Antioxidant Activity of *Jatropha Curcas* Linn. Bark Extract on Aspirin Induced Gastric Ulcers

***Mohammad Azamthulla[1], Anbu J.[2] and Anita Murali[3]**

*****Corresponding Author E-mail: mohammadazamthulla@gmail.com

Contributors:

[1]Assistant Professor,
[2]Professor, [3] Professor and Head
Department of Pharmacology,
Faculty of Pharmacy, M.S.
Ramaiah University of Applied
Sciences, Gnanagangothri
Campus , New BEL Road, M S R
Nagar, Bangalore, Karnataka,
India - 560 054

Abstract

Aim: The antiulcerogenic effects of the aqueous extract of Jathropa curcas Linn. on aspirin induced ulceration in rats with respect to antioxidant status in the gastric mucosa has been investigated. **Merthodology**: Oxygen free radicals are considered to be important factors in the pathogenesis of gastric ulcer. The level of lipid peroxides, which were elevated highly in rats with acute gastric mucosal injury, was taken as an index of oxidative stress .The activities of antioxidant defense enzymes were also decreased considerably by oral gastric administration of aspirin. **Results:** The decreased levels of antioxidant enzymes and increased mucosal injury were altered to near normal status upon pretreatment with Jathropa curcas Linn. extract when compared to the ulcer induced rats. The results indicate that Jathropa curcas Linn. extract may exert its gastro protective effect by a free radical scavenging action**. Conclusion**: Our observations suggest that Jathropa curcas Linn. extract may have considerable therapeutic potential in the treatment of gastric diseases.

Key Words: *Aspirin, Antioxidant Enzymes, Jathropa curcas Linn, Ulcer Healing*

1. INTRODUCTION

Oxidative stress and free radical mediated processes have been implicated in the pathogenesis of gastrointestinal disorders.[1] Nonsteroidal anti-inflammatory drugs are recognized as the most common etiologic factors associated with gastric ulcer.[2] Aspirin injures the gastrointestinal mucosa and because oxygen-derived free radicals mediate injury of this mucosa, oxy-radicals may play a pathogenetic role in the evolution of aspirin-induced erosive gastritis.[3] Aspirin induced ulcer has been used as a model for the evaluation of antiulcerogenic agent.[4] Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and wellbeing. Their role is twofold in the development of new drugs: (1) they may become the base for the development of a medicine, a natural blue print for the development of new drugs or; (2) a phytomedicine to be used for the treatment of diseases.[5] Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world.[6] *Jatropha curcas* Linn belong to the family Euphorbiaceae and are used in traditional folklore medicine to cure various ailments in Africa, Asia and Latin America.[7] *Jatropha curcas* Linn is commonly called physic nut, purging nut or pig nut. Previous studies have reported that the plant exhibits bioactive activities for fever, mouth infections, jaundice, guinea worm sores and joint heumatism.[8] Investigated and reported the anti-parasitic activity of the sap and crushed leaves of *Jatropha curcas* Linn. The water extract of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity.[9] Previous works have shown that many *Jatropha* species possess antimicrobial activity.[10] Therefore, the present study was conducted to evaluate the antiulcerogenic effect of *Jatropha curcas* Linn. in terms of its antioxidant status on aspirin induced gastric mucosal damage.

2. MATERIALS AND METHODS

Collection of plant Material and Extraction

The young barks of *Jatropha curcas* used for the investigation were collected from Bangalore in the month of October. The collected barks were cleaned from dust and other materials and then it was dried under the shade for 15 days. After confirming the dryness, the barks were chopped and pulverized in an electric grinder. The powdered plant materials were subjected to maceration separately. The powdered plant material was soaked in water for four days. Stirring of the mixture was done twice daily. After the fourth day, the mixture was filtered and the marc

was pressed. This process was repeated 3 times and then heated on the water bath until dry extract was obtained. Thus obtained extract of barks of Jatropha curcas Linn. was labeled and stored in the desiccator for further usage.

