

Sains Malaysiana 48(3)(2019): 629–635  
<http://dx.doi.org/10.17576/jsm-2019-4803-16>

## *In vivo* Analgesic, Antipyretic and Anti-inflammatory Activities of Ethanol Extract of *Pericampylus glaucus* in Experimental Animals

(Aktiviti Analgesik, Antipiretik dan Anti-Radang secara *in vivo* Ekstrak Etanol daripada *Pericampylus glaucus* pada Haiwan Uji Kaji)

MUHAMMAD KIFAYATULLAH\*, HAROON RAHIM, NAEEM ULLAH JAN,  
KAMRAN AHMAD CHISHTI, IRFAN ULLAH & SUDAIR ABBAS

### ABSTRACT

*Pericampylus glaucus* is a common Malaysian plant used traditionally in the treatments of joint pain, abdominal pain and headache. Hence, the present research was aimed to evaluate ethanolic extract of *Pericampylus glaucus* for analgesic, antipyretic and anti-inflammatory activities in experimental animals. The central and peripheral analgesic activity was determined by acetic acid induced writhing and hot plate method by examining the number of writhing and paw licking or jumping time. Meanwhile, the antipyretic activity was determined by Brewer's yeast- test that induced pyrexia and carrageenan- that induced hind paw inflammation was used for anti-inflammatory activities. The ethanolic extract of *Pericampylus glaucus* at doses (300 and 600 mg/kg b.wt) and Ibuprofen (100 mg/kg (b.wt) was used as a reference drug in the whole experiment. Intraperitoneal administration of *Pericampylus glaucus* produced significant ( $p < 0.01$ ) inhibition in writhing response in acetic acid induced writhing test and dose-dependent ( $p < 0.001$ ) prolonged paw licking in hot plate test as compared to control (normal saline treated group). Similarly, significant ( $p < 0.001$ ) attenuation in lowering rectal temperature was noted in animal groups that were treated with ethanolic extract of *Pericampylus glaucus* at different doses. The attenuation was, almost the same as produced by ibuprofen treated group. Furthermore, *Pericampylus glaucus* extract also produced significant reduction in hind paw edema ( $p < 0.001$ ), 4 h after administration of carrageenan and inhibition was 60.19% and 42.17% as compared to control (normal saline treated group). The findings of this study indicated that *Pericampylus glaucus* possess significant analgesic, antipyretic and anti-inflammatory activities and could possibly be used in the management of fever, pain and inflammation.

**Keywords:** Analgesic; anti-inflammatory; antipyretic; ethanolic extract; *Pericampylus glaucus*

### ABSTRAK

*Pericampylus glaucus* adalah tumbuhan yang biasa digunakan secara tradisi dalam rawatan sakit sendi, sakit badan dan sakit kepala. Oleh yang demikian, penyelidikan ini bertujuan untuk menilai ekstrak etanol *Pericampylus glaucus* untuk aktiviti analgesik, antipiretik dan anti-radang pada haiwan uji kaji. Aktiviti analgesik pusat dan periferal ditentukan oleh asid asetik bergelut teraruh dan kaedah plat panas dengan memeriksa kadar bergelut dan menjilat tapak kaki atau masa lompatan. Manakala aktiviti antipiretik ditentukan melalui ujian yis Brewer yang mengaruh pireksia dan karaginan - yang mengaruh inflamasi tapak kaki belakang digunakan untuk aktiviti antiradang. Ekstrak etanol *Pericampylus glaucus* pada dos (300 dan 600 mg/kg b.wt) dan ibuprofen (100 mg/kg b.wt) telah digunakan sebagai dadah rujukan pada uji kaji keseluruhan. Pemberian intraperitoneum *Pericampylus glaucus* menghasilkan perencatan tindak balas bergelut yang signifikan ( $p < 0.01$ ) dalam ujian bergelut asid asetik dan berkeperluan dos ( $p < 0.001$ ) menjilat tapak kaki berpanjangan dalam ujian plat panas berbanding kawalan (kumpulan terawat salin normal). Begitu juga, signifikan ( $p < 0.001$ ) pelemahan hidrologi merendahkan suhu rektum telah dicatat dalam kelompok haiwan yang telah dirawat dengan ekstrak ethanolic *Pericampylus glaucus* pada dos yang berbeza. Pengcilaan ini hampir sama seperti yang dihasilkan oleh kelompok yang dirawat menggunakan Ibuprofen. Selain itu, ekstrak *Pericampylus glaucus* juga menghasilkan pengurangan ketara dalam edema di simpul telapak belakang ( $p < 0.001$ ), 4 jam selepas pemberian carrageenan dan kesan 60.19 dan 42.17% berbanding kawalan (kelompok salina normal terawat). Keputusan kajian ini menunjukkan bahawa *Pericampylus glaucus* mempunyai nilai analgesik, antipiretik dan anti-radang yang penting dan mungkin boleh digunakan untuk merawat demam, sakit dan inflamasi.

