Antioxidant and Anti-Inflammatory Effect of Fruit Juice of *Annona Muricata L* (Soursop) During Ischemia Reperfusion Injury in Rats

Mustafa Taha Mohammed ,Sarah Isam Abbas

ABSTRACT:

OBJECTIVE:

The study included knowledge The chemical components of the Soursop juice and determine whether Soursop prevents ischemia-reperfusion induced brain injuries in rats.

METHODS:

The chemical components of the prepared fruit extract were detectedits components of glycosides, proteins....etc and we were Determination of trace element and the estimation of the biochemical parameters: Blood samples were harvested from the rats,. Serum IL-10,Adiponectin and oxidative stress levels of Malondialdehyde (MDA) and Glutathione (GSH)were estimated.

RESULTS:

The results showed that the extract contain : glycosides, proteins, saponins, tannins, various phenolic compounds alkaloids , flavonoids, steroids and vitamine C . The results also showed that there were high concentrations of B, Fe , Zn respectively and low concentrations of Mn, Cu, pb,Cd respectively ,very low concentrations Ni ,Co , Se . The levels of oxidative enzymes and Inflammatory cytokines in group 3 were significantly lower than those in the group 2.Our results suggest that soursop treatment protects the rat brain against ischemia-reperfusion induced brain injuries.

CONCLUSION:

The present study confirm that the fruit extracts of Soursop *(Annona Muricata L.)* posses *in vivo* anti-inflammatory and antioxidant activity because of its content (glycosides , tannins , saponins ,proteins ,various phenolic compounds ,alkaloids , flavonoids, steroids and vitamine C)

KEY WORDS :AML , IL-10 , Adiponectin, MDA ,GSH, soursop, ischemia/reperfusion

INTRODUCTION:

Annona muricata L (AML) commonly known as graviola or Soursop, belongs to the family of Annonaceae. It is a typical tropical tree with heart shaped edible fruits and widely distributed in most of tropical countries[(1)]. The leaves are lanceolate with glossy and dark green in color had been traditionally used to treat headaches, hypertension, cough, asthma and used as antispasmodic, sedative and nervine for heart condition[(2)]. Previous reports over the years have demonstrated that the leaf, bark, root, stem, and fruit seed extracts of *Annona muricata* are anti-bacterial[(3)], antifungal[(4)] and anti-malarial [(5)]. Its leaves extract were also found to possess antioxidant[(6)] and molluscicidal properties [2].

Recently, it has also been reported to exhibit anti-inflammatory and analgesic effects[(7)]. Among the chemical constituents found in the leaf of *Annona muricata* are alkaloids[8],essential oils[(9)]and acetogenins[(10)]. Annonaceous acetogenins, from *Annona muricata L* were found to be a promising new anti-tumor and anticancer agent in numerous *in vitro* studies. These acetogenins demonstrated to be selectively toxic against various types of the cancerous cells without harming healthy cells [(11)]. Due to the presence of various medicinal properties, the present study was an endeavour to investigate the chemopreventive effect of *A. muricata* L leaves, if any on two-stage mouse skin papillomagenesis model.

MATERIALS AND METHODS:

Collection of samples:

The *Annona Muricata L.* were collected from market of Baghdad, Iraq. The fruit were transported to the laboratory biochemistry in department of chemistry /College of Science /Al-Mustansiriya University, washed,

Department of Chemistry, College of Science, Al-Mustansiriya University.

cleaned to remove all traces of dust and insects then Squeezed , filtrated ,dried ,weighed and placed in airtight bottles and stored to be used for extraction.

Experimental Design:

Thirty-six male Wistar rats weighting 200-230g were used in this experimental study. All animals were maintained under standard conditions. Rats were deprived of food, but not water, for 24 h before surgery. Animals were divided into three groups, sham group (Group 1), ischemia-reperfusion I/R group (Group 2), andAML (Aqueous extract)treatment group (Group 3). All rats were anesthetized with 40-50 mg / kg of thiopental sodium. AML was given to the rats in treatment group, before ischemia and before reperfusion at a dose of 200 mg/kg by intraperitoneal route. We chose the dose of this agent according to reported studies about I/R and AML .Rats in the I/R group were infused only with saline.

Surgical procedure:

The Experimental cerebral ischemia induced occlusion of the two carotid arteries for 30 minutes. Reperfusion was introduced by releasing the snare gently for a period of 60 min. In control animals reproduced anesthesia, skin incision and separation of the arteries without subsequent ligation of vessels. All surgical procedures were carried out under general anesthesia (intra introduction of thiopental sodium at a dose of 40-50 mg / kg). During ischemia, the animal's body temperature was maintained at normal levels (37 ° C) using warm table lamp. After, decapitation of rats was used to study [12].

