Academic Journal of Life Sciences
ISSN(e): 2415-2137, ISSN(p): 2415-5217
Vol. 6, Issue. 8, pp: 101-107, 2020
URL: https://arpgweb.com/journal/journal/18
DOI: https://doi.org/10.32861/ajls.68.101.107

Academic Research Publishing Group

Original Research **Open Access**

In Vivo and *in Vitro* Anti-Inflammatory Activities of Extracts of *Pandiaka angustifolia* (Vahl.) Hepper (Amaranthaceae) Used in Traditional Medicine in Burkina Faso

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Article History
Received: September 5, 2020
Revised: September 26, 2020
Accepted: October 4, 2020
Published: October 7, 2020

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Abstract

Background: *Pandiaka Angustifolia* Valh Hepper (Amaranthaceae) whole plant is used in folk Burkinabe's medicine to treat ailments with an inflammatory component. Previous studies revealed the antioxidant capacity, xanthine oxidase, and lipoxygenase inhibitory activities of the plant, but to the best of our knowledge, its anti-inflammatory activities were not reported before. Therefore, this study was designed to evaluate the anti-inflammatory and analgesic activity of *P. Angustifolia* hexane and aqueous extracts using *in vitro* enzymatic methods and *in vivo* methods and verify the best anti-inflammatory extract implication in KATP pathways. **Experiments:** acute toxicity of the plant was conducted under OECD 423 guidelines. Phospholipase and cyclooxygenases were pro-inflammatory enzymes used to evaluate in *vitro* anti-inflammatory effects of plant extracts while carrageenan induced edema method was used to evaluate the anti-edematous activity and acetic acid inducing writhing method to evaluate the non-morphine analgesic effect of herbal mixture. ATP sensitive K^+ channel assay was performed *in vivo* using the glibenclamide as ATP-sensitive potassium channel (K_{ATP}) blocker. **Results:** enzymatic inhibition assays revealed that both hexane and aqueous extracts of *P. angustifolia* were good inhibitors against sPLA2 activity with IC_{50} values of 14.23 ± 0. 72 µg/mL and 11.56 ± 0.11 µg/mL, respectively. Aqueous extract presented the best inhibition for COX-1 (IC_{50} = 24.76 ±0. 51 µg/mL) while hexane extract concentration that inhibit 50% of COX-2 was lesser than those of aqueous extract. *P. angustifolia* aqueous extract orally administrated to NMRI mice caused no death at the dose of 3000 mg/kg b.w indicating that the plant toxicity is low. While hexane extract was unable to reduce Carrageenan-induced edema, ethanolic extract were significantly active when extract was orally administrated. Non-morphine analgesic activity evaluation revealed that ethanolic extract was more efficient on writhing reduction than hexane extract. Nociception effect of the plant is linked with its effects on K^+ ATP sensitive channels. **Conclusion:** Results indicate that the anti-inflammatory potential of *P. angustifolia* may be due to its polar phytoconstituents and observed pharmacological activities provide the scientific basis for the medicinal use of the plant in the treatment of ailment associated with inflammation.

Keywords: *Pandiaka angustifolia*; Enzymatic activity; Anti-inflammatory activity; Analgesic activity.

1. Introduction

Human beings are constantly exposed to noxious and pathogens, that are responsible of different ailments [1, 2]. Inflammation, a biological response of vascularized tissues to injuries (infections and damaged tissues) is characterized by increased blood flow to the tissue causing increased temperature, redness, swelling and pain. It is considered as a secret killer and has been linked with diseases like asthma, heart attacks, cancers, Alzheimer's and other diseases [3].

The inflammatory reaction is a succession of coordinated biological events that aims to circumcise and eliminate pathogens and noxious that injure every single component of the body [4]. It implicates several mediators and effectors sourced from inflammatory enzymatic activation [5]. Phospholipase ($sPAL_2$) and cyclooxygenases (COX-1 and COX-2) are among the majors inflammatory enzymes that generate respectively arachidonic acid (A.A.) through sPAL2 activation and prostaglandins (PGs) generated through COXs activity [6]. Mediators of inflammation like PGs are implicated in the apparition of cardinal signs of inflammation and they have a direct link with the opening of

K^+ channels particularly in peripheral nervous hyperexcitability [7]. Blockade of these channels opening is one of the solutions used to soothe chronic pain [8].

