



Phytochemical and Biological Evaluations of Methanolic Extract of *Amaranthus graecizans* subsp. *silvestris* (Vill.) Brenan

Saiqa Ishtiaq^{1*}, Tahir Ali^{1,2}, Bashir Ahmad², Fareeha Anwar²,
Muhammad Shaharyar Khan Afridi¹ and Humera Shaheen¹

¹Punjab University College of Pharmacy, University of the Punjab, Lahore, Pakistan.

²Riphah Institute of Pharmaceutical Sciences, Riphah International University, Lahore, Pakistan.

Authors' contributions

This work was carried out in collaboration between all authors. Authors SI, TA and MSKA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BA and FA managed the analyses of the study. Author HS managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2017/31698

Editor(s):

(1) Nawal Kishore Dubey, Centre for Advanced Studies in Botany, Banaras Hindu University, India.

Reviewers:

(1) Carmen Lizette Del Toro Sanchez, Universidad De Sonora, Mexico.

(2) Mohammad Jamshed Siddiqui, International Islamic University, Malaysia.

Complete Peer review History: <http://www.sciencedomain.org/review-history/18402>

Original Research Article

Received 20th January 2017
Accepted 12th March 2017
Published 29th March 2017

ABSTRACT

Amaranthus graecizans subsp. *silvestris* (AMGRS) has traditionally been used a folk medicine in the treatment of inflammation, gonorrhea and piles without scientific evidence. Therefore, methanolic (MeOH) extract of AMGRS was studied for anti-nociceptive, anti-inflammatory and enzymatic inhibition activities in addition to phytochemical screening to ascertain the realism of the folk claims. Anti-nociceptive activity was screened by hot plate test, tail immersion test and acetic acid-induced abdominal writhing in mice with different doses of MeOH extract of AMGRS *i.e.* 100, 300 and 600 mg/kg. Anti-inflammatory activity was evaluated using carrageenan-induced paw edema model in rats with different doses of MeOH extract of AMGRS *i.e.* 100, 300 and 600 mg/kg. While, anticholinesterase and protease inhibition was carried to screen MeOH extract for its relevant therapeutic uses. Hot plate technique and tail immersion method demonstrated dose related antinociceptive response starting from 300 mg/kg to maximum effect seen at 600 mg/kg

*Corresponding author: E-mail: Saiqa.pharmacy@pu.edu.pk;

dose. Acetic acid induced writhing produced 47% pain protection during 1st phase (0-15 minutes) at 600 mg/kg dose. Likewise, carrageenan induced rat paw edema test showed 42% reduction in edema at 4th h after oral administration of dose (600 mg/kg). The MeOH extract showed mild anticholinesterase inhibition (24.29±0.57) while exhibited significant protease inhibition activity (60.52±0.18). The present investigation suggests that this plant possesses substantial antinociceptive, anti-inflammatory and protease inhibition activities.

Keywords: *Amaranthus graecizans* subsp. *silvestris* (Vill.) Brenan; antinociceptive; anti-inflammatory; anticholinesterase; protease.

1. INTRODUCTION

During the last few decades, traditional system of medicine has received more global importance because of its versatile benefits. In developing countries, various estimates showed that majority of the population still relies greatly on traditional medical practitioner and medicinal plants to fulfill basic health care needs. The switching of interest towards alternative medicines was mainly due to availability of cost effective medicines with less side effects [1,2].

The current Amaranthaceae family is composed of almost 175 genera and 2,000 species [3]. Plants of Amaranthaceae family also have economic value. Species like *Salicornia herbacea* and *Beta vulgaris* are cultivated for their use as vegetables around the globe. Other varieties of *Beta vulgaris* i.e. fodder beet and sugar beet are widely processed in food industry. The seeds of *Chenopodium quinoa* (Quinoa) and *Amaranthus* sp. (Amaranth) are eatable and being consumed as pseudocereals [4].

In the tropical, subtropical and temperate climate, numerous species of Amaranthaceae family have been utilized as medicinal plants by native population. The recent pharmacological studies reported that plants belonging to Amaranthaceae are good therapeutic agents to cure variety of ailments due to the presence of diversified phytochemical constituents [5,6].

Amaranthus is one of the important genus of Amaranthaceae family. The seeds and roots of many species of genus *Amaranthus* have shown beneficial effects as antihypertensive, antihyperlipidemic as well as antioxidant agents. Some species are helpful in patients with constipation, anemia, fever, hemorrhage and kidney disorders. In Ghana and Ethiopia different species are used as analgesic agent and as a tapeworm expellant, respectively. The ash of stems of different plants of genus *Amaranthus* are used to treat wounds and cancers [7]. It has

been reported that the extract of seeds of *A. spinosus* are used as a dressing for fractured bones [8]. In Southeast Asia, a decoction of the root is utilized to treat gonorrhoea and is likewise linked as an emmenagogue and antipyretic. The Nepalese and few Indian tribes apply *A. spinosus* to trigger premature birth. In numerous nations, the wounded leaves are viewed as an emollient. The leaves are additionally utilized for nerve bladder irritation gastroenteritis, joint pain, abscesses and for the treatment of snakebites. The plant sap is utilized to treat convulsions and ophthalmia in youngsters. During summer, *A. spinosus* bark extract is taken to avert malaria [7].