Animals

Adult male albino rats of Wistar strain weighing 150-200g were purchased from the small animal house of veterinary college, NIMHANS, Bangalore. They were maintained under standard laboratory conditions with standard pelleted diet (M/s. Hindustan Lever Foods, Bangalore, India) and water ad libitum. All animal experiments were carried according to the guidelines of Institutional Animal Ethics Committee.

Selection of Doses

The acute toxicity study was carried out in adult female albino rats weighing about 150-200g by as per OECD 425 guidelines.[11] For the assessment of anti-oxidant activity, 3 dose levels were chosen in such a way that middle dose was approximately one tenth of maximum dose administered during acute toxicity studies (i.e. 1/10th of 2000mg/kg body weight-200mg/kg body weight) and a high dose which was twice that of one tenth dose (i.e.2X 200mg/kg-400mg/kg body weight) and a lowose which was 50% of one tenth dose (50% of 200mg/kg-100mg/kg body weight).

Experimental Procedure

The rats were divided into four groups of 6 animals each. The animals were kept fasting for 24 hours prior to the experiment but water was permitted ad libitum.

Group I - Animals received aspirin 400mg/kg body orally.
Group II- Animals received aspirin (400mg/kg body wt) + 100mg/kg of *Jatropha curca* Linn. extract orally for 7 days.
Group III- Animals received aspirin (400mg/kg body wt) + 200mg/kg of *Jatropha curc*a Linn. extract orally for 7 days.
Group IV- Animals received aspirin (400mg/kg body wt) + 400mg/kg of *Jatropha curca* Linn. extract orally for 7 days.

For biochemical estimation gastric mucosal tissue was taken from the antral portion of the stomach. The gastric mucosa was scrapped with a scrapper, weighed and homogenized in ice cold phosphate buffer (pH 7.2) to prepare the mucosal homogenate. The homogenate was centrifuged at 3000 rpm for 10min and the supernatant was used for further studies.

Biochemical Estimation

Protein was estimated by the method of Lowry et al using Bovine serum albumin as standard.[12] Lipid peroxidation products were estimated by assaying malondialdehyde formation according to the method.[13] The determination of total tissue sulphydryl (thiol) group (reduced glutathione level) was carried out according to the method of Ellman.[14] The autooxidation of epinephrine was noted by determining the activity of superoxide dismutase (SOD) by the method of Misra.[15] Catalase (CAT) activity was measured by following decomposition of H_2O_2 according to the method of Berr's and Sizer.[16] Glutathione peroxidase (GSH-Px) was assayed by the method of Rotruck et al using H_2O_2 as substrate.[17] Glutathione-S-transferase activity (GST) was measured using 1-chloro 2,4-dinitrobenzene as substrate according to Habig.[18]

Statistical Analysis

All data will be expressed as mean ± SD. Student's t-test will be performed for each experimental group. Data will be compared by analysis of variance (ANOVA) and only values with $P<0.05$ will be considered as significant.

3. RESULTS

Jatropha curca Linn. extract administered in doses 100, 200, 400mg/kg body wt. orally, caused a dose dependent decrease in ulcer index in aspirin induced rats (Table 1). Although a dose of 400mg/kg showed maximum ulcer protection (72.70%), but revealed toxic symptoms such as diahorrea and weight loss,

where as a dosage of 200mg/kg body wt of *Jatropha curca* Linn. extract given for 7 days markedly inhibited the number of lesions and showed ulcer protection (70.12%) without any toxic symptoms, which is safer for further biochemical studies. Activities of GSH, Lipid peroxides (LPO), SOD, CAT, GSH-Px, GST and protein levels in gastric mucosal tissue are shown in (Table 2). There was a significant increase in lipid peroxidation ($p<0.001$), decrease in antioxidant enzyme and protein levels in aspirin induced rats when compared with those of control rats (Group I). *Jatropha curca* Linn. extract pretreated rats (Group III & IV) maintained the protein level, antioxidant enzyme status and LPO levels at near normalcy when compared to ulcerated animals.. The solubility was calculated using the dissolution factor and regression equation from the standard graph.