Conventionally non-steroidal anti-inflammatory drugs (NSAIDs), steroidal anti-inflammatory drugs (SAIDs) and therapy based on monoclonal antibodies are used to treat inflammatory diseases [9]. Unfortunately, their frequent use lead to unwanted sides effects like digestive iatrogenic and kidney pathologies. Most of them are known to dysregulate the lipids, proteins and carbohydrates metabolism. Likewise, they do not allow complete health recovery, but simply attenuate the symptoms in many cases [9, 10].

Medicinal plants which are known and were used before the implementation of conventional medicine constitute therefore the new field of investigation for new molecules with more efficiency and less side effects. Most of the medicinal plants already investigated for their anti-inflammatory potential were identified based on their ethnomedicinal uses. *P. angustifolia* Valh Hepper (*P. angustifolia*), a plant belonging to Amaranthaceae botanical family is used in folk Burkinabe medicine for the formulation of invigorating beverage for parturients, as a spasmolytic, in the treatment of gonorrhea, adnexitis, in the treatment of inflammation of the female genital tract and in the treatment of malaria [11].

This study aimed to contribute to a better knowledge of the biological potential of *P. angustifolia*, by evaluating *in vitro* its ability to inhibit phospholipase ($sPAL_2$) and cyclooxygenases (COX-1 and COX-2), enzymes involved in the inflammatory process and *in vivo* to evaluate anti-edematous and analgesic effects and to verify extracts implication in K_{ATP} pathway.

2. Materials and Methods

2.1. Plant Material

The whole plant of *P. angustifolia* was harvested in January 2016 from natural habitat at Yaagma catholic sanctuary (30 km northern periphery of Ouagadougou, Burkina Faso). Botanical identity was assessed in the laboratory of biology and vegetal ecology (University Joseph KI-ZERBO, Ouagadougou, Burkina Faso) and a voucher specimen (CI: 16889) was deposited. Plant material was dried at room temperature, pulverized and defatted with petroleum ether in the proportion 1:10 (w/v) and residual marc were conserved for future uses.

2.2. Chemicals

Chemical were from analytical grade. Different solvents, acetylsalicylic acid, carrageenan, dimethylsulfoxide (DMSO), hydrocortisone, quercetin, acetaminophen, acetic acid, sodium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid, Carrageenan and acetylsalicylic acid were supplied by Sanofi Winthrop Industry (France). 1,2-diheptanoilthio-glycerophosphocholine (1,2dHGPC), secretory phospholipase A2 (sPLA2) from bee venom and 5,5′- dithiobis-2- nitrobenzoic acid (DTNB) were obtained from Cayman Chemical Co. (MI, USA). For the colorimetric inhibition of COX-1 and human COX-2, Screening Kit (Item No. 560131) manufactured by Cayman Chemical, USA was used.

2.3. Experimental Animals and Housing

Naval medical research institute (NMRI) female mice (7 to 8 weeks old, 25 to 35 g body weight) provided by the animal housing facility of the University Joseph KI-ZERBO, were used. Mice were kept in an environmentally controlled breeding room (20-25 °C, humidity, 12 h photoperiod), fed with standard laboratory food and water *ad libitum*. Mice were fasted 17 h before experiments. Animals were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Burkina Faso (Ethics committee acceptance CE-UOI-2018-05) and all procedures were in compliance with the European Council Directive of 24 November 1986 (86/609/EEC) [12]. All evaluations were performed between 9 a.m. and 16 p.m.

3. Experimental Methods

3.1. Preparation and Extraction of Plant Material

The extraction consisted of steeping a mass 50 g of defatted plant material in 500 mL of hexane. After mechanical stirring for 48 hours, the mixture was filtered using Whatman N°1 filter paper and concentrated in a rotary evaporator (Buchi Rotavapor RE-3, Buchi Labortecknic AG, Switzerland) at an approximative temperature of 35 °C, concentrated filtrate was stored at 4°C until further use, while the residual marc was reused after a drying period of 8 h for a new extraction using water as solvent.

