADH is then no longer oxidized and the citric acid cycle ceases to operate because the concentration of NAD+ falls below the concentration that these enzymes can use. Another way these chemical compounds can carry on their antitumor activity is inducing of reactive oxygen species (ROS) generation and reducing intracellular glutathione levels. Oxidative stress induced by reactive oxygen intermediates including superoxide, hydrogen peroxide are known to cause apoptotic cell death in the pathogenesis of diverse human diseases, including cancer, diabetes and neurodegenerative disorders. GSH (Glutathione) is an anti-oxidant and decreased intracellular levels of GSH (Glutathione) are associated with enhanced susceptibility to ROS mediated apoptosis. In addition down regulation of Bcl-2 is known to sensitize the cells to apoptotic death. In fact the protooncogene Bcl-2 is known to inhibit apoptotic and necrotic cell death [10].

5. Various Cancer Cell Lines and a Detailed Computational Study on its Potent Anti-Cancerous Leads:

Plants became the basis of traditional medicine system throughout the world for thousands of years and continue to provide mankind with new remedies. Here, we present a research study on a

medicinal plant, Graviola, a native of North America but rarely grown in India. It has a wide potent anticancerous agents coined as Acetogenins which play a key role towards many varieties of cancer, Acetogenins are potent inhibitors of NADH oxidase of the plasma membranes of cancer cells. Potent leads were taken for the study through literature survey, major types of cancer targets were identified, the natureceuticals and the cancer protein were subjected to docking analysis, further with the help of the dock score and other descriptor properties top ranked molecules were collected, commercial drug was also selected and identified as a Test compound for the study. Later, the phytochemicals were subjected to toxicity analysis. Those screened compounds were then considered for active site analysis and to find the best binding site for the study. R Programming library was used to find the best leads. Phytochemicals such as Anonaine, Friedelin, Isolaureline, Annonamine, Anomurine, Kaempferol, Asimilobine, Quercetin, and Xylopine were clustered and the highly clustered compounds such as Annonamine , Kaempferol termed to be a potential lead for the study. Further study on experimental analysis may prove the potentiality of these compounds. In the experimental analysis, Graviola leaves were collected, and the extracted components were tested against the HeLa cell line and PC3 cell line. HeLa cells treated with 75 µg of a crude leaf extract of A. muricata showing 80% of cell inhibition [27].

Phytochemical screening and anti-ovarian cancer properties of *Annona muricata* (Annonaceae) leaves, seed and fruit:

Ukwubile had conducted study which was aimed determining the phyto-constituents and potency of ethanol extract of seed of *Annona muricata* Linn on ovarian cancer tissues. Preliminary phytochemical screening of seeds revealed that it contains alkaloids, saponins, terpenes, flavonoids, anthraquinones, tannins, and cardiac glycosides. From the fractionation using column chromatographic technique, the fraction with alkaloids and flavonoids showed more anti -ovarian cancer activity when compared to other fractions. Mean survival time (MST) and percentage increase in life span were highest in group 4 with values 23 ± 0 .33 and 28 ± 0.30 respectively at 1000 mg / kg body weight (b.w) ($P< 0.05$, $n = 8$). 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 Ehrlich's Ascites Carcinoma cell (EAC) control groups with value 2.12 ± 0.10. Viable tumour cell counts (x 107 cells / ml) were 7.78 ± 0.18 (group 3 100 mg/kg), 5.85 ± 0.23 (group 4 1000 mg/kg) and 4.90 ± 0.015 group 5 (vinblastin 0.8 mg / kg standard drug) and values are statistically different from the EAC control (group 2) with value 12.25 ± 0.01 ($P< 0.05$, ANOVA). Non-viable tumour cell counts (x 10 7 cells/ ml) were on the increase as the doses increased; 0.90 ± 0.24 (group 3 100 mg/kg), 1.47 ± 0.21 (group 4 1000 mg/kg) and 1.63 ± 0.81 (group 5 vinblastin 0.8 mg/kg standard drug) while the EAC control (group 2) was 0.8 ± 0. 02 and values were compared. All

haematological parameters showed increase at the doses (intraperitoneal) investigated except total WBC white blood cells which slight decrease in values among the groups. Biochemical parameters in EAC- bearing Swiss female albino mice showed significance reduction in level of lipid perioxidation and increase in catalase and protein contents when compared to EAC control group ($P< 0.05$, ANOVA). The study therefore showed that seed ethanol extract of A. muricata Linn. had anti-ovarian cancer properties on the experimental animals and can therefore serve as a medication for ovarian cancer problems in females [28].

T47D BREAST CANCER CELL LINES:

Retno Widiastuti Aditiyono & his co workers in 2012 from Department of Medicine Jenderal Soedirman University Indonesia reported anti breast cancer activity of leaf extract of *Annona muricata*. Cancer treatment is medically still caused by problems because of its side effects are great. The plant that is empirically trusted by societies to have anticancer properties are the leaves of the soursop (*Annona muricata*). The breast cancer is the second leading cause of death in women after cervical cancer. The soursop (*Annona muricata*) is a traditional medicinal plant which is empirically by the people of Indonesia are used for anti-inflammatory and anti-tumour. This study aims to determine the cytotoxic effects from extracts of leaves of soursop and fraction results in cancer cells T47D. The research was carried out by extraction using ethanol and fractionation by column chromatography method that used various solvents were n-hexane, chloroform, ethyl acetate and methanol. Cytotoxic test performed by the method of MTT 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide assay and apoptosis tests performed by the method of Double Staining.