Chemical detection of the plant components:

The chemical components of the prepared fruit extract were detected using different tests as shown in Table .1.They included: glycosides, alkaloids, saponins, phenolic compounds, tannins, flavonoids , proteins ,steroids and Vitamine C [13] .

Determination of trace element:

10 ml were taken from fruit extract (juice) of *Annona muricata* L. and mixed with 10 drops of concentrated nitric acid and 2 ml of 60% prechloric acid in a conical flask, the mixture was kept for 24hrs covered with watch glass . and the trace elements were determined [14]by (Shimadzu AA-670, Flame Atomic Absorption Spectrophotometer).

Biochemical analyses:

Blood samples were harvested from the rats. Serum IL-10,Adiponectin [15] and oxidative stress levels of Malondialdehyde (MDA) and, Glutathione (GSH)[16].

RESULTS:

The results in Table.1showed , the juice Soursop gave positive tests for (glycosides, proteins, saponins, tannins, various phenolic compounds, alkaloids , flavonoids, steroids and vitamine C) similar results are also obtained by other studies[17].The concentrations of trace elements in *Annona muricata L.* presented in Table.2 .It shows ,the concentrations of (B , Zn , Fe) are (20.22 , 0.688 , 0.264) ppm, respectively and the concentrations of(Mn ,Cu) are (0.217,0.19) ppm, respectively , concentrations (pb , Cd ,Ni ,Co) are (0.13,0.0872,0.074,0.05) ppm respectively but (Se) was very low(0.0001) ppm .In Table 3., ischemia reperfusion (I/R) caused Inflammatory cytokines has been reported in ischemic reperfused brain, leading to tissue damage as production and indicated by increased levels of IL-10 and Adiponectin (in the I/R group) .The IL-10 and Adiponectin levels in the Soursop treatment group were significantly lower than those in the I/R group. They were significantly higher in the I/R group than those in the control group. As expected, ischemia reperfusion (I/R) caused production of oxygen free radicals. It has been reported in ischemic reperfused brain, leading to tissue damage as indicated by increased levels of MDA (in the I/R group) and GSH decrease MDA levels in the soursop treated group were significantly lower than those in the I/R group but GSH was increased. They were significantly higher in the I/R group than those in the control group. The results are summarized in Table 4

Table 1: **Chemical components analysis for fruit extract of Soursop.**

Components	Reagents	Note	Result fruit extract
Glycosides	Iodine test Molish test Benedict test	Blue ppt. Violet ring Orange ppt.	Ve+ Ve+ Ve+
Proteins	Folin-Ciocalteau reagent	Blue color	Ve+
Saponins	Fast stirring Mercuric Chloride	Dense foam for long time White ppt.	Ve Ve +
Phenolic compounds	Aqueous%1 Ferric chloride	Green ppt.	Ve+
Tannins	Aqueous%1 Ferric chloride Lead acetate%1	Green ppt. Preface yellow ppt.	Ve+ Ve+
Flavonoids	aqueous%1 Ferric chloride Ethanol hydroxide alcohol	Green ppt. Yellow ppt.	Ve+ Ve+
Alkaloids	Mayer's reagent Wagner reagent Picric acid	white ppt. Brown ppt. Yellow ppt.	Ve+ Ve+ Ve+
Steroids	Libermann-burchar Libermann's reagent	Green ppt. Blue color	Ve+ Ve+
Test for Fats and Oils	Solubility test		Ve+
Test for Vitamine C	Ascorbic acid	Yellow ppt	Ve+

Table 2: The concentration of trace elements content of fruit juice of Soursop.

Trace elements	symbol	Concentration(µg/ml)PPM
Boron	B	20.22
Zinc	Zn	0.688
Iron	Fe	0.264
Manganese	Mn	0.217
Copper	Cu	0.19
Lead	Pb	0.13
Cadmium	Cd	0.0872
Nikel	Ni	0.074
Cobalt	Co	0.05
Selenium	Se	0.0001

Table 3: Clinical parameters in control , I/R and I/R+ Soursop ,rats (n=12 ,mean ±SD)

parameters	control	I/R	I/R+soursop	p
IL-10 (pg/ml)	3.8±0.3	8.9±0.9	6.4±0.6	0.001
Adiponectin (µg/ml)	9.1±0.5	53.6±4.1	38.7±3.2	0.001

IL-10 (pg/ml) ,Adiponectin (µg/ml)

Table 4: Clinical parameters in control , I/R and I/R+ Soursop ,rats (n=12 ,mean ±SD)

parameters	control	I/R	I/R+soursop	p
MDA (µmole/l)	3.26±0.27	4.71±0.63	3.91±0.46	0.001
GSH (nmole/l)	1.93±0.4	0.96±0.2	1.24±0.03	0.001

