An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

EVALUATION OF *ANNONA SQUAMOSA LINN* LEAF EXTARACT ON EXPERIMENTALLY INDUCED CEREBRAL ISCHEMIC REPERFUSION INJURY IN ALBINO WISTAR RATS

Md.Sultan Ali*[1], Riyazunnisa D[2], B Venkata Ramana[3], K.Suresh[1]

[1]Department of Pharmacology, Safa College of Pharmacy, Kurnool, Andhra Pradesh, India
[2]Department of Pharmacology, Sri Lakshmi Venkateswara Institute of Pharmaceutical Sciences, Proddatur, Andhra Pradesh, India
[3]Department of Pharmacology, Prabhath institute of pharmacy, Nandyal, Andhra Pradesh, India
***Corresponding author E-mail:** kurnool_ali@yahoo.co.in

ARTICLE INFO

Key Words

Annona Squamosa,
Cerebro protective,
SGOT, SGPT

ABSTRACT

In the present investigation the Effect of *Annona squamosa* leaf extracts was evaluated in experimentally induced cerebral ischemia reperfusion injury in rat model pre treatment with Aqueous and Ethyl acetate. The protective effect of the aqueous extract of Annona squamosa may be due to the presence of flavonoids, Saponins, triterpenoids and tannins. In ethyl acetate extract of *Annona squamosa* the protective effect may be due the presence of flavonoids and triterpenoids. There was a dose dependent increase in cerebral protection in terms of reduction of infarct size of brain tissue, Decrease in White Blood Cell count, SGOT, SGPT levels in serum compare with Disease control. In this experiment Ethyl acetate extract was shown good result as compare to Aqueous extract. Furthermore investigation is needed to find out the particular constituent which is responsible this protective activity.

INTRODUCTION:

Ischemia is defined as reduction in blood flow that results in decreased supply of oxygen and nutrients to the tissue. Ischemia can occur to any part of the body. Heart attacks and strokes are the results of Ischemia. Cerebral ischemia is defined as the lack of oxygen and nutrient-rich blood flow in the areas of the brain. In USA the incidence of stroke occurs more than 700000/year, of which 20% of the patients will die within the 1st year. This number has risen up to 1 million per year by the year 2050. The complications includes stroke, Parkinsonism, coma sometimes leads to death. The leaf extract of Annona Squamosa Linn the plant have been used for insecticide, anthelminitic, styptic, externally used as suppurant. Unripe and dried Fruit work as antidysenteric. Bark is used as powerful astringent, antidysenteric and vermifuge. Rootbark, leaves and stems gave isoquinoline alkaloids. Powdered seeds are used to kill head-lice and fleas but care should be taken that the powder does not come in contact with the eyes as this causes great pain. Two acetogenins, annoreticuin and isoannoreticuin, isolated from the leaves, were found to be selectively cytotoxic to certain human tumours. The leaves and stems also gave alkaloids dopamine, salsolinol and coclaurine.

Taxonomy:

Kingdom	Plante
Order	Magnoliales
Family	Annonaceae
Genus	*Annona* L
Species	*squamosa*

Materials and Method:

