



Research Article

ANTI ULCER PROPERTIES OF ETHANOL EXTRACT OF AMARANTHUS HYBRIDUS LEAVES IN WISTAR RATS

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ABSTRACT

This study examined the anti ulcer properties of ethanol extract of *Amaranthus hybridus* leaves on Non Steroidal Anti-inflammatory Drug (NSAID) and alcohol induced gastric ulcer in rat models. Ethanolic nitrate extracts from *Amaranthus hybridus* leaves were administered orally at different doses (20, 40, 60, 80, 100, and 120 mg/kg) for a week in different doses. Diclofenac oral dose of 30 mg/kg diclofenac (an NSAID) and 80% alcohol induced ulcers. This effect was compared using 20 mg/kg cimetidine as positive standard and 2 ml/kg normal saline as control. This study found that *Amaranthus hybridus* leaves extract at the doses of 100 mg/kg and 120 mg/kg significantly protected albino rats against diclofenac and alcohol induced gastric ulcer. Comparing the ulcer protection percentage with a standard drug (Cimetidine), it was found that the *Amaranthus hybridus* leave extract protected at 34% while Cimetidine protected at 80%. It was concluded that *Amaranthus* hybrids, a dietary nitrate protects against diclofenac-induced gastric ulcers likely through the conversion of nitrates to nitrites by the activity of bacteria in the oral cavity and stomach.

Keywords: Anti- ulcer, *Amaranthus hybridus*, NSAID, Alcohol.

Introduction

Non steroidal anti inflammatory drugs (NSAIDs) are widely used for the treatment and management of pains which is associated with risks of gastrointestinal toxicity and damage (Jarosz *et al.*, 2017). For example a study by Peter *et al.* (2017) showed that diclofenac significantly increase ulcer scores in treated rats. High intake of alcoholic substances has been recorded among adolescents and adults all over the world. Similarly in Nigeria alcoholic substances predominantly consumed include palm wine, ordinary beer, wine Champagne and Ogogoro (Obi and Akpenna, 2015; Eniojukan and Chichi, 2014). Alcohol consumption of more than five drinks at a time significantly increases the risk of gastric ulcer in moderate drinkers and non-drinkers (Henry *et al.*, 1993). *Amaranthus* is a broad genus of about 60 species of short-lived herbs that breed mostly in the temperate and tropical regions. *Amaranthus hybridus* belongs to the family Amaranthaceae. Common names include; bush green (English), Tete-arowejeja (Yoruba) (Emmanuel and Folasade, 2011). *Amaranthus* is highly nutritious, both the grain amaranth and leaves are utilized for human as well as for animal food. Traditionally the plant was used to treat a variety of ailments. Alegbejo (2013) reported that a tea made from the leaves was thought to stop bleeding from ulcers and cure diarrhea. Also leaves of *Amaranthus hybridus* possess antibacterial effect, cleansing effect and also help to reduce tissue swelling. *Amaranthus viridi* is used for stomach problems and in pregnant women to alleviate labor pains (Mark, 2003). Another study by Ghosh *et al.*, 2013 revealed anti peptic ulcer activity of *Amaranthus spinosus* L. (Family: Amaranthaceae) leaves in ethanol induced gastric ulcers and cysteamine induced duodenal ulcers in rats. The leaves of *Amaranthus spinosus* are reported to produce inhibition of prostaglandin biosynthesis *in vitro* (Ibewuiké *et al.*, 1997). The plant is used as a laxative, diuretic, anti-ulcer, and antipyretic (Kirtikar and Basu, 2001).

Aim of Study

Current study examined the anti-ulcer properties of ethanol extract of *Amaranthus hybridus* leaves on a Non steroidal anti – inflammatory drug (NSAID) and alcohol induced gastric ulcer in rats.

Materials and Methods

Plant Materials

The fresh mature plants of *Amaranthus hybridus* were collected from an irrigation farm in wadata, Makurdi Local Government Area of Benue State and was identified and authenticated at Department of Botany, University of Agriculture, Makurdi by a taxonomist. A voucher specimen of the leaf was kept in the department for future references. The leaves of *Amaranthus hybridus* were then air-dried at room temperature until they were completely dried. After that the leaves were pounded into coarse powder using wooden mortar and pestle. The coarse powder was then passed through a 2 mm² sieve to get a uniform particle size and then used for extraction. For the ethanol extraction, 50g of the powdered *Amaranthus hybridus* leaves was soaked in 200ml of 80% ethanol in a maceration flask and was left to stand for 36 hours at room temperature with occasional shaking (Okoli *et al.*, 2002). The Filtrate was collected using a Whatman No.1 filter paper and was to evaporate to dryness in a steady air current and the residues collected after which the percentage yield of the extract was calculated.