There are three species of *Amaranthus* are reported in Pakistan i.e. *Amaranthus angustifolius* Lam., *Amaranthus blitum* var. *graecizans* (L.) Moq. *Amaranthus silvestris* var. *graecizans* (L.) Boiss. [9]. Herein, we selected *Amaranthus graecizans* subsp. *silvestris* to analyze the nature of phytochemicals present in methanolic extract. Moreover, antinociceptive and anti-inflammatory activities were evaluated using mice and rats models, respectively.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh plant parts were collected from Chakwal, authenticated by Dr. Muhammad Ajaib, Department of Botany, Mirpur University of Sciences and Technology (MUST), Pakistan against voucher specimen of GC. Herb. 2329. The collected material was cleaned, dried and then powdered and preserved in amber colored bottles.

2.2 Chemicals

Aspirin, Methanol, Tris buffer, Distilled water, Phosphate buffer, Galanthamine, N-benzoyl-DL-arginine-p-nitroanilide hydrochloride

(BAPNA), trypsin, 1.0 mM HCl, Acetic acid, Phenylmethanesulfonyl fluoride, acetyl cholinesterase, (PMSF) DMSO₄, and DTNB. All chemicals used were of analytical grade. The reagents used were freshly prepared in laboratory.

2.3 Experimental Animals

Swiss albino mice and Wistar rats of either sex were purchased from Riphah International University, Lahore Pakistan. All the animals were kept in animal house at controlled temperature $22 \pm 1^\circ\text{C}$ under 12 h of dark and light cycles. Animals had free access to food and water *ad libitum*. The protocols followed to carry out the project was approved by the Animal Ethical Committee of Punjab University College of Pharmacy, prepared by National Institute of Health (AEC/UCP/1041/4313).

2.4 Preparation of Methanol Extract

The powder of AMGRS (500 g) was macerated in 700 mL of 90% methanol for seven days. The macerated material was shaken periodically to facilitate extraction. After seven days, the sample solution was first filtered with *Whatmann* No. 1 filter paper. The MeOH extract was evaporated by using rotary evaporator at temperature lower than 40°C . The extract was collected and stored in air tight container which was placed in refrigerator for further investigations [10].

2.5 Phytochemical Screening

The phytochemical screening of the plant was carried out according to the standard screening tests and conventional protocols as described by Ishtiaq et al. [11].

2.6 Biological Evaluations

2.6.1 Analgesic activity

Analgesic activity of MeOH extract of AMGRS was determined by using three different methods as described below;

- Hot plate method
- Tail immersion method
- Acetic acid induced writhing method

Swiss albino mice of either gender weighing about 22 – 25 g were selected for this activity. Animals were categorized into five following groups (n=5) for all above stated methods.

Group 1 = Normal saline 10mL/kg/day orally and was labeled as negative control.

Group 2 = Aspirin 300 mg/kg/day orally as positive control.

Group 3 = MeOH extract of AMGRS 100 mg/kg/day given orally.

Group 4 = MeOH extract of AMGRS 300 mg/kg/day given orally.

Group 5 = MeOH extract of AMGRS 600 mg/kg/day given orally.

2.6.1.1 Hot plate method

Hot plate method was used to study analgesic activity of MeOH extract of plant according to standard method with slight modifications [12]. Animals were fasted 12 h before the commencement of experiment. Animals were pre-tested by keeping the temperature of hot plate at $55 \pm 0.1^\circ\text{C}$. The time taken in seconds for any type of animal response (paw licking and jumping) was noted. The animals showing latency time greater than fifteen seconds were not included in the study. Standard drug and MeOH extracts of AMGRS were given orally using different doses as mentioned in study design. After 30 minutes of administration of dose, mice were allowed to place on the hot plate and the latency time was recorded in seconds. A cut-off time of 60 second was set for all mice to prevent any damage to tissue. Latency time was recorded at predefined time intervals of 0.5 h, 1.0 h, 1.5 h, 2.0 h, 2.5 h, 3.0 h, 3.5 h, 4.0 h, and 4.5 h. Latency time of treated group was compared with control group.

2.6.1.2 Tail immersion method

Tail immersion method was used to describe the analgesic effect of the MeOH extract of AMGRS according to selected method with little modifications [13]. Only those animals were selected for this experiment who responded within 15 seconds to stimulus. Food of the animals was withdrawn five hours prior to the beginning of test. Grouping and dosing of the animals were same as described in study design. The tail of each animal was dipped in water bath thermostatically maintained at temperature of $50 \pm 5^\circ\text{C}$ and the time until tail flicking occurred, was noted with the help of stopwatch at 0.5 h, 1.0 h, 1.5 h, 2.0 h, 2.5 h, 3.0 h, 3.5 h, 4.0 h, and 4.5 h after the treatment. The temperature of water bath was sustained at $50 \pm 5^\circ\text{C}$. The results of the tested extract groups were compared with standard drug groups. The maximum time for tail

immersion was set 120 seconds as cut-off limit to escape each animal from tissue damage.