A. Cytotoxic Test Ethanol Extract: Cytotoxicity test is a qualitative and quantitative tests to determine how cell death. The method used to see cytotoxic effects of ethanol extract of leaves of the soursop on T47D (Human ductal breast epithelial tumor cell line) breast cancer cells is the MTT assay. The principle of the MTT assay is a spectroscopic method is by determining the absorbance value of formazan. MTT will be absorbed into the cell and entered into the system of cell respiration in mitochondria. The action of the enzyme active mitochondria in cells was metabolize tetrazolium salts, resulting in termination of tetrazolium ring by dehydrogenase enzymes which lead to tetrazolium formazan transformed into water-insoluble but soluble in SDS 10% and the purple coloured. Formazan formed is colored purple will be proportionate to the number of living cells (Pebriana et al., 2008). Cells that die dissolved in water and remain yellow because the mitochondria of cells that die are not respiration tetrazolium ring is disconnected so it can not reduce MTT reagent to formazan and the color is still yellow. The observations made by microscopic showed that the number of formazan formed in control wells with media more than the formazan formed in the wells treated test compound. This suggests that the treatment of ethanol extract of leaves of the soursop on

T47D breast cancer cells can lead to death. Cells that are dead will not be affected by the MTT reagent. Characteristic morphology of living cells is round with a protected cell wall that shines and stuck to the bottom plate, while the dark-colored cells that die and are not attached to the base plate. After addition of MTT and incubated for 4 hours of diving, added SDS in 10% HCl. The reason the use of SDS 10% as it can dissolve the formazan crystals and the results of MTT reaction did not cause precipitation. After settling for a night, then used an ELISA (enzyme-linked immunosorbent assay) reader to determine absorbance values. 595nm wavelength is used because it is the maximum wavelength in order to obtain sensitive and specific measurements. Absorbance value of the parameters obtained from the cytotoxic test was IC50 values, i.e. values that produce inhibitory concentrations of cancer cells by 50%. Apoptosis assay results are analyzed in a qualitative description. The results showed that the ethanol extracts of leaves of the soursop has a cytotoxic activity with IC50 (half maximal inhibitory concentration**)** values of 17.149 µg / mL.

B. Apoptosis Test: Apoptosis is programmed cell death mechanism that is important in multicellular organisms to maintain equilibrium. Tests performed to determine the mechanism of apoptotic cell death is through the mechanism of apoptosis. In T47D cells that were given the test compound indicates that berflouresens orange cells, whereas cells that are not given the test compound indicates green berfloures cells. The apoptosis assay results by the method of double staining above show that cancer cells are treated T47D ethanol extract of leaves of soursop (A) and tamoxifen (B) some berflouresens orange. This indicates that the test compound can induce apoptosis. In control cells berflouresens still look bright green oval which means the cells do not undergo apoptosis because the cells live only absorb acridine orange [29].

Table 11: The mean absorbance, percentage inhibition of T47D cells and IC50 values from ethanol extract of leaves of *A. muricata*

Test Materials	Concentration (µg/mL)	Mean absorbance	Living Cells (%)	Retardation (%)	IC50 (µg/mL)
Ethanol extract A.muricata	500	0.161	7.71	92.29	
	250	0.129	2.34	97.66	
	125	0.141	4.36	95.64	17.149
	62.5	0.138	3.8	96.2	
	31.25	0.457	57.28	42.72	
Tamoxifen	50	0.253	0	100	
	25	0.262	0.19	99.81	
	12.5	0.889	71.62	28.38	13.38
	6.25	1.07	92.29	7.71	
	3.125	0.167	91.95	8.05	

Ethanol extract of leaves of soursop (*Annona muricata*) has a cytotoxic activity in T47D breast cancer cell lines with IC50 of 17.149 µg/mL and can induce apoptosis as seen in Table 11 [29].

6. Chemopreventive Potential:

AH Roslida & his coworkers investigated the chemopreventive potential of Soursop leaves on chemically induced skin Papillomagnesis. Human beings have been exposed to a variety of carcinogenic agents which may act as initiator and promoter to the tumour formation. In fact, the initiation of carcinogenesis may occur many years before it is being promoted. Thus, the chemopreventive agents are preferable to slow, reverse or completely halted multiple steps in the carcinogenesis process. Therefore, a new science of chemoprevention has appeared as an attractive alternative to control malignancy. Two-stage skin carcinogenesis in mice model is used extensively to investigate the epithelial carcinogenesis which consists of initiation phase and promotion phase. Initiation phase is an irreversible reaction activated by initiator 7,12-dimethylbenza(α)anthracene (DMBA 100ug/100ul acetone) while promotion stage which is reversible and long term is triggered by repetitive application of promoter croton oil (1% in acetone/ twice a week)). In accordance development of anti-tumour-promoter is considered the most effective method in cancer chemoprevention due to the reversible nature of the promotion phase.

It can be concluded that A. muricata acts as a modulator of two stage skin paillomagenesis in ICR mice since it prevents the tumour formation, delayed the tumour promotion and progression, elicited by DMBA/croton oil. Since A.muricata is a plant rich in acetogenins, it can be suggested that the synergistic effects of phytochemicals present in this plant including acetogenins may be the underlying principle behind the chemopreventive potential of A.muricata. . A large numbers of agents including natural and synthetic compounds have been identified to possess potential cancer chemopreventive value, inhibiting mutagenesis, hyperproliferation or induce apoptosis or differentiation, which are critical characteristics of chemoprevention. Similarly, the present findings clearly indicate that topical administration of A.muricata L leaves during initiational as well as tumour promotional stage of papillomagenesis significantly reduced the occurrence of skin papillomas induced by DMBA/croton oil in mouse skin after 12 weeks without causing any toxic effect. AMLE (*Annona muricata* leaf extract) extracts in any stages appreciably decreased tumour burden by 58-100%, tumour volume by 20-100% and tumour incidence by 25-100% in the AMLE–treated experimental groups (Group IV, V and VI) as compared to the carcinogen treated control. Interestingly, At 100 and 300 mg/ kg, AMLE completely inhibited the tumour development in all stages. Histopathological study revealed that tumour growth from the AMLE-treated groups showed only slight hyperplasia and absence of keratin pearls and rete ridges. The results, thus suggest that the *A.muricata* leaves extract was able to suppress tumour initiation as well as tumour promotion even at lower dosage [30].