Fresh leaves of *Annona squamosa* were collected locally during the month of November to January. The taxonomic identification of these plant materials were authenticated by professor in Botany. These leaves were dried in shade, powdered coarsely, weighed and stored in a clean, dry and air tight container. Then this coarse powder was subjected for defatification with n-Hexane after conducted for successive extraction with distilled water and ethyl acetate solution. The extracts of powdered *Annona squamosa* leaves were prepared by sequential Soxhalation extraction with aqueous at 100^0c and Ethyl acetate at 60^0c by Soxhlet extractor, coarsely powdered *Annona squamosa* leaves were packed in a thimble &inserted in the Soxhlet apparatus, the extraction process with each solvent was continued for 24 hours, and the drug to solvent ratio of 1:3 was maintained after extraction the residues was evaporated on water bath at required temperature to get a solid mass. Nature, Yield of extract as in Table No: 01. Adult male or female Wister rats, weighing 200 to 250gm were used in the study. The study protocol was reviewed and approved by the institutional animal ethical committee (IAEC). They were housed in an air conditioned room and were kept in standard laboratory conditions under natural light and dark cycle (approximately 14 hr light/ 10 hr dark) and maintained humidity 60+5% and an ambient temperature of $25+2^0$c. The rats were divided into 5 groups each group contains 6 animals among 5 groups one is controlled and 2 groups are treated with aqueous extract of annonna Squamosa with 250 mg/kg, 500mg/ kg respectively. Another 2 groups are treated with ethyl acetate extract of plant with 250 mg kg and 500 mg/kg respectively. Rats were anaesthetized with Thiopentone sodium at a dose of 45mg/kg body weight and shifted to the surgery table. Check anesthesia level intermittently. Cerebral ischemia was induced by temporary bilateral carotid artery occlusion followed by reperfusion. Blood samples were collected by carotid artery for estimation of WBC count, these Blood samples were centrifuged at 4500rpm for 15 minutes. The supernatant was separated and used for estimation of SGOT and SGPT levels by Autoanalyser.

BIOCHEMICAL ESTIMATIONS:

1. Estimation of infarct size.
2. Histopathological changes.
3. Assay of White blood cell count.
4. Assay of Aspartate Transaminase (AST) Serum.
5. Assay of Alanine Transaminase (ALT) serum.

1. **Estimation of infarct size:** animals were sacrificed with excess dose of anesthesia. Brain was excised from skull rapidly and subsequently. It was sliced to 0.1cm thick sections and the slices were incubated in 1%T.T.C solution prepared in P_H7.4 phosphate buffer for 30 minutes at 37^0c.In viable cerebrum, TTC is converted by dehydrogenase enzymes to a red formazan pigment that stains tissue to dark red. The infracted cerebrum that does not take TTC stain where the dehydrogenase enzymes are drained off remains pale in color. Fig: 01
2. **Histopathological Changes:** After the completion of experiment the brain tissue was removed immediately and fixed in 10% formalin solution. Cross section (5µm thick) of the fixed tissues was cut. These sections were stained with hematoxylin and eosin (H & E) and visualized under light microscope to study the light microscopic architecture of the brain tissue.
3. **Assay of WBC count:** Total leukocyte count was determined by Haemocytometer method. The blood diluted and loaded into Neubars chamber and observed under 10x objective microscope. The number of cells counted for each blood sample was multiplied by 50 to obtain the total white blood cell count per microlitre of blood

S.no	Disease control	EAEAS 250mg/kg	EAEAS 500mg/kg	AEAS 250mg/kg	AEAS 500mg/kg
1	65.6	21.5	10.5	32.6	20.8
2	64.2	24.2	12.2	35.0	21.3
3	67.8	20.9	10.2	34.8	20.6
4	68.0	19.8	9.8	32.9	18.8
5	67.1	21.4	10.9	35.2	22.4
6	67.5	19.2	11.5	33.7	20.1
Mean±SD	66.7±1.49	21.16±1.74	10.85±0.88 ***	34.03 ± 1.12	20.66 ± 1.2 ***