2.6.1.3 Acetic acid induced writhing method

This method was also performed to measure the analgesic activity of MeOH extract of AMGRS [14]. The animals were fasted for six hours before the beginning the experiment. Grouping and dosing of tested extract groups and controlled or standard drug groups were according to the pattern given in study design. After thirty minutes of treatment, each animal was treated with 1 mL of acetic acid (1%, v/v in normal saline, 10 mL/kg) intraperitoneally. After 5 minutes of treatment with acetic acid, each animal was observed separately and the numbers of abdominal constrictions or writhes were counted for 30 minutes. The significance of findings of treated groups was compared with the results of control and standard groups. The percentage inhibition of writhes was calculated by formula given below:

$$\text{Percentage inhibition} = \frac{(A - B)}{A} \times 100$$

Where, A = Average number of writhes of the control group, B= Average number of writhes of the test group.

2.6.2 Anti-inflammatory activity

2.6.2.1 Carrageenan-induced rat paw edema method

The carrageenan-induced paw edema method was used to test the anti-inflammatory activity of MeOH extract of AMGRS according to standardized method few alterations [15]. Standard drug and tested plant extract were given orally using different doses. Rats were divided into five groups and tagged as:

- Group 1** = Normal saline 10 mL/kg/day orally and was labeled as negative control.
- Group 2** = Aspirin 300 mg/kg/day orally as positive control.
- Group 3** = MeOH extract of AMGRS 100 mg/kg/day given orally.
- Group 4** = MeOH extract of AMGRS 300 mg/kg/day given orally.
- Group 5** = MeOH extract of AMGRS 600 mg/kg/day given orally.

After an hour of oral intake of tested extracts and standard drug, 0.1 mL of carrageenan (1% solution in distilled water) was utilized to induce edema into the left hind paw of each rat. Digital vernier caliper was used to measure the size of paw of rats individually at 0 h, 1 h, 2 h, 3 h and 4 h after the treatment with carrageenan. The percentage (%) inhibition was calculated by using formula given below.

Percentage Inhibition =

$$\frac{\text{Edema of Control} - \text{Edema of Treated}}{\text{Edema of Control}} \times 100$$

The results of tested groups were compared with that of controlled groups.

2.6.3 Enzymes inhibition assays

2.6.3.1 In-vitro acetyl cholinesterase inhibition assay

The spectroscopic method used for the evaluation of anticholinesterase activity was developed by Ellman et al. [16]. Mixed 2.81 mL of phosphate buffer (pH=8) with 30uL of methanolic extract *A. graecizans* subsp. *silvestris*, 30 uL enzyme and 30 uL DTNB. This reaction mixture was incubated for about 10 minutes at 25°C. Then, added the 30 uL of acetylcholine iodide as a substrate. The hydrolysis of acetylcholinesterase was monitored after 35 min at 412 nm through UV/VIS spectrophotometer. The concentration of stock solution of MeOH extract was 1 mg/mL and for synthetics, was 5 mg/mL. Galanthamine was used as a positive control, while the negative control of the samples were established without adding substrate. All the reactions were performed in triplicate and calculated the mean and standard deviation. The extracts with percentage inhibition more than 50% were subjected to further dilutions to calculate IC₅₀. The percentage inhibition was calculated by using the following formula:

$$\text{Percentage inhibition} = \frac{(A - B)}{A} \times 100$$

Where; A = activity of the enzyme without test compound and B = activity of enzyme with test compound.

2.6.3.2 In-vitro proteases inhibition assay

Proteases inhibition assay was performed on the methanolic extract of *A. graecizans* subsp.

silvestris according to method with some modifications [17].

Tris buffer (100 mM): pH 7.5 was adjusted with HCl (5 M). Trypsin stock solution of concentration 0.002 mg/mL with tris buffer. BApNA was dissolved in 20 mg/ml in DMSO₄. 0.3 mL of enzyme and 0.1 mL of inhibitor was incubated at 37°C for 15 minutes then 0.6 mM substrate was added and final volume was adjusted to 2.5 mL with buffer. The reaction was quenched by adding 200 µl (30% acetic acid) and the absorbance was measured at 410nm using UV/VIS spectrophotometer. PMSF was used as positive inhibitor. Trypsin inhibitory potential was calculated by using the following formula.

Percentage inhibition =

$$\frac{\text{Absorbance (Blank)} - \text{Absorbance (Test)}}{\text{Absorbance (Blank)}} \times 100$$

2.7 Statistical Analysis

Graph pad prism was utilized to analyze the data obtained from experiments. All the results were represented in terms of \pm SEM. Statistical analysis was performed to find out variance by two-way ANOVA (analysis of variance) followed by Bonferroni post-test for comparison. $p < 0.05$ was considered as statistically significant.

3. RESULTS

3.1 Phytochemical Analysis

Phytochemical screening revealed presence of alkaloids, carbohydrates, saponins, flavonoids, phenol, steroids, and glycosides in the sample whereas proteins and triterpenoids were absent in the sample.

3.2 Analgesic Activity

3.2.1 Hot plate method

Analgesic potential of MeOH extracts of AMGRS was determined by hot plate method. Aspirin 300 mg/kg was used as a positive control and it showed the maximum latency time when compared with its control group and showed statistically significant ($p < 0.05$). At early hours, AMGRS 600 mg/kg showed almost same analgesic potential when compared with Aspirin 300 mg/kg but at late hours, aspirin had greater activity than AMGRS extract (600 mg/kg). AMGRS extract of 600 mg/kg dose showed significant ($p < 0.05$) analgesic effect in comparison to 100 mg/kg and 300 mg/kg dose.