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Table 12: Effect of *Annona muricata* Extract on Mouse Skin Papillomagenesis during Peri-Initiation Phase and Promotion Phase

Group	Peri-Initiation Phase					Promotion Phase				
	Body weight (g)		Tumor			Body weight (g)		Tumor		
	Initial	Final	Incidence (%)	Burden	Volume	Initial	Final	Incidence (%)	Burden	Volume
I	(Vehicle)									
	25.7 ± 1.14	33.8 ± 1.27	0.00[a]	0.00[a]	0.00[a]	27.4 ± 0.83	30.9 ± 1.5	0.00[a b]	0.00[ab]	0.00[ab]
II	(Carcinogen)									
	27.2 ± 0.89	34.1 ± 1.84	44.44	6.00 ± 1.47[b]	18.71 ± 12.52	24.9 ± 1.41	36.9 ± 1.44	60.00	3.5 ± 1.9	5.83 ± 1.94
III	(Curcumin 10 mg/kg)									
	28.1 ± 0.53	38.0 ± 0.76	30.00	3.00 ± 1.53[a]	8.70 ± 3.630	28.0 ± 0.81	34.4 ± 0.86	40.00	3.5 ± 0.65	6.7 ± 5.29
IV	(AMLE 30mg/kg)									
	22.9 ± 0.66	30.3 ± 1.80	33.33	3.00 ± 1.00a	14.79 ± 11.65	23.2 ± 1.09	28.3 ± 1.58	28.57[a]	1.5 ± 0.5[a]	0.52 ± 0.00[ab]
V	(AMLE 100mg/kg)									
	26.6 ± 0.85	24.7 ± 1.41	0.00[a]	0.00[a]	0.00[a]	26.8 ± 0.92	33.8 ± 1.35	0.00[a b]	0.00[ab]	0.00[ab]
VI	(AMLE 300mg/kg)									
	27.3 ± 0.56	29.5 ± 1.65	0.00[a]	0.00[a]	0.00[a]	21.4 ± 1.13	34.4 ± 0.86	0.00[a b]	0.00[ab]	0.00[ab]

*Values expressed as mean ± S. E. M, a (Group II) at p<0.05, b(Group III) at p<0.05: significance level between treated group and carcinogen control: (Group II) at p<0.05 [30].

Table 13: Effect of *Annona muricata* Extract on Mouse Skin Papillomagenesis During Peri-Initiation Phase/Promotion Phase

Group	Body weight (g)		Tumor		
	Initial	Final	Incidence (%)	Burden	Volume
I	(Vehicle)				
	27.4 ± 1.19	37.3 ± 0.94	0.00[a]	0.00[a]	0.00[a]
II	(Carcinogen)				
	24.1 ± 1.10	32.3 ± 1.54	55.55[b]	4.40 ± 1.72[b]	3.59 ± 1.34[b]
III	(Curcumin 10 mg/kg)				
	28.7 ± 0.92	36.2 ± 2.63	14.29[a]	1.00[a]	1.05[a]
IV	(AMLE 30mg/kg)				
	26.1 ± 0.80	27.4 ± 0.98	0.00[a]	0.00[a]	0.00[a]
V	(AMLE 100mg/kg)				
	22.2 ± 0.57	37.2 ± 1.28	0.00[a]	0.00[a]	0.00[a]
VI	(AMLE 300mg/kg)				
	28.2 ± 0.79	36.0 ± 1.12	0.00[a]	0.00[a]	0.00[a]

**Values expressed as mean ± S. E. M, a (Group II) at p<0.05, b(Group III) at p<0.05: significance level between treated group and carcinogen control: (Group II) at p<0.05 [30].*

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7. Antineoplastic Potential:

R Ashok Kumar & his coworkers in 2012 reported that the confirmation the presence of therapeutically active antineoplastic compounds in the n-butanolic leaf extract of *Annona muricata*. Plant-derived compounds have played an important role in the development of several clinically useful anticancer agents. Lesser side effects may make naturally occurring compounds a better choice than synthetic compounds. In many cases, the actual compounds isolated from the plants might not serve as the drug, but they serve as leads to the development of potential anticancer agents. The present study was aimed to evaluate the cytotoxic potential of n-butanolic leaf extract of *Annona muricata* L. on WRL-68 (normal human hepatic cells), MDA-MB-435S (human breast carcinoma cells) and HaCaT (human immortalized keratinocyte cells) lines by XTT ({2,3-bis (2-methoxy-4-nitro-5- sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide} assay. Prior to cytotoxicity testing, the extract was subjected to phytochemical screening for detecting the presence of compounds with therapeutic potential. Their relative antioxidant properties were evaluated using the reducing power and DPPH*radical scavenging assay. Since most of the observed chemo-preventive potential invariably correlated with the amount of total phenolics Furthermore, HPLC (High Performance Liquid Chromatography) analysis of the extract, also reported in this study, revealed the presence of a variety of phenolic compounds like flavonols, polyphenols and flavones, which could have been responsible for its observed antineoplastic potential. Correlation studies indicated a strong and significant ($P < 0.05$) positive correlation of phenolic compounds with free radical scavenging potential. The results revealed that the extract was moderately cytotoxic to normal cells with a mean IC50 value of 52.4 µg when compared with those obtained for cancerous cells (IC50 values of 29.2 µg for MDA-MB-435S and 30.1 µg for HaCaT respectively). The study confirms the presence of therapeutically active antineoplastic compounds in the n-butanolic leaf extract of *Annona muricata*. Isolation of the active metabolites from the extract is in prospect. The extract at its lower doses exhibited a significant cytotoxicity on MDA-MB-435S and HaCaT cells with the IC50 values of 29.2 and 30.1 µg respectively, while it exhibited only a moderate cytotoxicity towards WRL- 68 with a comparatively higher IC50 value of 52.4 µg, clearly indicating differential cytotoxicity, at least at the lower doses tested. However, at the highest tested dose, the cytotoxic effect of the extract was similar on both normal and cancer cell lines. This might be due to the influence of this particular extract over the normal cell turnover mechanisms which ultimately results in cell death.

Table 14: Phytochemical Screening of n-butanolic Leaf

Phytochemical tests		Leaves
1	Saponins	-
2	Flavonoids	+
3	Terpenoids	+
4	Tannins	+
5	Steroids	-
6	Phlobatannins	-
7	Oil	-
8	Cardiac glycosides	+
9	Reducing sugars	+
10	Anthraquinones	-

The phytochemical analysis of the n-butanolic leaf extract of *Annona muricata* revealed the presence of flavonoids, terpenoids, tannins, cardiac glycosides and reducing sugars. Whereas, the extract showed the absence of saponins, steroids, phlobatannins, oil and anthraquinones tested which is presented in Table 14 [7].

Table 15: Major Phenolic Compounds Present in n-butanolic Extract of *Annona muricata* by HPLC

Phenolic compounds	λ^a (nm)	EtR^b (min)	RtR^c (min)
A. Flavonols			
1. Quercetin	370	39.8	40.6
2. Robinetin	370	35.6	36.4
B. Polyphenols			
1. Gallic acid	250	6.1	5.8
C. Flavones			
1. Apigenin-6-C-glucoside	320	39.1	38.6
2. Vitexin	320	35.3	35.2
3. Luteolin 3',7-di-O-glucoside	320	30.8	31.6

[a]wavelength for determination, [b]experimental retention time, [c]standard retention time.