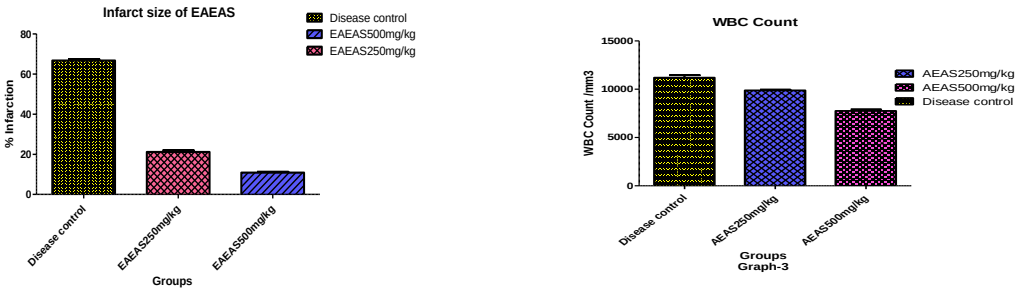


Fig 1: Graphical representation of AEAS and EAEAS

S.no.	Disease control	EAEAS 250mg/kg	EAEAS 500mg/kg	AEAS 250mg/kg	AEAS 500mg/kg
1	12100	8900	6800	9600	7200
2	11600	8960	7250	9750	7350
3	11800	9040	7640	9860	7640
4	10900	9150	7860	9920	7880
5	10500	9180	8300	10080	8150
6	10300	9250	8900	10100	8300
Mean±SD	**11200±737.5**	**9080±135.79**	**7791.66±747.1 *** **	**9885±192.639**	**7753.33±436.7 *** **

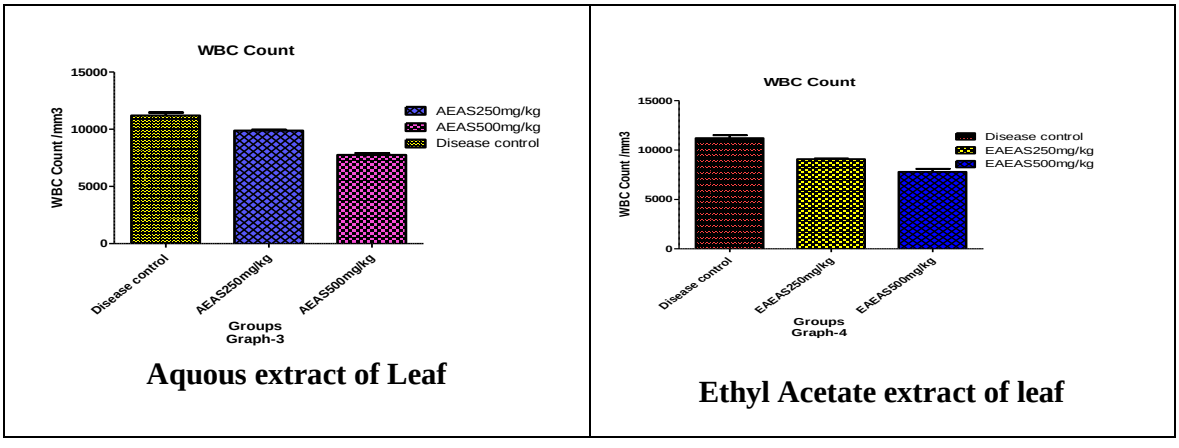


Fig 2: Graphical representation of WBC Count

S.no.	Disease control	EAEAS 250mg/kg	EAEAS 500mg/kg	AEAS 250mg/kg	AEAS 500mg/kg
1	109.4	72.3	34.2	81.4	38.6
2	110.6	71.8	30.6	84.6	36.4
3	112.4	70.9	31.4	83.7	39.2
4	113.6	72.8	32.9	80.9	40.6
5	109.9	71.4	29.8	84.4	38.7
6	110.2	70.5	30.6	85.2	41.4
Mean±SD	**111.01±1.63**	**71.61±0.86**	**31.58±1.65 *****	**83.36±1.78**	**39.15±1.74 *****