**Kata kunci:** Analgesik; anti-radang; antipiretik; ekstrak etanol; *Pericampylus glaucus*

## INTRODUCTION

Controlling pain, fever and inflammation is one of the largest challenges for clinical medicines (Ricciotti & Fitz Gerald 2011). The unpleasant experience, associated with perception of sensation that disrupts a person's life is termed as pain (Ferrell et al. 1991). Inflammatory mediators released in body due to stimulation of mast cell that activate nociceptors (receptor producing pain) and result pain in body (Dray 1995). Furthermore, G protein-coupled receptors are also involved in the development of oedema, inflammation, fever and pain (Ricciotti & Fitz 2011). Pyrexia is a medical sign primarily associated with increase in body that is then characterised by fatigue, depression, lethargy and anorexia (Kifayatullah & Waheed 2014). Pro-inflammatory mediators (cytokines) produced by damaging tissues, which enhance the production of prostaglandin E2 (PGE2) (Spacer 1994). Inflammation is a normal response of mammalian tissue in body against invading microorganisms. However, during this response blood cells and plasmic fluid accumulates in the body that leads to edema (Munford & Pugin 2001). The inflammatory response involves a multigrade initiation of enzymes, release of mediators, leakage of fluid, tissue breakdown and regeneration (Rock & Kono 2008). The common therapy for controlling fever, pain and inflammation are use of NSAIDs. However, a number of side effects of NSAIDs have been documented with their uses in human being (Hernández-Díaz & Rodriguez 2000). In order to avoid the side effects natural of product-based new antipyretic, analgesic and anti-inflammatory agents have been endeavoured. *Pericampylus glaucus* belongs to the family of Menispermaceae, found in Southeast Asian region and is claimed to be effective as traditional medicine for treating various diseases (Kifayatullah et al. 2016). Traditionally, roots of *Pericampylus glaucus* have been used to lower glucose level, fever and cholesterol level (Ong et al. 2011). Various parts of the plant have been harnessed elsewhere worldwide to stop-bleeding, treat snake bites, swelling, sore throat, joint pain, headache and abdominal pain (Muhammad et al. 2016). In addition, pure compounds such as alkaloids and terpenoids isolated from the plant have been reported for having significant potentials against HBV, cancer and HIV-1 virus (Yan et al. 2008; Zhao & Cui 2009). The ethanolic extract of *Pericampylus glaucus* possessed radical scavenger effect *in-vitro* (Kifayatullah et al. 2015a), besides anti-diabetic and anti-hyperlipidemic effects (Kifayatullah & Sengupta 2016). The active compound 2-[(trimethylsilyl)oxy]-Methyl Ester (cas) Methyl-o-trimethyl-silylsalicylate from plant has been proven for having hypoglycaemic effects (Kifayatullah et al. 2017). However, to the best of our knowledge, literature review showed that there is no published data until now was reported for the plant regarding the effectiveness of *Pericampylus glaucus* in the treatment of inflammation, fever and pain. Based on this evidenced, this study was therefore aimed to validate the analgesic, antipyretic and anti-inflammatory activities of *Pericampylus glaucus* in animal models.

## MATERIALS AND METHODS

## DRUGS, CHEMICALS AND INSTRUMENTS

The standard Ibuprofen of Sigma-Aldrich, was purchased from Bio Syn Tech Malaysia Group. Rotary evaporator model: (N-1000 (EYELA Tokyo Co., LTD.), Japan, Digital thermometer (Holden medical B.V, Netherland) and Hot plate (AREC, VELP Scientific) were used. The other chemicals and reagents used were of analytical grade of Astral Laboratory chemicals and Sigma-Aldrich Co St. Louis, MO 63178 USA. Carrageenan's and Brewer's yeast used were purchased from the local market.

## COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

The plant, *Pericampylus glaucus*, was collected from Kampung Jeram, Kedah, Negeri Sembilan, Malaysia and authenticated by Ms Tan Ai Lee from the Forest Research Institute Malaysia (FRIM). It was then assigned with herbarium number (FRIM/394/490/5/18(118)). The leaves were washed and dried in shade for a period of three weeks and converted into coarse powder using mechanical grinder.

## PREPARATION OF PLANT EXTRACT

An amount of 100 g of coarse powder was extracted by continuous hot extraction method using ethanol as solvent in the soxhlet apparatus for a period of 48 h. The collected extract was then concentrated under reduced pressure in rotary evaporator at 40-45°C and preserved in desiccator for pharmacological activities.

## PRELIMINARY PHYTOCHEMICAL SCREENING

Previous research from literature review confirmed the presence of various phytochemicals in ethanol extract of *Pericampylus glaucus* except resin and anthraquinone by using Mayer's, Froth, Fehling, Lead acetate, Acetone water and Bontrager's test (Kifayatullah et al. 2015a), before determination of analgesic, antipyretic and anti-inflammatory activities.

## EXPERIMENTAL ANIMALS

Male and female Balb/c mice with average weight of 25-30 g were used in this study and kept in animal holding facility centre of Lincoln University College, Malaysia. The study that involved the use of animal was performed in fulfilment with protocols and the guidelines that were approved by the animal ethics committee of Lincoln University College, Malaysia with Ref No: PHARM/2016/MK/02-Aug/11/Nov 2016-Dec 2016.

## ORAL ACUTE TOXICITY STUDY

OECD 423 guidelines were used for confirming the safety and toxicity of ethanol extract up to 4000 mg/kg (b.wt) in female Balb/c mice (Kifayatullah et al. 2015b). Hence, two

doses of extract 300 and 600 mg/kg (b.wt) were chosen in the study and diluted in normal saline before use.

#### PHARMACOLOGICAL TESTS ANALGESIC ACTIVITY

The peripheral analgesic effects of ethanol extract of *Pericampylus glaucus* against acetic acid induced writhing was performed according to the procedure of (Kifayatullah) with some modification (Kifayatullah & Ishrat 2014). The Balb/c mice were randomly distributed into 4 groups of 5 animals per each cage. Group 1 served as normal control that received only normal saline at dosage of 10 mL/kg (b.wt). Group 2 was designated as a positive (standard), received the Ibuprofen at dose of 100 mg/kg (b.wt) intraperitoneally (i.p) while group 3 group and 4 were given ethanolic extract at dose 300 and 600 mg/kg (b.wt), respectively, and were designated as test group. For induction of pain 1 h after treatment, a volume of 0.2 mL of 0.6% v/v of acetic acid solution was injected intraperitoneally to all animals group and the total number of writhing was counted for duration of 20 min just 05 min after intraperitoneal (i.p) administration of acetic acid solution. The number of writhing in all treated group was compared to normal group (untreated group). The % of inhibition of writhing for all treated group was determined from the mean writhing count of normal control group by following equation (Kifayatullah & Ishrat 2014).

$$\% \text{ age of inhibition} = \frac{N_c - N_t}{N_c} \times 100$$

where  $N_c$  is the average number of writhing of normal control (normal saline) per group; and  $N_t$  is the average number of writhing of test per group (extract and standard). The same procedure of grouping was applied for determining the central analgesic activity of ethanol extract of *Pericampylus glaucus* by placing each animal on hot metal plate maintained at 55°C to measure the latency time (Kifayatullah & Ishrat 2014). Group 1 served as normal control received only normal saline at dosage of 10 mL/kg (b.wt). Group 2 was kept as positive control (standard) treated with Ibuprofen at dose of 100 mg/kg (b.wt). Group 3 and 4 were designated as test groups for ethanolic extract, were treated i.p at dose rate of 300 and 600 mg/kg (b.wt), respectively. The time between placement and shaking or licking of the paws or jumping was recorded as the index of response latency. The response of latency for each animal group used in this study was noted down in hot plate for 3 h and the interval was thirty min followed with cut off time 15 s in order to prevent damage of tissue.