Table 15 shows the phenolic compounds identified in the extract along with the respective retention times (Rt). The extract also contained unknown compounds evident from the HPLC data whose characterization is in prospect [7].

In conclusion, the study concludes that the n-butanolic leaf extract of *Annona muricata* might have potential for the development of therapeutically active compounds, which could serve as precursors and/or chemical templates for the design of an effective, more potent and safe antineoplastic drug which may be more potent than existing drugs of its class. These encouraging preliminary results provide a scientific basis for further characterization of individual compounds from this extract [7].

8. Wound Healing Activity:

Padmaa M Paarakh & her co workers reported the wound healing capacity of *Annona muricata* extract**.** A wound which is disrupted state of tissue caused by physical, chemical, microbial or immunological insult ultimately heals either by regeneration or fibroplasias. Healing progresses

through three general stages (1) inflammatory, (2) proliferative and repair and (3) remodelling stages. During inflammatory stages, as a result of injury, the blood circulation in the local area are reduced which leads to local hypoxia, acidosis and low pH. Following the inflammatory response to injury or wound, a tremendous proliferation of cells takes place which is actually responsible for the process of repair. The cellular responses include blood supply, surface covering and reproduction of collagen which ultimately helps to bind the wound margin and development of permanent functional tensile strength. In the healing of the excised wounds, the mechanism of wound contraction plays an important role. The term contraction implies reduction in the wound size and is believed to take place as a result of movement of wound edges towards the centre. Generally the wound contraction continues for 10- 15 days and therefore it stops. It has been shown that circular wound contract at a relatively slower rate than the square/rectangular ones. Most studies of the wound healing were made on excised surface wound and were concerned with the rate and extent of epithelization. In large open wounds, it is recognized that proper debridement of dead and devitalized tissue was essential for prompt healing.

Table 16: Effect of *A. muricata* extract on open wound. Values are expressed in mean ± SEM Day Area in sq cm

Day	Area in sq cm	
	Control group [n=7]	Treated group [n=7]
1st day	9.21 ± 0.75	9.98 ± 0.35
4th day	8.36 ± 0.68	8.01 ± 0.65[a]
7th day	6.68 ± 0.51	5.28 ± 0.39[b]
12th day	2.35 ± 0.47	1.14 ± 0.13[a]

[a]P< 0.05 compared with control group; [b]P< 0.001 compared with control group.

From the Table 16, it is clear that the 4 % alcoholic extract of *Annona muricata* as an ointment applied daily for a period of 12 days significantly reduced the area of open wounds as compared with those of control group of rats. The actual healing seems to proceed from the 4th day onwards from the day of wounding. The observation revealed a better healing pattern as percentage reduction in the wound area of the treated group (19.74, 47.09 and 88.58%) than that of the control group (9.22, 27.47 and 74.47%) on 4th, 7th and 12th day respectively. The result obviously indicates the wound healing property of *Annona muricata* extract [31].

Antioxidant activity, fatty acids profile and determination of tocopherols:

Débora Maria Moreno Luzia & Neuza Jorge from Department of Food Engineering and Technology, São Paulo State University, São Paulo, Brazil in 2012 had done the study to analyze the soursop seeds as to its composition, to evaluate the antioxidant potential of seeds extract and characterize the oil extracted from them, regarding the fatty acids profile and content of tocopherols.And they found out that the soursop seeds constituted significant sources of lipids, proteins and carbohydrates and

can therefore be used in food and feed, and offer relevant antioxidant activity of phenolic compounds. The oil seeds are a good source of unsaturated fatty acids, especially oleic and linoleic acids and they have significant amounts of total tocopherol [32].

In vitro antioxidant studies in leaves of Annona species

Baskar and others studied antioxidant potential of leaves of three different species of Annona by using different in vitro models eg. 1,1-diphenyl-2-picryl hydrazyl (DPPH), 2,2-azinobis-(3-ethylbenzothizoline-6-sulphonate) (ABTS), nitric oxide, superoxide, hydroxy radical and lipid peroxidation. The ethanolic extract of A.muricata at 500 g/ml showed maximum scavenging activity (90.05%) of ABTS radical cation followed by the scavenging of hydroxyl radical (85.88%) and nitric oxide (72.60%) at the same concentration. However, the extract showed only moderate lipid peroxidation inhibition activity. In contrast, the extract of A.reticulata showed better activity in quenching DPPH (89.37%) and superoxide radical (80.88%) respectively. A.squamosa extract exhibited least inhibition in all in vitro antioxidant models excepting hydroxyl radical (79.79%). These findings suggest that the extracts of A. muricata possess potent in vitro antioxidant activity as compared to leaves of A. squamosa and A.reticulata suggesting its role as an effective free radical scavenger, augmenting its therapeutic value. This may be attributed to the presence of acetogenins, which probably play a role as an effective free radical scavenger and hence an effective antitumorous agent [33].

Evaluation of in vitro anti oxidant activity of *Annona muricata* bark

Ahalya and others evaluated the anti oxidant activity of Ethanolic bark extract of *Annona muricata*. They carried out in vitro study of anti oxidant activity by DPPH (1, 1-Diphenyl 2picrylhydrazyl), Hydroxyl radical scavenging assay and reducing power method with Gallic acid as the standard in all the three methods. The results revealed that the extract of *Annona muricata* possess significant antioxidant activity [34].

Table 17: Effect of Ethanolic Extract of *Annona muricata* bark on different antioxidant models [34]

Concentration (µg/ml)	%Inhibition		
GALLIC ACID	DPPH	HYDROXYL METHOD	REDUCING METHOD
1	23±1.24	55.6±0.72	0.0075±0.002
2.5	37.6±1.51	65.3±1.9	0.0201±0.004
5	60 ±0.47	70±0.28	0.0412±0.004
IC50	3.5µg/ml	0.5µg/ml	
ANNONA MURICATA (TEST DRUG)(µg/ml)	DPPH	HYDROXYL METHOD	REDUCING METHOD
50	23.6±0.27	-	0.0795±0.008
100	37.6±1.08	47.3±0.21	0.1256±0.001
300	95±0.47	64.5±0.70	0.3390±0.001
500	-	77.3±1.10	0.5765±0.001
IC50	109µg/ml	120µg/ml	

Hepatoprotective activity of *Annona muricata* Linn Polyalthia cerasoides Bedd.