3.2. *In vitro* Enzymatic Inhibition

3.2.1. Phospholipase A2 ($sPLA_2$) Inhibition Assay

$sPLA_2$ inhibition activity was assayed using the method described by D'Almeida, *et al.* [13], with slight modifications. Briefly, test samples (enzyme activity in presence of an inhibitor) were prepared after mixing 50 µL of buffer Tris-HCl (10 mM, pH 8) with 10 µL of enzyme $sPAL_2$ (1µg/mL) and extracts of plant dissolved in DMSO was used at different concentration to determine the concentration that inhibit 50 % of enzyme activity, while positive control sample (enzyme activity without inhibitor) was prepared using the same reagent except plant extract which was replaced by hexane or water . The reaction was initiated by the addition of 150 µl of 1,2-diheptanoylthio-glycerophosphocholine (1,2-dHGPC) (1.66 Mm). After 15 min of incubation in a 96 wells microplate, 10 µL of DTNB (10 mM) were added then the mixture absorbance was recorded using a microplate reader (Epoch, BioTeck

instruments, USA) at 405 nm. 1,2-diheptanoylthio-glycerophosphocholine (1,2-dHGPC) and Triton X-100 were used as substrates, their reconstitutions were achieved to a final concentration of 1.25 mM using buffer Tris–HCl (10 mM pH 8) with $CaCl_2$ (10 mM), KCl (100 mM) and Triton X-100 (0.3 mM). Different tests were realized in triplicate and the percentage of $sPAL_2$ inhibition was calculated using the following formula:

$$\textbf{\% of inhibition} = \frac{\textbf{ATS-ACS}}{\textit{ACS}} \textbf{x100}$$

ATS: Absorbance in the presence of tested sample (inhibitor)
ACS: Absorbance without inhibitor

3.2.2. Cyclooxygenase Inhibition Assay

The inhibition of COXs was performed using a commercially available colorimetric COX (ovine) inhibitor screening assay kit (Cayman Chemical Company, New York, NY, USA). All the inhibitors were dissolved in an appropriate solvent. The COX activity was evaluated using N, N, N', N'-tetramethyl-phenylenediamine (TMPD) as a co-substrate, with arachidonic acid as described by the manufacturer. The TMPD oxidation was monitored spectrophotometrically at 590 nm. Different concentrations of extracts were used to determine the concentration that inhibit 50 % of the enzyme activity.

3.3. *In Vivo* Assays

3.3.1. Acute Toxicity

The acute toxicity study was conducted under OECD 423 guidelines [14] with slight modifications. The mice were randomized into three groups and each group contained six animals. The 1th group (Control) received saline solution (NaCl 0.9%), the 2nd and the 3rd group received *P. angustifolia* aqueous extract dose of (2400 mg/kg b.w.) and (3000 mg/kg b.w.) respectively. After extract administration, the animals were observed continuously for the first 4 hours to detect eventual behavioral changes. Then, they were observed periodically for 72 hours for any mortality. Animals were maintained and weighed during the next two weeks. At the end of the study, mice were sacrificed by cerebral dislocation and organs (liver, spleen, kidney, lung, heart and brain) were removed, weighed and observed for possible morphological changes.

3.3.2. Anti-Edematous Activity

Anti-edematous activity was carried out according to the method described by Winter and Risley [15], with slight modifications as described by Emmanuel, *et al.* [16]. Briefly six batches of six mice were randomly used. The extracts of *P. angustifolia* was administrated *per os* at different doses 200,400 and 600 mg/kg b.w. to four test groups, 1 h before the injection of 50 µL of carrageenan (1% in NaCl 0.9 %) under the aponeurosis plantar of the hind paw at each mouse. Control group received only vehicle solution (physiological saline solution), while reference group received acetylsalicylic acid at the dose of 100 mg/kg b.w. Each treated paw volume was measured 1 h before the carrageenan injection and at time intervals of 1 h, 3 h and 5 h after the carrageenan injection using a plethysmometer (model Ugo Basil n 7141, Italy). The anti-edematous activity was evaluated as a percentage of reduction of the edema in treated mice compared to negative control using the following formula:

$$\textbf{inhibition \%} = \frac{\textbf{A - B}}{\textbf{A}} \textbf{x 100}$$

A represents the average difference paw edema volume in the negative control group and **B** represents the average difference of paw edema in treated group mice.