Results revealed that MeOH extracts of AMGRS showed significant ($p < 0.05$) dose dependent and time dependent effect at different doses *i.e.* 300 mg/kg and 600 mg/kg when compared them with standard. There was increase in response with the increase of dose. A maximum latency time of 13 seconds was observed with the dose of 100 mg/kg; the maximum latency time of 22 seconds was produced by 300 mg/kg dose; the maximum latency time of 28 seconds was produced by 600 mg/kg when compared with the control (Fig. 1).

3.2.2 Tail immersion method

Tail immersion method was also used to determine the analgesic effect of plant extract. Maximum analgesic effect with aspirin was significantly ($p < 0.05$) increased at the end of 2.5 h of test followed by decline in reading upto 4.5 h. Similarly, different concentrations of AMGRS showed significant ($p < 0.05$) protected effect against heat when compared to control. AMGRS extract at the dose of 600 mg/kg showed significant ($p < 0.05$) analgesic effect in comparison to 100 mg/kg and 300 mg/kg dose. Extract of 100 mg/kg dose showed maximum response time of 1.6 seconds; 300 mg/kg dose showed maximum latency time of 6.2 seconds; 600 mg/kg dose showed maximum latency time of 7.8 seconds. Maximum response time was noted in case of 600 mg/kg dose of extract when compared to 100 mg/kg and 300 mg/kg dose (Fig. 2).

3.2.3 Acetic acid induced writhing method

Acetic acid induced method was also used to evaluate the intensity of pain in peripheral region. Results of the analgesic activity of MeOH extracts of different concentrations of AMGRS are given in Fig. 3. Aspirin (300 mg/kg) was used as a positive control. The positive control group showed highly significant ($p < 0.05$) results compared with control group. Different concentrations of extracts of AMGRS *i.e.* 300 mg/kg, 600 mg/kg demonstrated significant ($p < 0.05$) responses at various time intervals. During 1st phase (0-15 min), aspirin (300 mg/kg) produced 57% pain protection against chemically induced pain. Similarly, different doses of methanolic extract of *A. graecizans subsp. silvestris* *i.e.* 100 mg/kg, 300 mg/kg and 600 mg/kg showed 12%, 36% and 47% of pain protection during 1st phase (0-15 min), respectively. Maximum pain protection was noted in case of 600 mg/kg dose of extract in response to 100 mg/kg and 300 mg/kg dose.

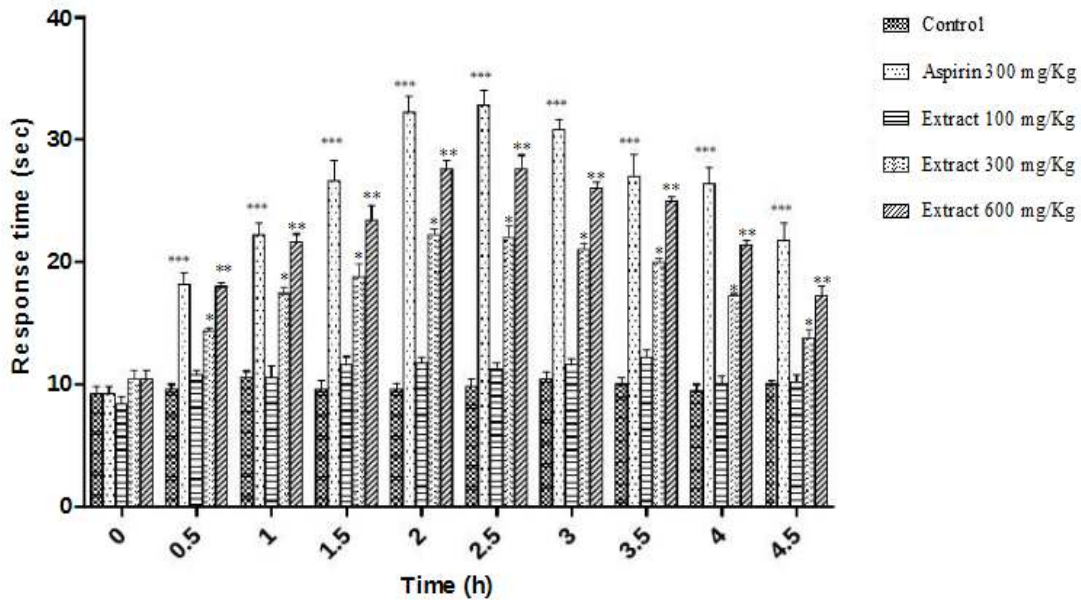


Fig. 1. Analgesic activity of the MeOH extract of AMGRS by Hotplate method at 100mg/kg, 300mg/kg and 600mg/kg doses. Aspirin was used as positive control. n=5. $p < 0.05^*$, 0.01^{**} and 0.001^{***} were considered statistically significant, very significant and highly significant respectively