The hepatoprotective effect of *Annona muricata* (Annonaceae) were monitored by estimating the serum transaminases (SGOT and SGPT), serum alkaline phosphatase (SALP), liver and brain lipid peroxidation (LOP) and their total protein content.

Table 18: Effect of drug treatment on serum transaminase level

Sl. No.	Groups	SGPT (ALT) U/ml	SGOT (AST) U/ml	Alkaline Phosphatase (KA units)
1	Control	35.56±6.12 (10)	118.60±17.80(10)	14.57±1.40(7)
2	Carbon Tetrachloride Control	1700.00±114.82 (10)	2010.00±67.47(10)	74.77±9.98(7)
3	A.muricata treated	765.00±91.86(7)a	1243.30±115.75(7)a	26.19±1.81(7)a
4	P.cerasoides treated	1197.14±118.92 (7)b	1425.71±196.33(7)c	40.71±3.87(7)b

aP<0.001; bP<0.01; cP<0.02 when compared to carbon tetrachloride control. Figures in parenthesis indicate the number of observations.

Table 19: Effect of drug treatment on protein and lipid peroxidation levels in liver and brain

Sl. No.	Groups	Protein mg/g tissue		Lipid peroxidation MDA nmole/mg	
		Liver	Brain	Liver	Brain
1	Control (7)	117.07±6.81	37.49±1.01	422.37±28.0	178.34±14.46
2	Carbon tetrachloride Control (7)	62.80±3.29	18.39±0.88	889.20±22.48	634.70±25.27
3	A.muricata treated (7)	101.70±4.02a	28.20±1.71a	492.00±18.72a	210.50±29.71a
4	P.cerasoides treated (7)	98.20±3.97a	22.40±2.01	639.00±29.97a	357.00±30.71a

aP<0.001 when compared to carbon tetrachloride control.

Table 20: Mean values of percentage production of drug treatment against CCl4 challenge

Treatment	SGPT	SGOT	SALP	Lipid peroxidation MDA nmole/mg		Total Protein mg/g tissue	
Group	U/ml	U/ml	KA units	Liver	Brain	Liver	Brain
A.muricata treated	56.20	40.54	80.70	85.09	92.30	71.68	33.71
P.cerasoides treated (7)	30.21	30.89	56.58	53.60	56.91	65.23	13.79

$$\% \text{ Mean protection} = \frac{\text{Value from drug treatment} - \text{Value from CCl4 treated}}{\text{Value from vehicle treated} - \text{Value from CCl4 treated}} \times 100$$

The alcoholic extracts of *A.muricata* and *P.cerasoides* (Tables 18 and 19) have afforded and overall protection against CCl4 induced toxicity, the activity being slightly less in the case of *P.cerasoides*. LPO (in both liver andbrain) and SALP levels were reduced significantly by both drugs (Tables 18 and 19) suggesting that the plant drug scavenges free radicals produced by CCl4 metabolism which could be the possible mode of action of these drugs as hepatoprotective agents.

The percentage protection afforded by the two drugs against increase in SGOT, SGPT, SLP, liver and brain LPO levels and decrease in liver and brain protein levels are given in Table 20. All these data suggest that the plant drugs possess possible antihepatotoxic activity, which are in tune with the fact that these drugs are prescribed by traditional medical practitioners in Indian to be administered in form of decoction against liver disorders [35].

9. Effect of Graviola on Kidney:

The Influence of *Annona muricata* Leaves Extract in Damaging Kidney Cell and Inducing Caspase-9 Activity:

Azim Yahia M.Dayeef and co-workers in 2013 from Indonesia investigated the efficacy of ethanolic extracts of *Annona muricata* leaves for its cytotoxicity potential and induction of apoptosis in tubular cells was investigated. Phytochemical screening verified presence of alkaloids, tannins, flavonoids, saponins, anthraquinones and cardiac glycosides. The present study was undertaken to investigate the leaf extract effects of *Annona muricata* Linn. on the level of serum creatinine and damage of tubular cell structure that effect of kidney cell functions and caspase-9 expression in glomerulus and tubular cells. The animals (n=20) Were grouped a (control), I, II, AND III (experimental). The experimental animals of group I were administered with 10 mg/kg body weight/ day *Annona muricata* extract in saline for 40 days, group II were administered with 20 mg/kg body weight/ day *Annona muricata* extract in saline for 40 days and III were administered with 40 mg/kg body weight/day *Annona muricata* extract in saline for 40 days and then we see the effect on creatinine serum concentration by ELISA method and glomerulus, tubular cells were fixed and processed to examine the histological changes and caspase-9 expression in glomerulus and tubular cells [36].

Effect of Different Doses of *Annona muricata* Ethanolic Extract on Creatinine Serum Concentration:

In the figure 5 below with increasing doses of *Annona muricata* extract increasingly high then drop down to the more creatinine serum concentration that causes kidney damage leading to renal failure