#### ANTIPYRETIC ACTIVITY

The antipyretic effect of ethanol extract of *Pericampylus glaucus* was evaluated based on Brewer's yeast induced pyrexia test (Kifayatullah & Waheed 2014). Healthy Balb/c mice of both sexes with average weight (25-30 g) were used in this study that was randomized into 4 groups of 5

animals each. Digital thermometer was used to record the rectal body temperature. Subcutaneous injection of yeast (20% w/v) diluted in normal saline was used for induction of pyrexia. The rectal temperature was recorded 17 h after subcutaneous (s/c) injection. Group 1 served as a normal control, received only normal saline at dose 10 mL/kg (b.wt), group 2 was kept as positive (standard), treated with yeast and Ibuprofen at 100 mg/kg (b.wt) intraperitoneally (i.p). Group 3 and 4 were administered intraperitoneal (i.p) with yeast and extract at 300 and 600 mg/kg (b.wt), respectively. The rectal temperatures of each group were recorded at 0, 1, 2, 3 and 4 h after administration of ethanolic extract and Ibuprofen.

#### ANTI-INFLAMMATORY ACTIVITY

Carrageenan-induced inflammation on the hind paw method was used to evaluate the anti-inflammatory action of ethanolic extract of *Pericampylus glaucus* that was explained earlier with some changes in procedure (Brahmbhatt et al. 2010). Balb/c mice (male and female) after an overnight fasting except free excess to water was distributed into 4 groups of 5 animals per cage. All the animals were injected with 0.1 mL of freshly prepared 1% (w/v) carrageenan on the sub plantar right hind paw of each mouse for induction of oedema. Group 1 served as normal control. Normal saline at dose of 10 mL/kg (b.wt) was given to 1 group and served as normal control. Group 2 was treated with Ibuprofen at dosage of 100 mg/kg (b.wt) and served as positive control. Group 3 and 4 were treated i.p with ethanolic extract of *Pericampylus glaucus* at dosage of 300 and 600 mg/kg (b.wt), respectively. Treatments with Ibuprofen and extract were performed 60 min before administration of carrageenan. Increase in paw thickness was measured with vernier caliper just before and after 1, 2, 3, and 4 h of carrageenan injection. The percentage of inhibition was calculated with respect to its vehicle- treated group (normal saline) by formula:

$$\text{Inhibition (\%)} = \frac{\text{Means oedema of control} - \text{means oedema of sample}}{\text{Mean paw oedema of control}} \times 100$$

#### STATISTICAL ANALYSIS

Values were expressed as mean  $\pm$  Standard Error of Means (SEM). Values were statistically analysed by two-way analysis of variance (ANOVA) followed by Bonferroni test. Probability ( $p$ ) levels of  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  were considered statistically significant.

## RESULTS

#### PRELIMINARY PHYTOCHEMICAL SCREENING

The presence of various phytochemicals in the ethanol extract of *Pericampylus glaucus* showed the existence of saponins, alkaloids, reducing sugars, flavonoids, tannins,

sterol, terpenoids except anthraquinones and resin. The results are summarized in Table 1.

TABLE 1. Phytochemical analysis of ethanolic extract of *Pericampylus glaucus*

Phytochemical Test performed Result		
Alkaloids	Mayer's	Present
Tannins	Lead acetate	Present
Saponins	Froth	Present
Flavonoid	Alkaline reagent	Present
Anthraquinone	Liebermann	Absent
Sterol	Fehling Acetone	Present
Resin	Acetone	Absent

#### ANALGESIC ACTIVITY

The effects of ethanol extract of *Pericampylus glaucus* and Ibuprofen on acetic acid induced writhing in Balb/c mice are summarized in Table 2. The result indicated that mice pre-treated with 300 and 600 mg/kg (b.wt) of extract produced significant reduction in writhing ( $p < 0.001$ ) as compared to normal control. The inhibition percentage was 30.28 % and 55.67% for ethanol extract at 300 and 600 mg/kg (b.wt), respectively. The positive control group treated with Ibuprofen at 100 mg/kg (b.wt) produced more potent inhibition (71.02%,  $p < 0.001$ ) in writhing response than ethanolic extract.