Acute Toxicity Studies

A pilot survey was carried out using nine (9) Albino rats of either sex. Three rats were placed in three groups and extract doses of 100, 1000 and 2000 mg/kg body weight was administered and observed for a period of three days. If mortality was observed in one animal,

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then the same dose was tagged "toxic" and was included in the LD₅₀ studies. After the pilot survey, another set of 28 rats were placed in seven groups of four animals (male and female) each and administered different concentrations of the extract orally in total doses of 500, 700, 900, 1100, 1300, 1500 and 1700 mg/kg body weight for a period of seven days. The drug dose was administered after the animals were kept fasting overnight and provided with only water. LD₅₀ was calculated using the mathematical method formula:

$$LD_{50} = LD_{100} - \sum \left(\frac{Dd - Md}{n} \right)$$

Where LD₅₀ = median lethal dose;
 LD₁₀₀ = dosage required to kill 100%
 Dd = Dose difference
 Md = Mean dead
 n = group population

Experimental animals

Wistar albino rats of either sex within the weight range of 150-250 g were obtained from the animal house of the college of health sciences, Benue State University, Makurdi. The rats were placed on a standard diet (as recommended by the animal house department of the University). They were also maintained under the standard condition of 12 hours light dark cycle. Fourteen groups of albino rats (150-250g) with each group consisting of three (3) animals were used. The first six groups were designated using Greek characters of ALPHA, BETA, DELTA, GAMMA, SIGMA, and KAPPA. The next six were designated using Hindu-Arabic numerals of 1, 2, 3, 4, 5, and 6. The remaining two groups were designated "Control" and "Standard" groups. The groups designated with Greek characters and Hindu-Arabic numerals were treated with 20, 40, 60, 80, 100, and 120 mg/kg of the *Amaranthus hybridus* extract respectively. All treatments lasted for seven days. The Control group was treated with 0.1ml of normal saline for a period of seven days. The Standard group was not treated with any drug during the seven day period. All doses were administered orally using the gastric gavage technique using cannulae and syringe. After the seven day treatment period, all the animals in the fourteen groups were fasted for twenty four hours with water provided *ad libitum*. The water supply was removed two hours before drug administration.

Chemicals and drugs

Diclofenac (Hovid Nigeria, Plc), cimetidine (GSK, Glasosmith Nigeria Plc) and 80% ethanol (BDH, Germany) were used for this study.

NSAID-Induced Gastric Ulcer

In the NSAID-induced ulcer model, the rats in groups Alpha, Beta, Delta, Gamma, Sigma, and Kappa were treated with diclofenac oral dose of 30 mg/kg. They were then left for one hour after which the stomach was dissected and examined for the presence of bleeding, adhesion, dilatations and ulcers.

Alcohol-Induced Gastric Ulcer

The fasted rats in groups 1, 2, 3, 4, 5, and 6 were administered oral doses of 2 ml/kg of 80% ethanol. They were then left for one hour after which the stomach was dissected and examined for presence of ulcers.

Standard Drug Administration

The animals in the control group were given 20 mg/kg of cimetidine orally. After a period of 1 hour 1 ml of 80% alcohol then administered. They were then left for one hour after which the stomach was examined.

Dissection/ Isolation

The animals were stunned and then sacrificed by cervical dislocation, one hour after the induction of ulcer using 30 mg/kg Diclofenac and 2 ml/kg of 80% ethanol. The abdomen was then carefully dissected. The Stomachs were isolated and carefully removed from the GIT. Isolated stomachs were then cut open along the greater curvature and pinned to an improvised soft board. Tyrode solution was used to maintain the isolated stomach during observation. The length of each gastric ulceration lesion was measured and the ulcer index was expressed as the sum of the length of the entire lesion in mm plus the haemorrhagic plus the pin point.

The following table was used as the main criteria for scoring each ulcer.

Table 1. Criteria for Ulcer Score

S/NO	ULCER TYPE	SCORE
1.	Pin-point	1
2.	Less than 3mm	2
3.	Greater than 3mm	4
4.	Haemorrhagic	5

Ethical Clearance

Prior to investigation, Ethical clearance was obtained from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Benue State University, Makurdi. Animals were handled in accordance with protocols approved by the institutional animal ethics committee (IAEC), as adopted by the Faculty of Basic Medical Sciences, Benue State University, Makurdi, Nigeria.

Statistical Analysis

Results obtained from the study were expressed as Mean \pm SEM (Standard Error of Mean). With P-value of less than 0.01 ($p < 0.01$) considered to be statistically significant, a one-way analysis of variance (ANOVA) was used to determine the mean differences for variables between groups

Results

Results for this study are presented in table as shown below.

Table 2. Result for Acute Toxicity Studies of ethanol extract of *Amaranthus Hybridus* leaves

Group	Extract Dose (mg/kg)	Dose Difference (mg/kg)	Dead	Mean Dead (Md)
1	500	-	0/4	0.0
2	700	200	0/4	0.0
3	900	200	¼	2.5
4	1100	200	¼	2.5
5	1300	200	2/4	3.0
6	1500	200	4/4	-
7	1700	200	4/4	-
TOTAL			12/28	8.0

Form the table above, the LD₅₀ was calculated using the mathematical method formula,

$$LD_{50} = LD_{100} - \sum \left(\frac{DdxMd}{n} \right)$$

Therefore, LD₅₀ = 1100 mg/kg

Table 3. Effects of various extract doses of *Amaranthus Hybridus* against Diclofenac induced gastric ulcer in rats

Group	Dose (mg/kg)	Ulcer index (mm)	% Ulcer protection
Alpha	20	30.33± 0.57	10.79
Beta	40	29.00± 1.00	14.70
Delta	60	27.33± 0.57**	19.61
Gamma	80	26.33± 0.57**	22.55
Sigma	100	22.33± 0.57**	34.32
Kappa	120	22.66± 1.00**	33.35

Values were mean ± SEM (n = 3) animals in each group. **p < 0.01 when compared to control (normal saline) group. Higher doses of the extract produced greater percentage of ulcer reduction.