3.3.3. Analgesic Activity

The analgesic effect of the extracts was evaluated according to the number of abdominal contortions induced by the intraperitoneal injection of acetic acid (0.6%) as described by Noufou [17]. Briefly, lots of seven were randomly constituted. The negative control group received distilled water while the reference group, acetaminophen (paracetamol) a dose of 200 mg / kg and the other lots received the aqueous (AEpa) and hexane extracts (HEpa) of the whole plant of *P. angustifolia* at doses ranging from 100, 200, 400 to 600 mg / kg. One hour after extracts or reference administration, the animals intraperitoneally received acetic acid 0.6% at the dose of 10 mL / kg. Five minutes after the injection of acetic acid, the number of contortions was counted in each mouse for 15 min. The analgesic effect was evaluated according to the following formula:

$$\textbf{Inhibition \%} = \frac{\textbf{Nb - Nt}}{\textbf{Nb}} \textbf{x 100}$$

Nb is the average of the number of contortions of the mice of the blank control group and Nt is the average of the number of contorted mice of the batch treated.

3.3.4. Non-morphine Analgesic Activity: Involvement of ATP-Sensitive K+ Channel Pathway

The non-morphine analgesic activity involving the K^+ channels was evaluated using the procedure described by Perimal [18], with slight modifications. Four groups of 7 mice were divided as follows: the control group receives only the vehicle (saline), the second group receives glibenclamide (an ATP sensitive K+ channel inhibitor) and the last two groups receive respectively the hexane and aqueous extracts at a dose of 400 mg / kg of body weight. All mice except the first were pretreated with glibenclamide (10 mg / kg) 15 min before administration of saline or both extracts. Mice were injected with acetic acid, 1 hour after treatment. Five minutes after the acetic acid injection the

number of contortions was recorded for 15 min. The analgesic effect was evaluated according to the following formula

$$\text{Inhibition \%} = \frac{Nb - Nt}{Nb} x\ 100$$

3.4. Statistical Analysis

The statistical analysis was performed with GraphPad Prism 5.03 for Windows (Graph Pad Software, Inc., California USA), using One-way of variance (ANOVA). All results were expressed as the mean ± S.E.M. Dunnett's test for comparisons with control group were used for analysis of differences between tested samples/groups and controls. Differences were considered significant when the p value was less than 0.05 ($p < 0.05$).

4. Results and Discussion

4.1. Acute Toxicity Effects of *P. Angustifolia*

The mice body weight and relative organ weight were significantly changed (Table I). In the acute toxicity assessment, no animal deaths were observed, no changes in the appearance of internal organs both in groups receiving the extract at the dose of 2400 mg/kg b.w. and of 3000 mg/kg b.w. as compared to control group. However, about 30 min after administration, all mice in the two extract treated groups presented behavioral changes, they were prostate, and presented limit interest in feeding. About 45 minutes after drugs administration animals regained their usual behaves. Therefore, the LD_{50} of aqueous extract is greater than 3000 mg/kg b.w. in mice based on OCDE 425/2008 guideline [19]. Hence, the results suggest that the extract is safe.

Extract effects on organs revealed that liver, lung, heart and brain were not affected by drug administration over the testing period. Their weight was statistically the same in treated group as in the control one. Therefore, the spleen and the kidney weight were found reduced in the tested group. Changes in the weight of internal organs were considered as an indicator of chemical exposure. Spleen play a capital role in immunity while kidney is the main blood filter very susceptible to toxicants because high volume of blood flows through it and it filter large amounts of toxins which can concentrate in its tubules. Results observed may indicate that in the aqueous extract of *P. angustifolia* exist compounds that affect their functions

4.2. *In Vitro* Pro Inflammatory Enzyme Inhibition by Extracts of *P. Angustifolia Secretory Phospholipase A2 (sPAL2) and COX-1 and COX-2 Inhibitor Activity Assay*

Phospholipases are family of enzyme responsible for hydrolyzing the *sn*-2 fatty acids of membrane phospholipids generating free fatty acids and lysophospholipids [6]. COXs and LOX further catalyze the metabolism of free arachidonic acids in different eicosanoids that are powerful mediators in the inflammatory process [20]. Numerous studies have established the physiopathology of many diseases such as cerebral illnesses [21], cardiovascular disorders [22], cancers [23], asthma, respiratory distress syndrome [24] and progression of tonsillitis disease [25] in the elevation of inflammatory enzymes levels. Thus, the activity reduction of the sPLA2 and COXs is a beneficial process towards the limitation of the inflammation process.