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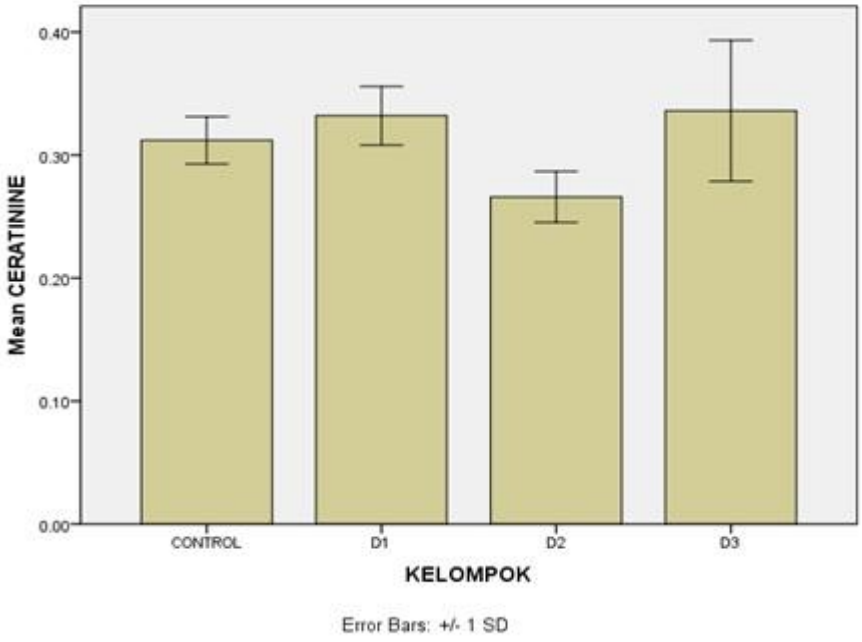
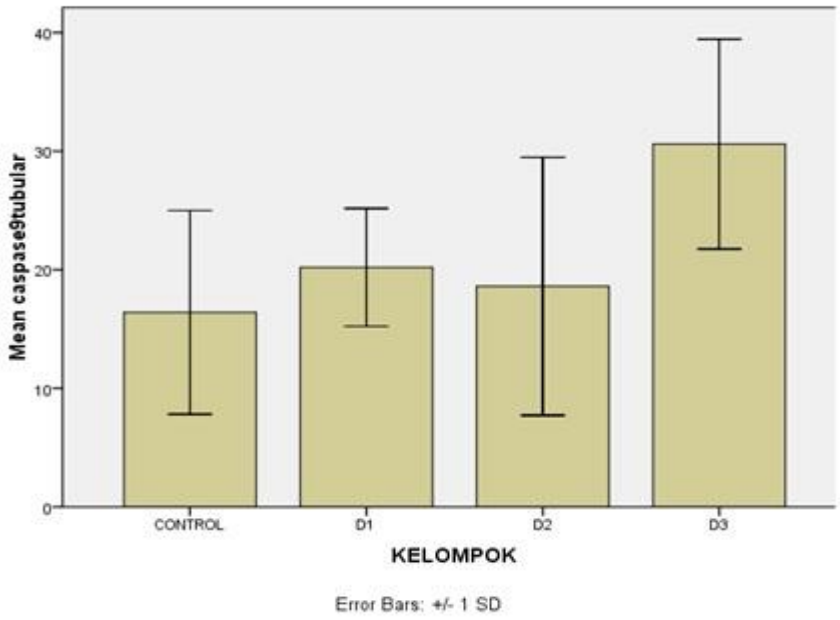
Fig 5: Bar Graph of mean of creatinine in serum mice for each group



Effect of Different Doses of *Annona muricata* Extract on Account Of caspase-9 in Tubular cell

In the figure 6 below with increasing doses of *Annona muricata* extract, caspase-9 activity also increase that effect in tubular cell and damage its structure.

Fig 6: Bar Graph of mean of caspase-9 tubular cell for each group



Effect of Different Doses of *Annona muricata* Extract on Account Of caspase-9 in Glomerulus cell

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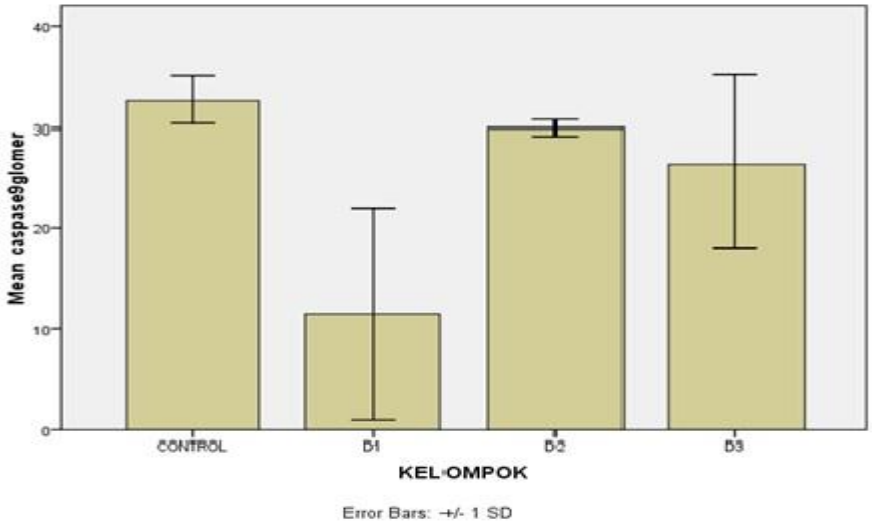
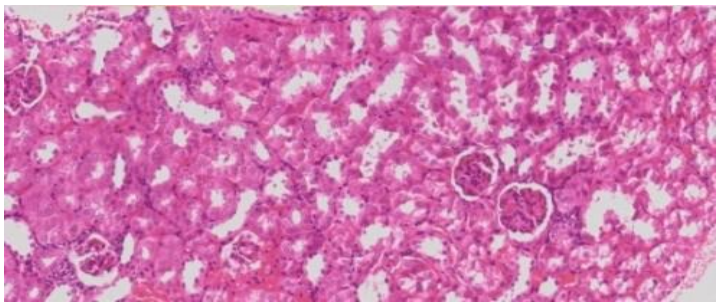
In the figure 7 below, for all doses of *Annona muricata* extract we found decrease of caspase-9 in glomerulus cell.

Fig 7: Bar Graph of mean of caspase-9 glomerulus cell for each group



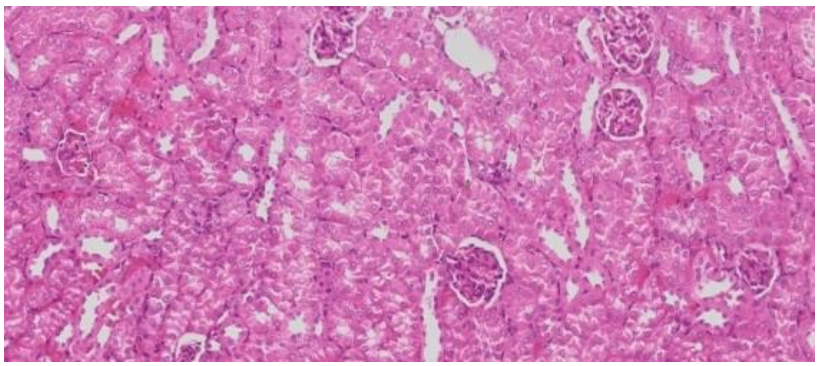
Group Effect of dose of *Annona muricata* extract on kidney cells (glomerulus and tubular).

Fig 8: Normal glomerulus and tubular cavity (by microscope 100X)



Control Group mice showing the glomerulus and tubular cells in the microscope whit out treatment by *Annona muricata* extract, where the tubular cavity some as normal. (figure 8) .