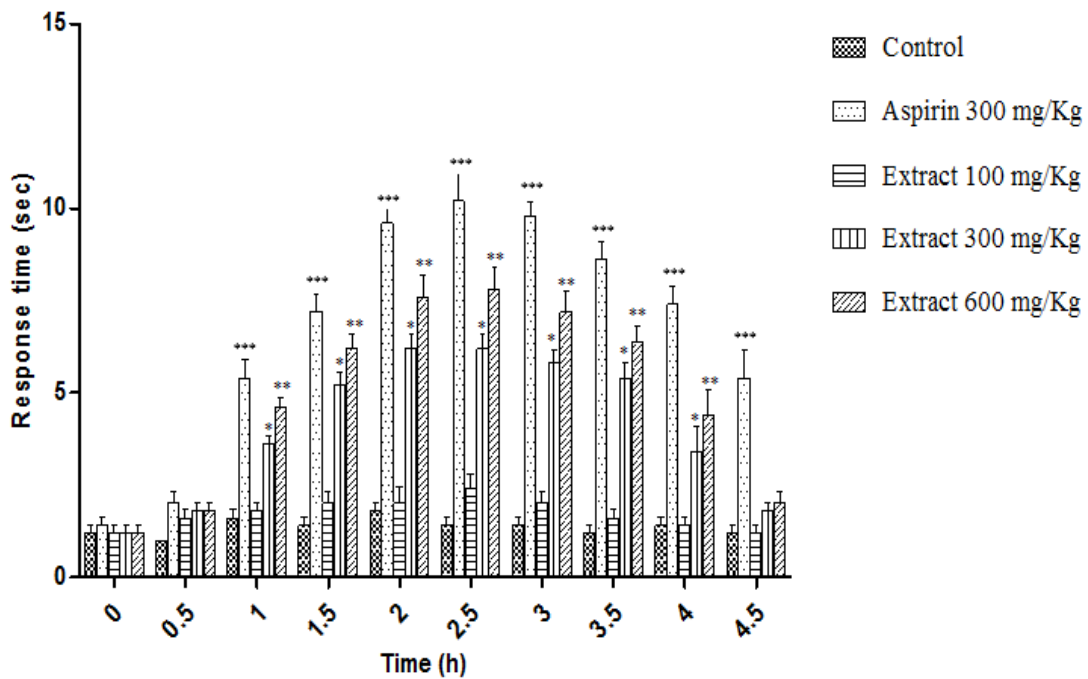


Fig. 2. Analgesic activity of the MeOH extract of AMGRS by tail immersion method at 100mg/kg, 300mg/kg and 600mg/kg doses. Aspirin was used as positive control. n=5. $p < 0.05^*$, 0.01^{**} and 0.001^{***} were considered statistically significant, very significant and highly significant respectively

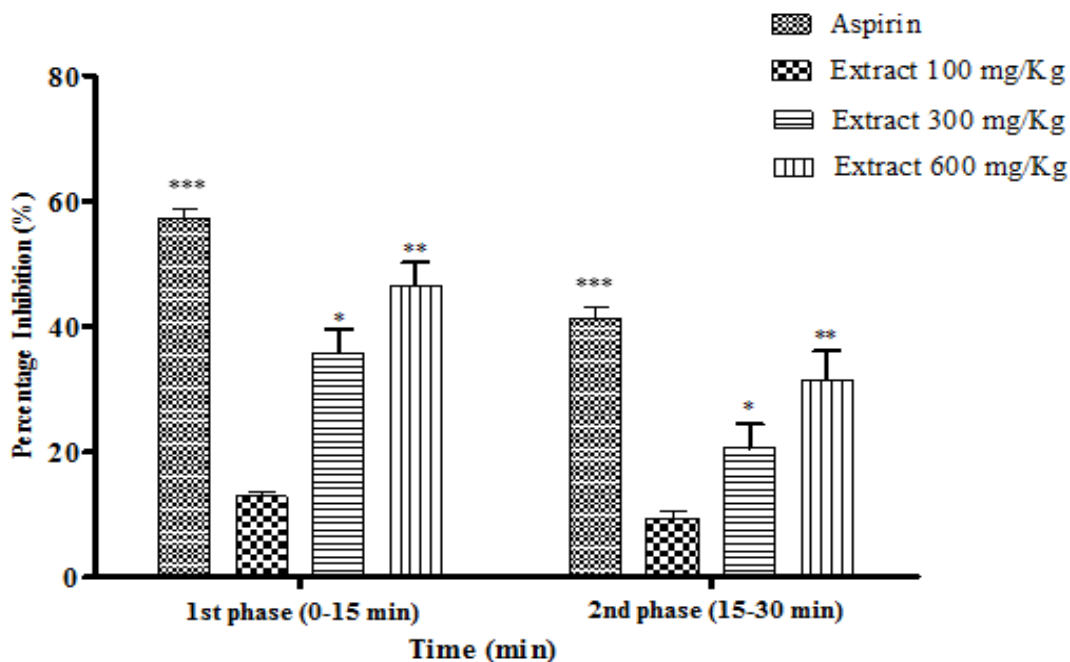


Fig. 3. Percentage (%) Pain protection of MeOH extract of AMGRS by using the acetic acid induced writhing method in mice. Aspirin was used as positive control. n=5. $p < 0.05^*$, 0.01^{**} and 0.001^{***} were considered statistically significant, very significant and highly significant respectively