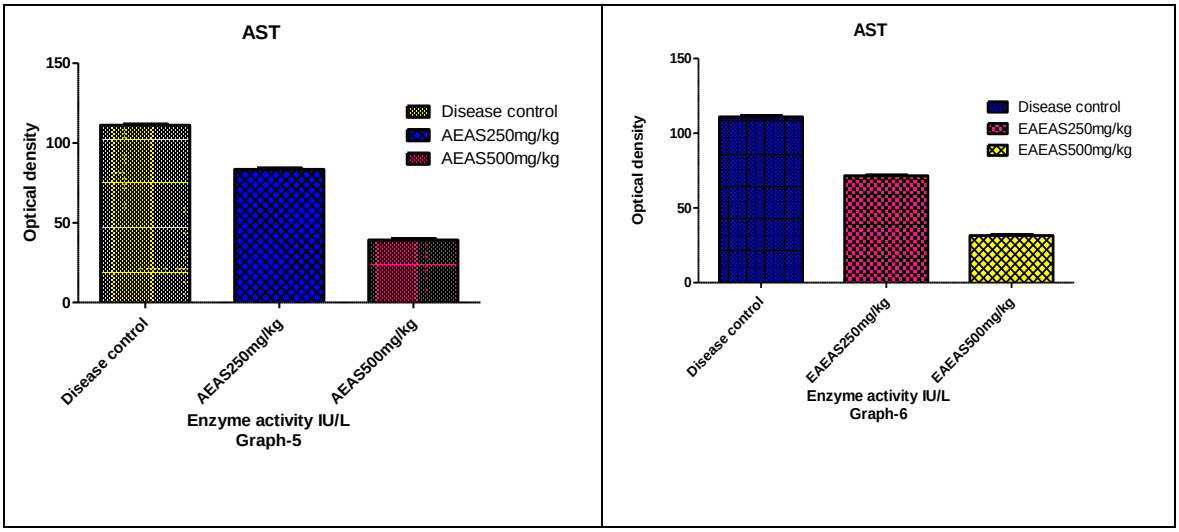


Fig 3: Graphical representation of AST

S.NO.	Disease control	EAEAS 250mg/kg	EAEAS 500mg/kg	AEAS 250mg/kg	AEAS 500mg/kg
1	75.2	52.6	35.6	65.6	42.1
2	69.4	50.8	34.2	63.4	40.8
3	67.3	51.5	33.8	62.1	42.4
4	72.9	50.2	34.7	65.4	41.8
5	70.5	52.1	35.2	64.8	40.5
6	69.8	50.4	34.8	63.6	39.8
Mean±SD	**70.85±2.79**	**51.26±0.96**	**34.71±0.65 *****	**64.15±1.35**	**41.23±1.02 *****