Fig 9: Effect of dose *Annona muricata* extract in 10 mg/kg.bw/day (by microscope 100X)



Group I mice after treatment by 10 mg/kg.bw/day *Annona muricata* extract showing a shrinkage of tubular cavity if compared with the above figure of glomerulus and tubular cells whit out treatment by *Annona muricata* extract. (figure 9)

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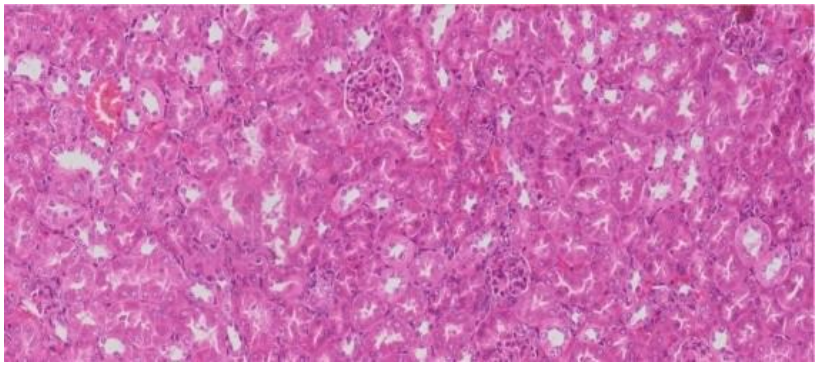
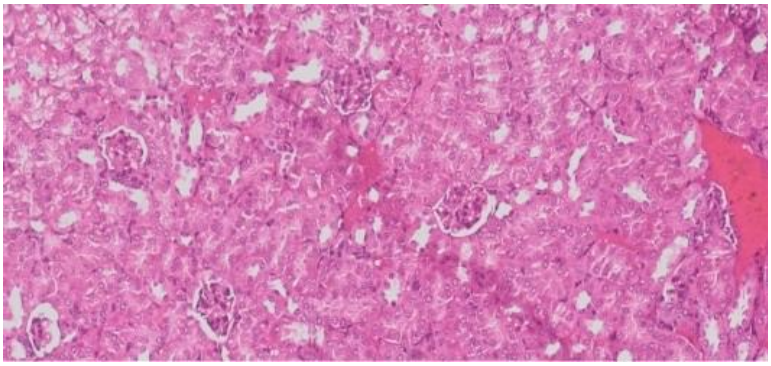
Fig 10: Effect of dose *Annona muricata* extract in 20 mg/kg.bw/day (by microscope 100X)



Group II mice after treatment by 20 mg/kg.bw/day *Annona muricata* extract, showing the change in the concentration of *Annona muricata* extract dosing will lead to increase damage of tubular cavity but is normal number of glomerulus cells. (figure 10) .

Fig 11: Effect of dose *Annona muricata* extract in 40 mg/kg.bw/day (by microscope 100X)



Group III mice after treatment by 40 mg/kg.bw/day *Annona muricata* extract, showing more severe damage of tubular cavity, but is normal number of glomerulus cells. Therefore increasing dose variation would see more suffer damage and was observed in the fourth image, where the function with *Annona muricata* extract dose 40 mg/kg increased damage of tubular cells number that effect of kidney cell function leading to renal failure. (figure 11)

- Control : control group with normal saline
- Group I : *Annona muricata* extract with doses 10 mg/kg.bw/day
- Group II : *Annona muricata* extract with doses 20mg/kg.bw/day
- Group III : *Annona muricata* extract with doses 40 mg/kg.bw/day

Annona muricata extract has the potential to causes damage to the kidney cell and function by prolonged exposure. The male kidney organs are severely damaged at doses 40 mg/kg.bw in mice. The effects include increase in creatinine serum concentration and damage structure tubular. It has

been shown to produce developmental toxicity in several doses, which cause kidney damage leading to renal failure [36].

Different Studies on *Annona muricata*:

• Cytotoxicity / Antileishmanial: Cytotoxicity and antileishmanial activity of *Annona muricata* pericarp: Extracts and fractionation led to the isolation of three acetogenins—annonacin, annonacin A and annomuricin A [37].

• Anti-Herpes Simplex Virus: Study showed the extract of AM to inhibit the cytopathic effect of HSV-1 on vero cells indicating an anti-HSV1 potential [38].

• Anti-Hyperlipidemia: Study of methanolic extracts of AM on serum lipid profiles in experimentally induced diabetic Wistar rats showed antihyperlipidemic activities with significant reductions in total cholesterol, LDL and VLDL and a significant increase in HDL and antiatherogenic index.

• Anti-depression: Graviola may have antidepressive activity due to its ability to stimulate serotonin receptors.

• Cytotoxicity: A crude hexane extract of *Annona muricata* L. gave a significant activity with an IC50 value of 0.8 pg/ml against CEM-SS cell line while the crude ethyl acetate (EA) extract also gave a significant activity with an IC50 value of 0.5 pg/ml but against HL-60 cell line.

• Acute and Subchronic Toxicity Studies / Antidiabetic: Toxicity studies were done on aqueous extracts of leaves. The extract did not produce any toxic effect in animal tissues at low and moderate doses but could cause kidney damage in higher doses. Lowering of plasma glucose level and positive effects on cardiovascular risk factors suggest good antidiabetic activity [39,11].

Toxicological evaluation of the lyophilized fruit juice extract:

Olufunsho Awodele & his co workers from Faculty of Basic Medical Sciences, Department of Pharmacology, College of Medicine, University of Lagos, Idi-Araba, Lagos, of Nigeria in 2013 reported that *Annona muricata* did not induce any significant toxic effect, indicating that it is safe in rats following oral administration for 60 consecutive days. *Annona muricata* fruit juice is widely consumed either raw or after processing in tropical countries because of its very juicy, creamy and sweet character including its medicinal importance. The safety of AM fruit was investigated in Sprague-Dawley rats for acute and 60-day subchronic toxicity effects.

In this research they found out that there was no mortality recorded up to 2000 mg/kg following acute administration. There were no significant changes in vital organ weights and haematological and biochemical parameters. However, significant ($p<0.05$) reduction in platelet count and packed cell volume was observed at 2000 and 400 mg/kg, respectively, which was reversed after cessation of treatment. Interestingly, subchronic oral administration of AM (80, 400 or 2000 mg/kg)

significantly ($p<0.001$) increased sperm count and motility in comparison to vehicle-treated control. AM long-term treatment induced significant *($p<0.05$, <0.01 and <0.001) increases in the levels of glutathione, superoxide dismutase (SOD) and catalase,* respectively, in the liver and kidney. Conversely, AM (2000 mg/kg) produced significant ($p<0.001$) increase in malondialdehyde level with decreased ($p<0.05$) SOD activity in the brain. The study established that AM did not induce any significant toxic effect, indicating that it is safe in rats following oral administration for 60 consecutive days. So there is no harm in consuming the *Annona muricata* as it did not induce any toxic effect [40].