The IC_{50} results obtained for sPLA2 inhibitor activity assay and COX-1 and COX-2 assay are expressed in Table 2. Both hexane and aqueous extracts of *P. angustifolia* showed significant inhibition against sPLA2 with respectively IC_{50} values of 14.23 ± 0. 72 µg/mL and 11.56 ± 0.11 µg/mL. Their activities were respectively 4.56 and 5.62 times greater than the activity of acetylsalicylic acid used as control. In the case of cyclooxygenases inhibition, the results obtained revealed that the aqueous extract (IC_{50}=34.76 ± 0. 51) was more inhibitory of COX-1 than the hexane extract. This was no longer the case for the inhibition of isoform 2 of cyclooxygenase where hexane extract was 3 time more inhibitor than aqueous extract. Our previous study on the phytochemistry of the plant revealed that the *P. angustifolia* content a large amount of phenolic compounds. Pharmacological effect of the plant may be due to the presence of phenolic compounds and compounds like in polar extracts.

4.3. *In Vivo* Anti-Edematous and Analgesic Effects of *P. Angustifolia*

4.3.1. Anti-Edematous Activity

The effects of aqueous extract on carrageenan induced -paw edema in mice have been shown in table 3. The different doses administrated orally inhibited significantly the edema induced after carrageenan injection. Oral administration of plant extract reduced edema rate dose dependently. The paw edema induced by carrageenan is an experiment animal model used to evaluate the acute anti-inflammatory activity of natural substances and drugs. The carrageenan injection provokes the activation of mast cells and the release of chemical mediators [26]. Mediators release after inflammation induction initiation occurs in three successive phases; first phase lasted for 1 hour that mediated by histamine, serotonin and the second phase was due to release of bradykinin during 2-3 hours; prostaglandins were produced during the last phase (4-6 h) [27]. The aqueous extract inhibited preponderantly the inflammatory response during the fist and the third phases of edema. At different doses, the oral administration of aqueous extract caused an important inhibition of paw edema induced by carrageenan injection. The maximal inhibition was noted at fifth hour after the oral administration (78.37% at 600 mg/kg). It suggests that the oral administration could more inhibit mediators of inflammation produced during the first phase (histamine and serotonin) and the prostaglandins biosynthesis during the last phase.

4.3.2. Analgesic Activity

The writhing induced by acetic acid injection is an experimental animal model used to evaluate the analgesic potential of pharmacological substances like NSAIDs. Substance P, histamine, serotonin and prostaglandins are among the mediators stimulating the nociceptive neurons after the injection of acetic acid in mice [28]. Results obtained showed that the oral administration of aqueous extract of *P. angustifolia* was able to inhibit significantly the writhing induced by acetic acid. Elevation of administrative doses causes less writhing inhibition indicating that the drug analgesic action was dose-dependent. The analgesic effect of aqueous extract of *P. angustifolia* may be due to the inhibition of the release of cytokines and pro-inflammatory mediators such as prostaglandins. Writhing number counted after the oral administration of the extract were reported in Table 4. Paracetamol, the reference product caused an inhibition more important than the aqueous extract at the same dose

4.3.3. Contribution of K_{ATP} Channel Pathway to the Analgesic Effect of Aqueous Extract

The alteration in peripheral analgesic effect of 400 mg/kg aqueous extract with pre-treatment of K_{ATP} channel blocker glibenclamide at the dose of 10 mg/kg (*i.p*) is demonstrated in figure 1. Glibenclamide did not affect the number of writhes in acetic acid-induced writhing test when administrated alone. Glibenclamide pre-treatment followed by aqueous extract significantly ($p<0.05$) reversed the decrease in the number of writhing induced by aqueous extract of *P. angustifolia*. Regarding the observed results, we can conclude to the possibility that aqueous extract of *P. angustifolia* may have peripheral analgesic effects because of its capacity to reduce the amount of substances that trigger nociception or their activities after the acid acetic injection.