Table 1. Effect of Jatropha *curca* Linn extract on gastric lesions induced by aspirin in rats

Groups	Dose	3 days Lesion score	% of Inhibition	7 days Lesion score	% of Inhibition
Group I	**Aspirin Induced ulcer** 400 mg/kg body. wt orally	$15.4 \pm 2.3_a$	-	$I5.4 \pm 2.3_a$	
Group II	Aspirin (400mg/kg body. wt) + 100mg/kg of *Jatropha curca* Linn. extract orally for 7 days.	$12.3 \pm 2.4_b$	20.12	$9.1 \pm 1.2_b$**	49.30
Group III	Aspirin (400mg/kg body. wt) + 200mg/kg of *Jatropha curca* Linn. extract orally for 7 days.	8.6 ± 0.96b**	44.15	4.6 ± 0.60b***	70.12
Group IV	Aspirin (400mg/kg body. wt) + 400mg/kg of *Jatropha curca* Linn. extract orally for 7 days.	7.4 ± 0.86b**	44.15	4.2 ± 0.54b***	72.70

All values are mean ± SEM, n = 6. P *curca* Linn. extract treated +aspirin values: *<0.05, **< 0.01***<0.001 Aspirin induced Vs (b) *Jatropha*

Table 2. Effect of *Jatropha curca* Linn. extract on number of lesions, lipid peroxidation, antioxidant enzymes and protein levels of gastric mucosa in rats

Parameters	Group I	Group II	Group III	Group IV
Number of lesions	-----	15.4 ± 2.3***	4.6 ± 0.60***	------
Protein (mg/100 mg tissue)	16.3 ± 1.6	7.9 ± 0.68***	14.8 ± 1.5***	16.1 ± 1.4NS
Lipid peroxide (nmoles of MDA/mg protein)	3.8 ± 0.31	9.7± 0.74***	4.2 ± 0.29***	3.9 ± 0.22NS
Superoxide dismutase (units/mg protein)	5.3 ± 0.32	3.4 ± 0.13***	4.9 ± 0.21***	5.5 ± 0.35 NS
Catalase activity (nm of H_2O_2 decomposed/min/mg protein)	6.8 ± 0.42	3.2 ± 0.28***	5.9 ± 0.45***	6.9 ± 0.44 NS
Glutathione peroxidase (µg GSH utilised/minute/mg protein)	158.2± 10.2	60.1±5.8***	142 ± 9.2***	165.4±10.9 NS
Glutathione-S-transferase (nmoles of CDNB conjugated/min,/mg protein)	4.6 ± 0.32	2.8 ± 0.19***	3.9 ± 0.29***	4.4 ± 0.31 NS

All values are mean ± SEM, n = 6. P values: ***<0.001; NS - Non-significant, Group II and Group IV was statistically compared with Group I. Group III was statistically compared with Group II.

4. DISCUSSION

The oxygen derived free radicals play a key role in the mechanism of aspirin induced acute gastric mucosal lesions. Aspirin induces the reactive oxygen metabolites in animal models, which may contribute to mucosal injury[19]. The role of these reactive oxygen species and the potential antioxidant protective effect of *Jatropha curca* Linn. extract on gastric mucosal tissue are topic of high current interest. Many reports have demonstrated that most injury of gastric mucosa can be reduced by pretreatment with scavengers of reactive oxygen species.[20] In the present study, *Jatropha curca* Linn. extract inhibited the increase in area of gastric mucosal lesions in aspirin induced ulceration in rats. These protective effects were observed at oral doses of *Jatropha curca* Linn. extract 200 & 400 mg/kg body wt (Group III & IV) and a recovery of 70.12% was observed within 7 days. It offers gastro protection against aspirin induced ulcer by significantly blocking lipid peroxidation. In the present study aspirin pretreatment significantly reduced the protein concentration of the stomach. It may be due to accumulation of toxic free