3.3 Anti-inflammatory Activity

3.3.1 Carrageenan-induced rat paw edema method

Anti-inflammatory activity of MeOH extract of AMGRS was evaluated by using carrageenan induced rat paw edema method. Results of standard and different doses of methanolic extract AMGRS were statistically significant ($p < 0.05$) when compared with control. Aspirin showed significant ($p < 0.05$) reduction (47%) in edema when it was compared with control. Similarly, different concentrations (300mg/kg and 600 mg/kg) of AMGRS showed significant ($p < 0.05$) dose dependent reduction in edema in comparison to control. However, dose of 100 mg/kg did not produced significant effect. Extract of 100 mg/kg dose presented 08%; extract of 300 mg/kg dose showed 32% and extract of 600 mg/kg dose gave 42% of protection against chemically induced edema after 3 hours of test. Inhibition of rat paw edema was observed greater with 600 mg/kg dose in comparison with 100 mg/kg and 300 mg/kg doses and it was statistically significant ($p < 0.05$). Results were tabulated and presented graphically (Fig. 4).

3.4 Enzyme Inhibition Assays

3.4.1 Acetyl cholinesterase inhibition assay

In-vitro anticholinesterase activity was performed using the methanolic extract. PMSF was used as a reference drug. The MeOH extract of *A. graecizans* subsp. *silvestris* exhibited the percentage inhibition of 24.29 ± 0.57 .

3.4.2 Protease inhibition assay

The percentage protease inhibition assay of MeOH extract of *A. graecizans* subsp. *silvestris* was 60.52 ± 0.18 .

3.5 Thin Layer Chromatography (TLC)

The MeOH extract was subjected to TLC and various bands were observed (Fig. 5).

4. DISCUSSION

The MeOH extract of the AMGRS showed analgesic and anti-inflammatory effects in all dose. The activities was evaluated against thermally and chemically induced pain to explore

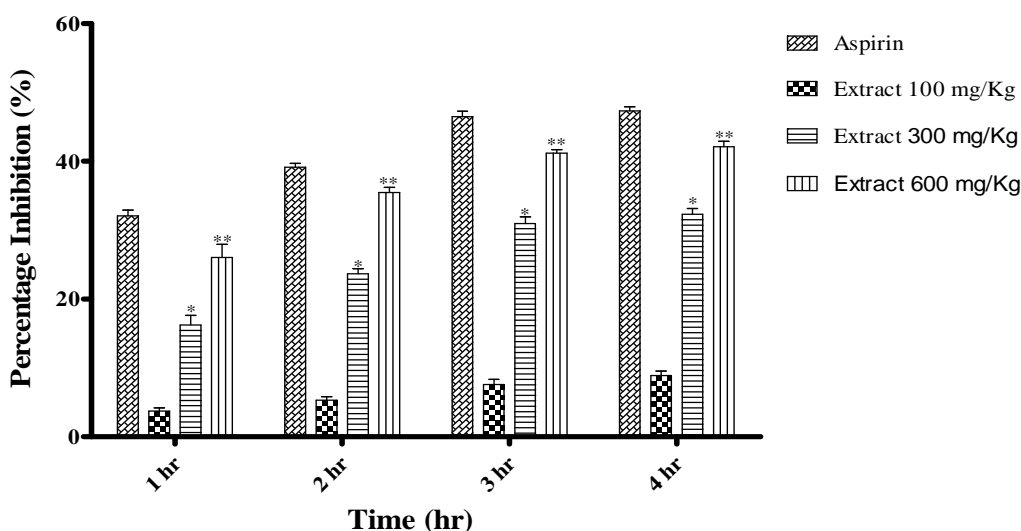


Fig. 4. Percentage (%) inhibition of MeOH extract of AMGRS on carrageenan induced rat paw edema method. Aspirin as positive control. $p < 0.05^*$, 0.01^{} and 0.001^{***} were considered statistically significant, very significant and highly significant respectively**

Table 1. Rf and hRf values of various components of methanolic extract of AMGRS with different mobile phases

Sr. no.	Mobile phase	Solvent front (cm)	Components distance (cm)	Visible light	UV- light	Rf	hRf (%)
1	Chloroform: Ethanol 95 : 05	4.7	0.4	Light brown	Blue	0.08	08
			1.5	No	Light pink	0.31	31
			1.9	No	Red	0.40	40
			2.1	Green	Pink	0.46	46
			2.3	No	Dark red	0.49	49
			2.7	No	Light purple	0.57	57
			3.4	No	Pink	0.72	72
			3.7	Green	Light green	0.79	79
			4.4	Green	Purple	0.93	93
			4.6	Light green		0.97	97

their central and peripheral pain inhibition potential. Hotplate method and tail immersion test models were used to investigate the centrally acting anti-nociceptive activity of the extract. MeOH extract of AMGRS at different dose levels *i.e.* 300 mg/kg and 600 mg/kg exhibited dose dependent analgesic effect in hot plate (Fig. 1) and tail immersion test (Fig. 2). It is possible that the central analgesic effect of the extract may be mediated by inhibition of prostaglandin and bradykinins synthesis.

Acetic acid induced writhing test is used to screen the peripheral analgesic effect of the tested substance. Acetic acid stimulates the

production of prostaglandins within peritoneum and activates local peritoneal pain receptors due to which abdominal constrictions (writhing) get started [18]. Since, the MeOH extract of AMGRS (300 mg/kg and 600 mg/kg) had significant analgesic effect in acetic acid induced writhing test (Fig. 3), it suggests that the extract has peripheral analgesic effect which may be achieved through inhibition of cyclooxygenase COX-synthesized prostaglandins.