5. Conclusion

The outcomes of this study reveal that aqueous extract of *P. angustifolia* has an anti-inflammatory potential that may be explained by its ability to inhibit pro-inflammatory enzymes phospholipase and cyclooxygenase. Anti-edematous activity of the plant and its analgesic activity may be due to its ability to inhibit the production of inflammatory mediators like histamine, serotine and prostaglandins, additionally, the analgesic effect of the extract may be due to the activation of K_{ATP} channels involved in nociception.

In the light of these findings and considering that *Pandiaka angustifolia* is a natural source of pharmacological actives compounds whose toxicity is low, could be use after the identification and withdrawal of compounds implicated in spleen and kidney possible disfunction in the management of inflammation. Results credit the medicinal use of this plant, but also reveal it possible effect on spleen and kidney. Further investigations will be initiate to identify and isolate pharmacological active compounds of this species.

Figure-1. The effect of 10 mg/kg glibenclamide pre-treatment on the analgesia induced by 400 mg/kg of aqueous extract of *P. angustifolia* in acetic acid writhing test. AEpa: Aqueous extract of *P. angustifolia*, GB, Glibenclamide***$p<0.001$; significant and differences based on the control group, & $p<0.05$; significant differences based on 400 mg/kg aqueous extract of *P. angustifolia*. One-way analysis of variance (ANOVA) followed by Tukey's HSD multiple comparison test was performed. Values expressed as mean ± S.E.M. (n = 8)