radicals in the mucosal cells.[21] *Jatropha curca* Linn. extract treated rats provided protection against the action of aspirin by increase in protein content of the gastric mucosal tissue. Oxygen handling cells have antioxidant enzymes such as CAT, SOD, GST, GPX and GSH which are the first line of cellular defense against oxidative injury, decomposing O_2 & H_2O_2 before they interact to form more reactive (OH-) radicals, SOD mainly act by quenching of superoxide and active oxygen free radical, produced in different aerobic metabolism.[22] SOD and CAT enzymes are highly specific in their catalytic mode of actions and it decreases the gastric mucosal damaging effect of aspirin.[23] Although the increase in catalase is necessary for effective antioxidant activity, the changes in CAT levels were significant.[24] Hence the antioxidant activity of *Jatropha curca* Linn. may be one of the important defensive factors involved in its ulcer protective effect. The role of GSH as an endogenous gastric antioxidant in mucosal protection, however, remains controversial since recent evidence has indicated an inverse correlation between gastric mucosal GSH levels and mucosal protection.[25] GST and GSH-Px are essential for maintaining a constant ratio of reduced glutathione to oxidized glutathione in the cell .The extract maintained the activity of GSH-Px almost at near normalcy by its ability to increase the level of reduced GSH, and to decrease lipid perioxidation. The enhancement in GST activity was observed in *Jatropha curca* Linn. treated gastric mucosal tissues compared to ulcerated tissues. Many research have proved that antioxidants may play an important role not only by protecting against gastric mucosal injury, but also by inhibiting progression of gastric ulcer.[26] The observed cytoprotective and antioxidative activity of the extract is attributed to the presence of biologically active phytoconstituents with having antioxidative nature.

5. CONCLUSION

In conclusion it can be said that *Jatropha curca* Linn. exhibit a protective effect through free radical scavenging properties and reduces oxidative damage caused by aspirin. These results provide additional support for the popular use of this plant as an antiulcer remedy in the Indian traditional medicine. A further detailed study on various other parameters of mucosal defensive factors could elucidate their exact mechanism of actions and their usefulness in the treatment of ulcer.

6. ACKNOWLEDGEMENT

Authors thanks Dean Dr. V. Madhavan, Dr. Anita Murali, HOD Department of Pharmacology and Dr. Anbu, Department of Pharmacology, Faculty of Pharmacy, Ramaiah University of Applied Science, Bangalore, Karnataka, India.

7. CONFLICTS OF INTEREST

There are no conflicts of interest among the authors

REFERENCES

[1] Salim AWS. Use of Scavenging Oxygen Derived Free Radicals to Protect Rat Against Aspirin and Ethanol Induced Erosive Gastritis. *J Pharma Sci* 1992; 81:41-49.

[2] Isenberg JI, Mc Quaid KR, Laine, Walsh JH. Acid Peptic Disorders. *Textbook of Gastroenterolgy. Edited by Yamada T. 2nd edition J.B. Lippincott. Philadelphia P.A.* 1995; 41:1347-1430.

[3] Adams JD. Burgers Medicinal Chemistry and Drug Discovery, 5th edition, H.E Wolff, *John Wiley and sons, New York*. 1996: p.261.

[4] Goel RK, Chakrabarti A, Sanyal AK. The Effect of Biological Variables on the Antiulcerogenic Effect of Vegetable Plantain. *Planta Medica* 1985; 51: 85-88.

[5] Iwu M. Handbook of African Medicinal Plants. *CRC Press, Boca Raton, FL*. 1993; 51-55.

[6] WHO. Traditional Medicine: Growing Needs and Potential. WHO Policy Perspectives on Medicines. *World Health Organization, Geneva* 2002:p.1-6.

[7] Burkill HM. The Useful Plants of West Tropical Africa Families EJ, *Royal Botanical Gardens Kew* 1994; 25: 90-94.