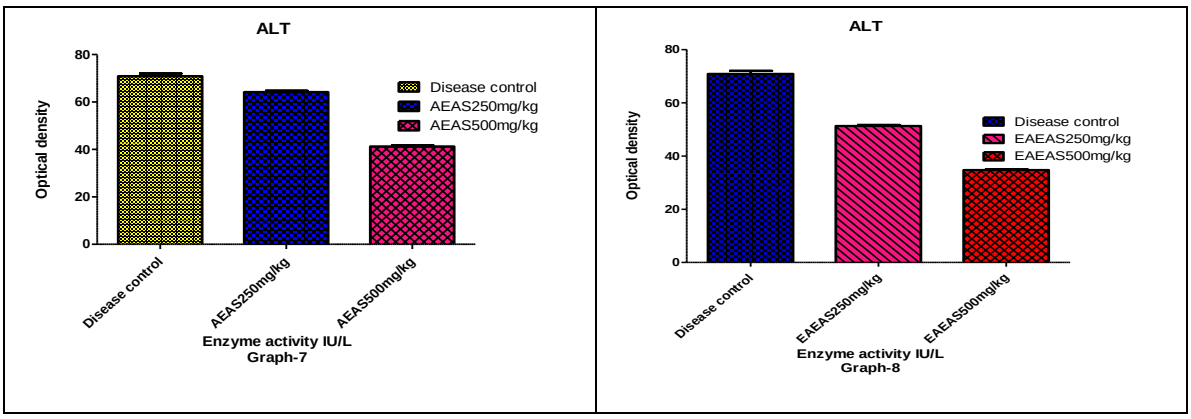
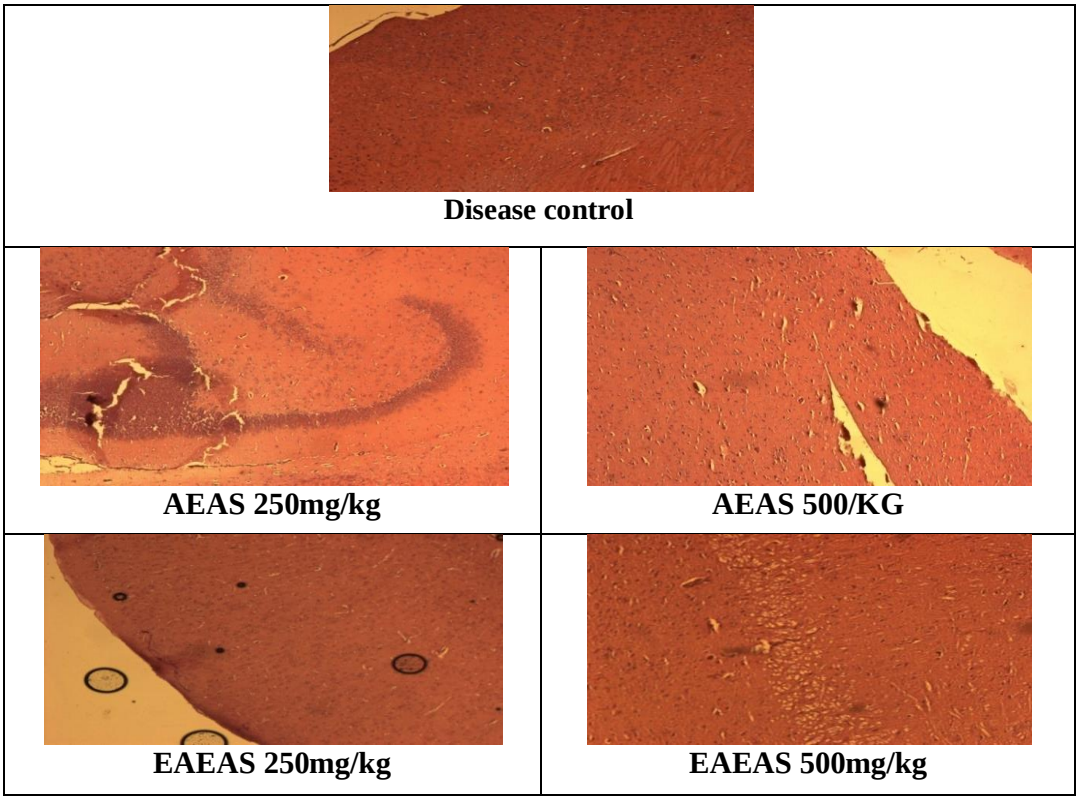


Fig 1: Graphical representation of ALT

HISTOPATHOLOGICAL REPORTS: The histological images were obtained by microscope with camera and were pictured as follows.



4. **Assay of Aspartate transaminase in serum:** SGOT kit is treated with blood serum of rats and observed the levels of transaminase enzyme released by Kidney, Brian, Heart and Skeletal Muscles.
5. **Assay of Alanine transaminase in serum:** Blood serum is treated with SGPT kit and observed the levels of ALT enzyme released by Liver and Skeletal Muscles.

RESULTS AND DISCUSSION:

The phytochemical screening indicates the Annonna Squamosa Linn Leaf Ethyl acetate extract gives only positive tests for steroids, Flavonoids and terpinoids. The infarct size was compared with control group verses Aquoeos extract and ethyl acetate extract of Annonna Squamosa which was tabulated in table No: 02. The WBC was compared with control group verses Aquoeos extract and ethyl acetate extract of Annonna Squamosa which was tabulated and graphically represented.in table No: 03.