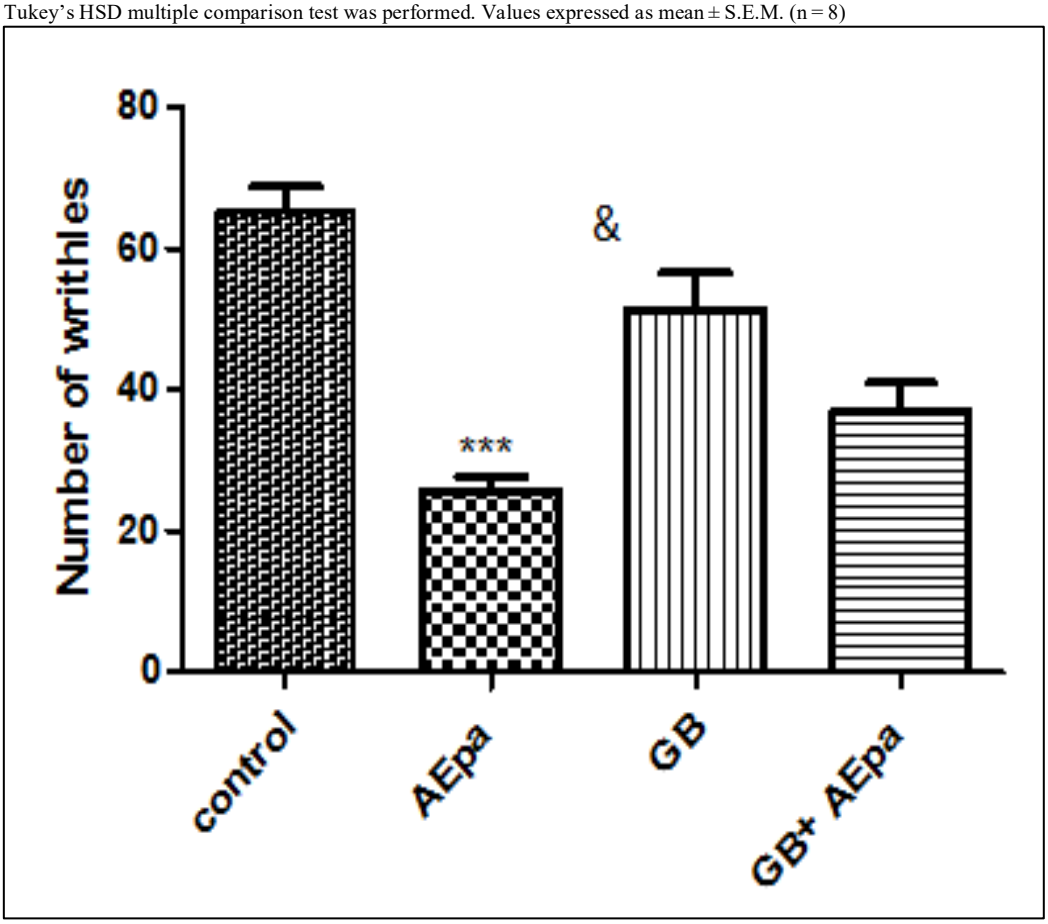

Table-1. Weekly weight (g) and relative organs weight (%) after two weeks

		Aqueous extract doses.		
Parameters		**2400 mg/kg b. w.**	**3000 mg/kg b. w.**	**Control group**
Body weight	1st day	25.78 ± 0.34	26.51 ± 0.37	29.10 ± 0.21
	7th day	29.33 ± 0.28	30.81 ± 0.41	30.98 ± 0.34
	14th day	29.67 ± 0.19	32.02 ± 0.11	33.17 ± 0.10
Organs weight	Liver	4.27 ± 0.26	4.42 ± 0.21	4.70 ± 0.09
	Spleen	0.38 ± 0.08	0.36 ± 0.07	0.44 ± 0.07
	Kidney	0.92 ± 0.05	0.84 ± 0.09	1.09 ± 0.05
	Lung	0.72 ± 0.09	0.71 ± 0.09	0.73 ± 0.07
	Heart	0.45 ± 0.02	0.47 ± 0.05	0.42 ± 0.03
	Brain	1.2 ± 0.13	1.25 ± 0.07	1.22 ± 0.06

Table-2. IC_{50} results of sPLA2, COX-1 and COX-2 by hexane and aqueous extracts of *P. angustifolia*

Samples	**sPLA2 IC_{50} (µg/mL)**	**COX-1 IC_{50} (µg/mL)**	**COX-2 IC_{50} (µg/mL)**
Hexane extract	14.23 ± 0.72^{c}	85.9 ± 1.01^{d}	61.59 ± 2.23^{b}
Aqueous extract	11.56 ± 0.11^{b}	34.76 ± 0.51^{c}	185.41 ± 1.41^{c}
Quercetin	0.95 ± 0.04^{a}	$15.8\ 9 \pm 2.27^{b}$	9.23 ± 0.42^{a}
indomethacin	nd	0.597 ± 0.17^{a}	8.80 ± 0.92^{a}
Acetylsalicylic acid	65 ± 1^{d}	nd	nd

Results are expressed as the mean values. Values showing the same letter are not significantly different ($p<0.05$) from another in the same column. nd: not determined

Table-3. Effect of administration of aqueous extract of *P. angustifolia* on carrageenan induced hind paw edema

Samples	**Doses (mg/kg b.w)**	**Increase in paw volume (mL)**			**Edema inhibition (%)**		
		1 h	**3 h**	**5 h**	**1 h**	**3 h**	**5 h**
control	--	0.26 ± 0.03	0.31 ± 0.04	0.37 ± 0.05	--	--	--
AEpa	200	0.19 ±0.05*	0.22 ± 0.04**	0.15 ± 0.04**	26.92	29.03	59.45
	400	0.14 ± 0.03**	0.17 ± 0.03**	0.12 ± 0.03**	45.15	45.16	67.56
	600	0.10 ± 0.06**	0.12 ±0.06**	0.08 ± 0.03**	61.53	61.29	78.37
Hdc	10	0.21 ± 0.03*	0.29 ± 0.04	0.25 ± 0.03**	20.83	17.65	32.43

Data are expressed as mean ± SEM (n= 3), AEpa; Aqueous extract of *P. angustifolia*, Hdc, hydrocortisone. ** p 0.001 significant from control (one-way ANOVA analysis followed by Dunnett' test).

Table-4. Effect of administration of aqueous extract on writhing induced by acetic acid (mean ±SEM, n =6)

Samples	**Doses (mg/kg b.w.)**	**Numbers of writhing**
Control	-	68.43 ± 2.00
Aqueous extract	100	49.24 ± 1.93**
	200	34.22 ±.2.02**
	400	26.19 ± 2.35**
Paracetamol	100	37.33 ±1.77**

** p 0.001 significant from control (one-way ANOVA analysis followed by Dunnett' test).

Conflict of Interest Statement

We declare that we have no conflict of interest.

Acknowledgement

Authors are grateful to APPEAR 75, TWAS 12-044 RG/BIO/AF/AC_G and IFS F/5374-1 projects for providing laboratory facilities that have made this work possible.

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