[8] Irvine FR. Woody Plants of Ghana (With Special Reference to their Uses). *2nd Ed. OUP, London* 1961:p.233-237.

[9] Matsuse Tl, Lim YA, Hattori M, Correa M, Gupta MP. A Search for Anti-Viral Properties in Panamanian Medicinal Plants-The effect on HIV and Essential Enzymes. *J Ethnopharmacol* 1999; (64):15-22.

[10] Aiyelaagbe OO. Antibacterial Activity of Jatropha Multifida Roots. *Fitoterapia* 2001; (72); 544-546.

[11] OECD 425 Guidelines. OECD Guidelines for Testing of Chemicals. 2001 Dec; 1/26:1-26
[12] Lowry OH, Roseburgh NJ, Farr AL, Randall RJ. Protein Measurement with Folin Phenol Reagent. *J Bio Chem* 1951; 193:265-278.
[13] Das UN, Kumar KV, Krishna Mohan L. Lipid Peroxidation, EFA in Patients with Diabetes Mellitus and Diabetic Nephropathy. *J Nut Med* 1994; 4:149-155.
[14] Ellman GL. Tissue Sulphydryl Groups, Arch. *Biochem Biophys* 1959:82-70.
[15] Misra HP, Fridovich I. The Role of Superoxide Anion in the Autooxidation of Epinephrine and a Simple Assay of Superoxide Dismutase. *J Bio Chem* 1972; 24:3170-3179.
[16] Beers RF, Sizer IW. A Spectrophotometric Method for Measuring the Breakdown of H2O2 by Catalase. *J Biol Chem* 1952; 95:133.
[17] Rotruck JT, Pope AL Ganther H. Selenium Biochemical Role as Component of Glutathione Peroxidase. *Science* 1979; 179:588.
[18] Habig WH, Pabst MJ, Jackpoby WB. Glutahione – S - Transferase. A First Enzymatic Step in Mercapturic Acid Formation. *J Bio Chem* 1974; 249:7130-7139.
[19] Mc Alindon ME, Muller AF, Fillipowicz B, Hawkey CJ. Effect of Allopurinol, Sulphasalazine and Vitamin C on Aspirin Induced Gastroduodenal Injury in Human Volunteers. *Gut* 1996; 38:518-524.
[20] Goel RK, Sairam K, Rao V. Role of Gastric Antioxidant and Antihelicobactor Pylori Activities in Antiulcerogenic Activity of Plantain Banana (Musa Spaientum Var. Paradisiaca). *Ind J Exp Bio* 2001; 39:719-722.
[21] Al-Bekairi AM, Quershi S, Ahmed M.M, Afzal M, Shah AH. A study of Uric Acid Pretreatment for the Protection of Rat Gastric Mucosal against Toxic Damage. *Food and Chem. Toxicol* 1992; 30:525-531.
[22] Mac Millan LA, Crow JP, Thompson JA. Peroxynitrite Mediated Inactivation of Manganese SOD Involves Nitration and Oxidation of Critical Tyrosine Residues. *Biochemistry* 1998; 37:1613.
[23] Halliwall B, Gutteridge MC. The Importance of Free Radicals and Catalytic Metal Ions in Human Diseases. Mol. *Aspects Med* 2001; 8:89-193.
[24] Mizui T, Sato H, Hirose F, Doteuchi M. Effective of Antiperoxidative Drugs on Gastric Damage Induced by Ethanol in Rats. *Life Sci* 1987; 41:755.
[25] Mutoh H, Ota S, Hirashi H, Ivey KJ, Terano A, Sugimoto T. Reduced GSH Protects Cultured Gastric Mucosal Cells from Suckling Rats against Acid. *Ame J Physiol* 1991; 261:65.
[26] Singh P, Bhargava VK, Garg SK. Effect of Melatonin and b-Carotene on Indomethacin Induced Gastric Mucosal Injury. *Ind J Physiol* 2002; 46:229-234.