Carrageenan-induced paw edema is well authenticated animal model to explore the anti-inflammatory activity of synthetic compounds as well as natural products. Paw edema induced by

carrageenan is consisted of two phases; the initial or first phase (1 h or 1.5 h) involves a non-phagocytic edema which is followed by a second phase or late phase (2 h – 5 h) with increased edema development that persisted up to 5 h [19]. During first phase, mediators such as histamine, bradykinin and serotonin are responsible for the development of edema due to their action on vascular permeability while prostaglandins involve in the progress of edema during second or late phase [20]. Results of anti-inflammatory activity of extracts AMGRS were appreciable in comparison with control group. In present study, the extracts of AMGRS (300 and 600 mg/kg) produced significant anti-inflammatory effect in both phases *i.e.* First and second phase. This effect might be due to the inhibition of synthesis of histamine and prostaglandins. It is also reported that the substance can decrease the volume of carrageenan induced paw edema by inhibiting cyclooxygenase [21].

tannins, carbohydrates, flavonoids, cardiac glycosides, sterols, lipids and alkaloids. Flavonoids are found to be very good inhibitors of prostaglandins which are responsible for late phase of inflammation and pain perception [22]. The analgesic and anti-inflammatory effects in current study can be attributed to the presence of flavonoids and tannins in the MeOH extract of AMGRS.

In-vitro enzyme inhibition assays (acetyl cholinesterase inhibition assay and proteases inhibition assay) were performed and results were calculated. Anticholinesterase activity is useful assay to screen the sample for its use in the treatment of Alzheimer's disease. Despite the unknown etiology of Alzheimer disease but is associated with impairment in cholinergic transmission. The elevation of acetylcholine amount through AChE enzyme inhibition has been accepted as the most effective treatment strategy against Alzheimer's disease. Therefore, large number of AChE inhibitors is used for symptomatic relief of Alzheimer disease. Similarly, proteolytic enzymes are necessary for the normal regulation of proteins, and any type of abnormality in the regulation of enzymes can cause initiation of different disorders for example in small intestine hydrolysis of protein peptide bond is catalyzed by trypsin [23].

Chemical characterization of MeOH extract of AMGRS was performed by TLC method using commercially available silica gel TLC plates. The MeOH extract was separated into about ten bands with the mobile phase chloroform and ethanol (95:05). Out of these ten bands five were visible in ordinary light and the rest of the five were seen when the chromatogram was observed under UV Lamp (Fig. 5). All of the separated phytoconstituents showed different colors under UV light, this revealed that the extract contains chemical components that are fluorescent in nature. The Rf values for all bands were calculated and it ranged from 0.08 to 0.97 as shown in Table 1.

5. CONCLUSION

The MeOH extract of AMGRS provides adequate and significant *in-vivo* analgesic and anti-inflammatory activity in the animal models, which is sufficient to justify the folklore use of this herb as anodyne. The results of enzyme inhibition assay for cholinesterase inhibitory effect ascertain its worth in the management of Alzheimer disease, while the protease inhibition

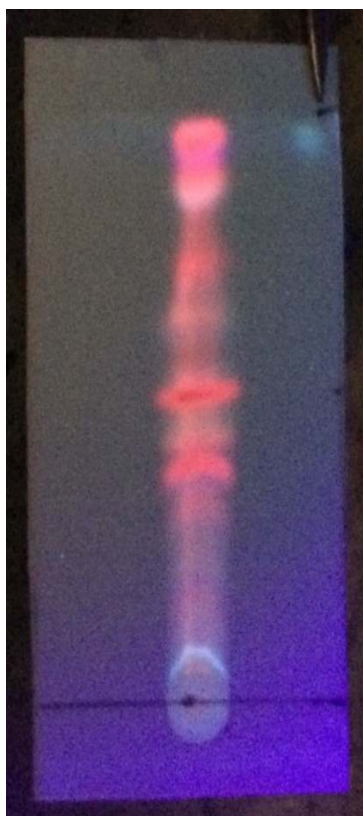


Fig. 5. Separation of phytochemicals on silica gel plate of MeOH extract of *A. graecizans* subsp. *silvestris* with mobile phase chloroform and ethanol (90:05)

Phytochemical analysis of MeOH extract of AMGRS exhibited the presence of saponins,

demonstrates its proteolytic effects. These therapeutic effects and folklore use of this herb can be associated with the presence of variety of phytoconstituents present in the extract. i.e. saponins, tannins, flavonoids, cardiac glycosides, sterols and alkaloids. The TLC quantification revealed the fluorescent components present in the extract.

CONSENT

It is not applicable.