Existing Practical Uses

Cancer research is ongoing on these important Annona plants and plant chemicals, as several pharmaceutical companies and universities continue to research, test, patent, and attempt to synthesize these chemicals into new chemotherapeutic drugs. In fact, graviola seems to be following the same path as another well-known cancer drug—Taxol. From the time researchers first discovered an antitumorous effect in the bark of the pacific yew tree and a novel chemical called taxol was discovered in its bark, it took thirty years of research by numerous pharmaceutical companies, universities, and government agencies before the first FDA-approved Taxol drug was sold to a cancer patient (which was based on the natural taxol chemical they found in the tree bark). With graviola, it has taken researchers almost ten years to successfully synthesize (chemically reproduce) the main antitumorous chemical, annonacin. These acetogenin chemicals have a unique waxy center and other unique molecular energy properties, which thwarted earlier attempts, and at least one major pharmaceutical company gave up in the process. Now that scientists have the ability to recreate this chemical and several other active acetogenins in the laboratory, the next step is to change the chemical just enough (without losing any of the antitumorous actions in the process) to become a novel chemical, which can be patented and turned into a new (patented) cancer drug. (Naturally occurring plant chemicals cannot be patented.) Thus far, scientists seem to be thwarted again—every time they change the chemical enough to be patentable, they lose much of the antitumorous actions. Like the development of taxol, it may well take government agencies like the National Cancer Institute and the National Institutes of Health to step forward and launch full-scale human cancer research on the synthesized unpatentable natural plant chemical (which will allow any pharmaceutical company to develop a cancer drug utilizing the research, as happened with taxol) to be able to make this promising therapy available to cancer patients in a timely fashion. In the meantime, many cancer patients and health practitioners are not waiting—they are adding the natural leaf and stem of graviola (with over forty documented naturally occurring acetogenins, including annonacin) as a complementary therapy to their cancer protocols. After all, graviola has had a long

history of safe use as an herbal remedy for other conditions for many years, and research indicates that the antitumorous acetogenins are selectively toxic to just cancer cells and not healthy cells—and in minuscule amounts. While research confirms that these antitumorous acetogenins also occur in high amounts in the fruit seeds and roots of graviola, different alkaloid chemicals in the seeds and roots have shown some preliminary in vitro neurotoxic effects.35 Researchers have suggested that these alkaloids might be linked to atypical Parkinson's disease in countries where the seeds are employed as a common herbal parasite remedy.36 Therefore, using the seeds and root of graviola is not recommended at this time. The therapeutic dosage of graviola leaf, (which offers just as high of an amount of acetogenins as the root and almost as much as the seed) is reported to be 2–3 g taken three or four times daily. Graviola products (capsules and tinctures) are becoming more widely available in the U.S. Market, and are now offered under several different manufacturer's labels in health food stores. As one of graviola's mechanisms of action is to deplete ATP energy to cancer cells, combining it with other supplements and natural products that increase or enhance cellular ATP may reduce the effect of graviola. The main supplement that increases ATP is a common antioxidant called Coenzyme Q10 and for this reason, it should be avoided when taking graviola. 15 And some recent studies have described how extracts of Soursop reduces blood sugar in diabetics by improving insulin production, Improves cardiovascular health by reducing blood fats, treat drug resistant cancer, stop diarrhoea in children, among others. Nigerian researchers have evaluated the effects of methanolic extract of Soursop leaves on the pancreatic islet cells of streptozotocin induced- diabetic rats. The results of the study indicated that Soursop extract treatment decreased the blood glucose concentration of diabetic rats due to the regeneration/proliferation in the pancreatic Beta cells (Beta-cells). The pancreas is a gland organ in the digestive and endocrine system of vertebrates. Beta cells (beta-cells, Beta-cells) are a type of cell in the pancreas in areas called the islets of Langerhans. They make up 65-80 per cent of the cells in the islets. Beta cells make and release insulin, a hormone that controls the level of glucose in the blood [41].

CONCLUSION:

The review presented here, is not a complete one, but gives a broad aspect on various studies done on wound healing , antimicrobial, antiovarian, antioxidant, chemopreventive, allelopathic, effect on kidney , effect on liver, its toxological evolution, its phytochemical & pharmocological activities.& summarised some of the findings regarding other medicinal properties & various studies on chemical constituents of Graviola. It is a natural fruit that has enormous uses especially in the treatment and prevention of cancer. Most of the experiments thus far carried out have been preclinical (animal or in vitro studies).Randomized clinical trials have to be carried out to know the exact effect in human

diseases. If, in the future, medicinal values of Graviola can be adequately assessed, especially of its anticancer activity, this fruit could have significant medicinal value.

Abbreviations	
NADH	nicotinamide adenine dinucleotide phosphate
MDR	multi-drug resistance
ATP	Adenosine tri-phosphate
COX	cyclooxygenase
LOX	lipooxygenase
DM	Diabetes Mellitus
ROS	reactive oxygen species
GSH	Glutathione
MST	Mean survival time
T47D	Human ductal breast epithelial tumor cell line
AMLE	*Annona muricata* leaf extract
WRL-68	normal human hepatic cells
MDA-MB-435S	human breast carcinoma cells
HaCaT	human immortalized keratinocyte cells
HPLC	High Performance Liquid Chromatography
ELISA	enzyme-linked immunosorbent assay
MTT 3-	4,5-di- 2-yl-2,5-diphenyltetrazolium bromide
IC50	Inhibitory Concentration
HaCaT	human keratinocyte
THF	tetrahydrofuran
THP	tetrahydropyran
PC-3	prostate adenocarcinoma
PACA-2	pancreatic carcinoma

COMPETING INTERESTS:

The authors declare that they don't have any competing interests.

AUTHOR'S CONTRIBUTION:

Trupti Sawant was involved in preparing manuscript. Dr. Dayanand Gogle participated in discussions of views represented in the paper. Both authors have read and approved the final manuscript.

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