Table 4. Effects of various extract doses of *Amaranthus Hybridus* against 80% alcohol induced gastric ulcer in rats

Group	Dose (mg/kg)	Ulcer index (mm)	% Ulcer protection
1	20	30.00± 1.00	9.09
2	40	28.33± 0.57	14.15
3	60	26.00± 1.00**	21.21
4	80	23.66± 0.57**	28.30
6	100	21.66± 1.15**	34.36
8	120	21.66± 0.57**	34.36

Values were mean ± SEM (n = 3) animals in each group. **p < 0.01 when compared to control (normal saline) group. Higher doses of the extract produced greater percentage of ulcer reduction.

Table 5. Comparing effects of *Amaranthus hybridus* and cimethidine against diclofenac and alcohol induced gastric ulcer ulcers in rats

Group/Dose	Diclofenac Induced		Alcohol Induced	
	Ulcer index	% Ulcer protection	Ulcer index	% Ulcer protection
A.H (100mg/kg)	22.33 ± 0.57**	34.32	21.66 ± 1.05**	34.36
Cimetidine (20mg/kg)	6.66± 0.57**	80.41	6.00 ± 1.00**	81.81
N.S (2ml/kg)	34.00± 1.00	-	33.00± 1.73	-

A.H: *Amaranthus Hybridus*; N.S: Normal Saline; Values were mean ± SEM (n = 3) animals in each group. **p < 0.01 when compared to control group

Discussion

Amaranthus hybridus is a nitrate-rich vegetable generally consumed as food- either steamed or as a major component of soups and delicacies in Nigeria and other parts of the world. Nitrate-rich vegetables could ameliorate the injury Non Steroidal Anti-inflammatory Drug (NSAID) and alcohol induced gastric ulcer caused in rat models (Al-Harbi *et al.*, 1997). This study has found that *Amaranthus hybridus* leaves extract at the doses of 100 mg/kg and 120 mg/kg significantly protected albino rats against diclofenac and alcohol induced gastric ulcer. *Spinacia oleracea* commonly named as spinach also of the family of Amaranthaceae was found to have a potent ulcer protective activity at a dose 500 mg/kg and 1000 mg/kg, but at the dose 1000 mg/kg was more potent (Kore *et al.*, 2011). Other studies by Debiprasad *et al.*, (2013) and Ghosh *et al.*, (2013) established that powdered leaves of *Amaranthus spinosus* L. (family of the Amaranthaceae) could protect animal models significantly from formation of gastric ulcers induced by ethanol and duodenal ulcers

induced by cysteamine. Comparing the ulcer protection percentage with a standard drug (Cimetidine), it was found that the *Amaranthus hybridus* leave extract protected at 34% while Cimetidine protected at 80%. This present findings is similar to that of Mitra *et al.*, (2014) who compared anti ulcer activities of *Amaranthus spinosus* Linn with Ranitidine. Pretreatment of rats with *Amaranthus spinosus* Linn. leaves gave significant protection (25.25%, 63.12% and 66.78% by the doses 0.5 g/kg, 1.0 g/kg and 1.5 g/kg respectively) to the animals from forming ulcers by aspirin. Ranitidine (50 mg/kg), however, gave 72.76% protection. Cimetidine is a drug that blocks the production of acid by acid producing cells in the stomach. It belongs to the class of drugs called H₂ (histamine-2) blockers that also ranitidine. *Amaranthus hybridus* leave extract protects against diclofenac and alcohol induced gastric ulcers likely through the conversion of nitrates to nitrites by the activity of bacteria in the oral cavity and stomach and subsequent conversion to Nitric oxide which has a vasodilation activity of NO formed. Future studies will reveal the actual mechanism by which this leaves exert its anti ulcer properties.

Conclusion

Peptic ulcer diseases (PUD) is known to cause sore on the lining of the stomach, duodenum or esophagus. In this study, Ulcer spots were seen to be reduced after the intake of *Amaranthus Hybridus*. Treatment of stomach ulcers with *Amaranthus Hybridus* leave extract significantly improved and restored deranged ulcerations seen in rats

Recommendations

The *Amaranthus Hybridus* leaves are nitrate rich vegetable found to ameliorate gastric ulcer. It is therefore highly recommended as a dietary supplement in patient with Peptic Ulcer Disease (PUD)

Competing interests: Authors have declared that no competing interests exist

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