Malondialdehyde = MDA (µg/l) , Glutathione = GSH (nmole/l)

DISCUSSION:

Virtually all plants have one or more phytochemical resident in their leaf, stem, root, fruit and flowers. Fruit juice *A. muricata* contains phytochemicals including tannins, flavonoids, saponins and alkaloids which are known to exhibit medicinal as well as physiological activities. Flavonoid are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection .They have been found to be anti-microbial substances against wide array of microorganisms invitro[18]. They are also effective antioxidants and show strong anti-cancer activities[19].The presence of these phytochemicals in *A. muricata* could be contributory to its antioxidant activity observed in this investigation. In the present experiment the order of increasing relative abundance of these phytochemical in the fruit juice of *A. muricata* is Glycoside, Saponin ,Alkaloid, Flavonoid, Total phenolic compounds [20].

In the fruit extract (juice) of *Annona Muricata*, the concentration of Boron was 20.22ppm , Plenty of data supports the hypothesis that boron is an essential element and that it is involved in regulating parathormone action[21].

Zinc recorded a concentration of 0.688ppm cofactor in more than 100 enzymatic reactions and essential component of nuclear DNA binding proteins; serves in the expression of genes for metallothioneins. Zinc deficiency causes a block in protein and nucleic acid synthesis. The immune system, the skin and the gastro-intestinal tract are the tissues of the body with the highest rate of protein synthesis[22]that are affected by deficiency.

The concentration of Fe was 0.264ppm iron isessential component of haemoglobin and the cytochromes. Serves in the expression of genes for receptors of ferritin, trans ferritin and metallothioneins[23]

Manganese is required for the synthesis of mucopolysaccharides through the enzymes polymerase and galacto-transferase .It isessential cofactor in many enzymatic reactions Deficiency of Mn in the body may lead to central nervous system (CNS) disorders[24]. The concentration of Mn was 0.217ppm.

Copper isessential cofactor in several reactions concerning iron use, collagen synthesis, and suppression of free radicals. It Serves in the expression of genes for several enzymes[25]. The concentration of Cu in fruit extract of soursop ranged from(0.19ppm).Nickel essential trace element involved in the metabolism of several species of bacteria, archea, and plants. In these organisms, nickel is involved in enzymes that catalyze both non-redox (e.g., urease, glyoxalase I) and redox (e.g., hydrogenase, carbon monoxide dehydrogenase, superoxide dismutase)[26]reactions. The concentration of Ni was 0.074ppm. The concentration of Co was 0.05ppm. Cobalt is known to play an important role in thyroid metabolism in humans and essential in the structure of vitamin B_{12}.[27]

Selenium has an important role as an essential component of glutathione peroxidase which provides the active site. Absorption of selenium depends on the chemical form, the organic form being better absorbed than the inorganic. It is essential for enzyme reactions for glutathione and thyroxine)[28]. The concentration of Se in fruit extract of soursop was 0.0001ppm.

.Phytochemical screening done on the fruit extract *Annona muricata* L (AML) indicated that it contains alkaloids, saponins, flavonoids, tannins, triterpenes and steroid. Flavonoids and tannins have been reported to inhibit prostaglandin synthesis [29] . It is ubiquitously known that flavonoids have a great potential as anti-inflammatory agents [30] .Therefore, It can be postulated that these flavonoids may correlate appropriately for the present activities. The presence of other constituents in the extract such as tannins may give the synergistic effect to the flavonoids

In this study, experimental results show that the juice of soursop performs as

an anti-inflammatory agent in rats in ischemia reperfusion injury models. Priorly, AML has been reported on its anti-inflammatory and antinociceptive effects on different animal models[31].

Although the exact mechanisms of action of AML on the different biochemical variables examined in this study could not be established, a number of earlier investigators have shown that tannins and other polyphenolic compounds (e.g., coumarins), flavonoids, triterpenoid saponins, and a host of other plant secondary metabolitespossess hypoglycaemic, hypolipidaemic, hypotensive, anti-inflammatory, and other pharmacological and biochemical properties in various experimental animal models [32].*A. muricata* is known to contain ellagic acid, tannis, flavonoids, polyphenolic compounds, triterpenoids, β-sistosterol, and so on [33]. It is, therefore, not unreasonable to speculate that some of the above chemical constituents of the plant, especially the coumarins, flavonoids and triterpenoids, are probably responsible for the altered biochemical variables in the hepatic tissues, as well as the antidiabetic property of

AML, observed with plant's fruit extract in this study.

CONCLUSION:

The present study confirm that the fruit extract of Soursop *(Annona Muricata L.)* posses *in vivo* anti-inflammatory and antioxidant activity because of its content (glycosides , tannins , saponins ,proteins ,various phenolic compounds ,alkaloids , flavonoids, steroids and vitamine C).

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