The AST (Aspartate Transaminase) was compared with control group verses Aquoeos extract and ethyl acetate extract of Annonna Squamosa which was tabulated and graphically represented in table No: 04. The ALT (Alanine Transaminase) was compared with control group verses Aquoeos extract and ethyl acetate extract of Annonna Squamosa which was tabulated and graphically represented in table No: 05

CONCLUSION:

Experimental studies reveal that aqueous and ethyl acetate extract from *Annona squamosa* (250mg/500mg/kg) orally administered for 7 days produce a significant decrease in the infarct size, White Blood Cell count, SGOT and SGPT levels in the model of cerebral infarction reperfusion injury in albino Wister rats. In this experiment Ethyl acetate extract has been shown good protection as compare to aqueous extract in cerebral ischemia.

BIBLIOGRAPHY:

1. http://www.heart.org/HEARTORG/Conditions/HeartAttack/PreventionTreatmentofHeartAttack/Silent-Ischemi...
2. "Metabolic crisis without brain ischemia is common after traumatic brain injury: a combined micro dialysis and positron emission tomography study". *Journal of Cerebral Blood Flow & Metabolism*. Journal of Cerebral Blood Flow & Metabolism. 2009. Retrieved2009-03-05.
3. Y R Yadav MCh, Sanjay Pandey M Ch *et al* Hemispheric cerebral ischemia in traumatic brain injury. Indian journal of Neurotrauma, Vol.2, No.2: 135-138 2005.
4. Woranan Kirisattayakul, Jintanaporn Wattanathorn *et al*. cerebroprotective effect of Moringa oleifera against Focal ischemic stroke induced by middle cerebral artery occlusion. Oxidative Medicine and Cellular Longevity: vol 2013: 10.
5. Izumi Harukuni, MD, Anish Bhardwaj, MD has reported the mechanisms of brain injury after global cerebral ischemia. Neurol Clin 24 (2006) 1-21.
6. Mumby et al Global cerebral ischemia Behavioral neuroscience, 1996.
7. Introduction of ischemic stroke, UT southwestern medical center.
8. Otilia Margaritescu, Evaluates the Histopathological changes in acute ischemic stroke. Romanian journal of Morphology and Embryology 2019, 50(3):327-339.
9. Rodent models of focal cerebral ischemia.
10. Inflammatory mechanisms in ischemic stroke: therapeutic approaches.
11. David B. Arcinieges, MD, FANPA, CBIST Hypoxic-Ischemic brain injury
12. Rafieirad M, Zangeneh Nezhad Z And Eydipour Z Have Reported The Effects Of Oral Ferulago Angulate Hydroalcoholic Extract On Lipid Peroxidation Induced By Ischemia/Hypoperfusion In The Hoppocampus Rat. International journal of biology, pharmacy and allied sciences, Feb, 2014, 3(2): 157-164.
13. M. Nandagopal *et al*. have reported the Cerebroprotective effect of Root extract of Asparagus racemosus wild. In Global cerebral ischemia in rats. Journal of pharmacology and toxicology 6(1): 49-61; 2011.
14. S. Sindhura *et al* have reported the Evaluation of Stroke Preventive Effect of Ethanolic Extract of Ventilago Madrasaspatana Gaertn against Bilateral Carotid Artery Occlusion Induced Stroke in Rats. International research journal of pharmaceutical and applied sciences, 2014; 4(2): 1-6.
15. classification of cerebral stroke
16. Shinji Nagahiro *et al* pathophysiological and treatment of cerebral ischemia.
17. Bhardwaj A, Alkayed NJ, Kirsch JR, *et al*. Mechanisms of ischemic brain damage. Curr.Cardiol Rep 2003; 5:160–7.
18. Leist M, Nicotera P. Cell death: apoptosis versus necrosis. In: Welch KMA, Caplan LR, Reis DJ, et al, editors. Primer on Cerebrovascular disease. Philadelphia: Academic Press; 1997. p. 101–4.