ACKNOWLEDGEMENTS

The research project was funded by Punjab University College of Pharmacy, University of the Punjab, Lahore Pakistan. We are also thankful to Dr. Muhammad Ajaib for aiding in the collection and authentication of plant material.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Aburjai T, Hudaib M, Tayyem R, Yousef M, Qishawi M. Ethnopharmacological survey of medicinal herbs in Jordan, the Ajloun Heights region. *Journal of Ethnopharmacology*. 2007;110(2):294-304.
2. Al-Khalil S. A survey of plants used in Jordanian traditional medicine. *International Journal of Pharmacognosy*. 1995;33(4):317-323.
3. Müller K, Borsch T. Phylogenetics of amaranthaceae based on matK/trnK sequence data: Evidence from parsimony, likelihood, and Bayesian analyses. *Annals of the Missouri Botanical Garden*. 2005;66-102.
4. Stintzing FC, Kammerer D, Schieber A, Adama H, Nacoulma OG, Carle R. Betacyanins and phenolic compounds from *Amaranthus spinosus* L. and *Boerhavia erecta* L. *Zeitschrift für Naturforschung C*. 2004;59(1-2):1-8.
5. Mroczek A. Phytochemistry and bioactivity of triterpene saponins from Amaranthaceae family. *Phytochemistry Reviews*. 2015;14(4):577-605.
6. Rahmatullah M, Hosain M, Rahman S, Akter M, Rahman F, Rehana F, Kalpana MA. Antihyperglycemic and antinociceptive activity evaluation of methanolic extract of whole plant of *Amaranthus tricolor* L. (Amaranthaceae). *African Journal of Traditional, Complementary and Alternative Medicines*. 2013;10(5):408-411.
7. Achigan-Dako EG, Sogbohossou OE, Maundu P. Current knowledge on *Amaranthus* spp.: Research avenues for improved nutritional value and yield in leafy amaranths in sub-Saharan Africa. *Euphytica*. 2014;197(3):303-317.
8. Baral M, Datta A, Chakraborty S, Chakraborty P. Pharmacognostic studies on stem and leaves of *Amaranthus spinosus* linn; 2011.
9. Flora of Pakistan. Available: http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=250064248
10. Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry*. 2004; 85(2):231-237.
11. Ishtiaq S, Ahmad M, Hanif U, Akbar S, Kamran SH. Phytochemical and *in vitro* antioxidant evaluation of different fractions of *Amaranthus graecizans* subsp. silvestris (Vill.) Brenan. *Asian Pacific Journal of Tropical Medicine*. 2014;7:S342-S347.
12. Saleem U, Ahmad B, Ahmad M, Hussain K, Bukhari NI. Anti-nociceptive, anti-inflammatory and anti-pyretic activities of latex and leaves methanol extract of *Euphorbia helioscopia*. *Asian Pacific Journal of Tropical Disease*. 2015;5(4): 322-328.
13. Gupta AK, Parasar D, Sagar A, Choudhary V, Chopra BS, Garg R, Khatri N. Analgesic and anti-inflammatory properties of gelsolin in acetic acid induced writhing, tail immersion and carrageenan induced paw edema in mice. *Plos One*. 2015;10(8): e0135558.
14. Xu Q, Wang Y, Guo S, Shen Z, Wang Y, Yang L. Anti-inflammatory and analgesic activity of aqueous extract of *Flos populi*. *Journal of Ethnopharmacology*. 2014; 152(3):540-545.
15. Kakoti BB, Pradhan P, Borah S, Mahato K, Kumar M. Analgesic and anti-inflammatory activities of the methanolic stem bark extract of *Nyctanthes arbor-tristis* linn. *BioMed Research International*. 2013;6. (Article ID 826295).
16. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and

- rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*. 1961;7(2):88-95.
17. Jedinák A, Mučková M, Košťálová D, Maliar T, Mašterová I. Antiprotease and antimetastatic activity of ursolic acid isolated from *Salvia officinalis*. *Zeitschrift für Naturforschung C*. 2006;61(11-12):777-782.
 18. Muhammad N, Saeed M, Khan H. Antipyretic, analgesic and anti-inflammatory activity of *Viola betonicifolia* whole plant. *BMC Complementary and Alternative Medicine*. 2012;12(1):1.
 19. Mbiantcha M, Kamanyi A, Teponno RB, Taponjou AL, Watcho P, Nguenefack TB. Analgesic and anti-inflammatory properties of extracts from the bulbils of *Dioscorea bulbifera* L. var *sativa* (Dioscoreaceae) in mice and rats. *Evidence-Based Complementary and Alternative Medicine*; 2011.
 20. Maity TK, Mandal SC, Mukherjee PK, Saha K, Das J, Pal M, Saha BP. Studies on antiinflammatory effect of *Cassia tora* leaf extract (fam. Leguminosae). *Phytotherapy Research*. 1998;12(3):221-223.
 21. Bukhari IA, Khan RA, Gilani AH, Ahmed S, Saeed SA. Analgesic, anti-inflammatory and anti-platelet activities of the methanolic extract of *Acacia modesta* leaves. *Inflammopharmacology*. 2010;18(4):187-196.
 22. Chakraborty ARKB, Devi RK, Rita S, Sharatchandra KH, Singh TI. Preliminary studies on antiinflammatory and analgesic activities of *Spilanthes acmella* in experimental animal models. *Indian Journal of Pharmacology*. 2004;36(3):148.
 23. Shahwar D, Raza MA, Tariq S, Riasat M, Ajaib M. Enzyme inhibition, antioxidant and antibacterial potential of vasicine isolated from *Adhatoda vasica* Nees. *Pak. J. Pharm. Sci*. 2012;25(3):651-656.

© 2017 Ishtiaq et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://sciedomain.org/review-history/18402>