TABLE 2. Analgesic effects of *Pericampylus glaucus* and Ibuprofen in acetic acid-induced writhing in Balb C mice

Treatment	Count/min	Inhibition of writhing (%)
Normal Control	42.10±1.11	71.02
Ibuprofen 100 mg/kg (b.wt)	12.20 ±0.36 <sup>c</sup>	30.28
300 mg/kg (b.wt)	29.35 ±0.71 <sup>b</sup>	55.67
600 mg/kg (b.wt)	18.66 ±0.80 <sup>c</sup>	

Data are expressed as mean ± SEM,  $n=5$ , <sup>b</sup> $p < 0.01$ , <sup>c</sup> $p < 0.001$  significant in relative to control

Treatment with ethanolic extract of *Pericampylus glaucus* at 300 and 600 mg/kg (b.wt) significantly ( $p < 0.001$ ) prolonged the latency time as compared to negative control (normal saline treated group). The significant effects began as early as 30 min and last for 150

min. The increase in latency time was dose-dependent and the maximum effect was found at 150 min with latency time for 300 mg/kg (b.wt) was 38.95±0.01 while for 600 mg/kg (b.wt), the latency time was 44.70±1.34. After 150 min, the latency time began to decline for both dosages of ethanolic extract. The latency time for Ibuprofen was also the highest at 150 min (53.66±0.18) and then declined. The results are summarized in Table 3.

#### ANTIPYRETIC ACTIVITY

Treatment of Balb/c mice with ethanol extract displayed dose-dependent antipyretic effect against brewer's yeast induced pyrexia was shown by reduction in rectal temperature. The initial average rectal temperature of animals group was 34.7°C. The ethanol extract caused lowering of the body temperature up to 4 h following its administration. A significant incremental change in rectal temperature was noted after subcutaneous (s/c) injection of yeast peaking at about 17 h that was 38.10°C that was further decrease to 37.0°C 1 h after treatment with ethanolic extract at 600 mg/kg (b.wt) and subside after 4 h showing a sizeable reduction in rectal temperature that was almost same as showed by Ibuprofen. The ethanolic extract at 300 mg/kg (b.wt) produced attenuation in fever but was not statistically ( $p > 0.05$ ) significant up to 2 h and showed slight significant ( $p < 0.05$ ) after 3 h that become  $p < 0.01$  after 4 h of treatment as compared to control group (normal saline). Treatment with Ibuprofen at dose rate 100 mg/kg (b.wt) significantly ( $p < 0.001$ ) reduced rectal temperature that was induced by yeast 3 h after administration. Attenuation in rectal temperature after treatment with ethanolic extract at 300 and 600 mg/kg (b.wt) was nearly equally potent to Ibuprofen. The results are tabulated in Table 4.

#### ANTI-INFLAMMATORY ACTIVITY

Intraperitoneal administration of ethanol extract of *Pericampylus glaucus* produced significant dose-dependent reduction in hind paw edema induced by injection of carrageenan. After 3 and 4 h of carrageenan induction, ethanolic extract at 300 and 600 mg/kg (b.wt) showed significant anti-inflammatory activity ( $p < 0.001$ ) as compared to control. The carrageenan-induced paw edema significantly reduced by 54.36% and 60.19%, respectively, at 4 h of treatment as compared to that of control group. Inhibition of edema by Ibuprofen at 100

TABLE 3. Analgesic effects of ethanolic *Pericampylus glaucus* extract of in Eddy Hot plate mouse model

Treatment	0 min	30 min	60 min	90 min	120 min	150 min	180 min
Control (Normal saline)	8.1±1.1	7.1±1.2	9.1±0.01	8.3±1.0	9.5±1.8	10.70±0.8	7.5±1.3
Ibuprofen 100 mg/kg (b.wt)	9.2 ±1.1	24.4±1.39	30.2±2.2	37.9±1.2	46.0±1.7	53.6±0.1 <sup>c</sup>	49.7±1.4
300 mg/kg (b.wt)	10.2±0.9	17.0±1.7 <sup>a</sup>	23.3±1.2 <sup>b</sup>	28.3±1.4 <sup>b</sup>	34.5±1.0 <sup>c</sup>	38.9±0.01 <sup>c</sup>	33.2±0.1 <sup>c</sup>
600 mg/kg (b.wt)	10.6±2.0	21.3±1.8 <sup>b</sup>	29.3±1.2 <sup>b</sup>	32.1±1.9 <sup>c</sup>	37.2±1.5 <sup>c</sup>	44.7±1.3 <sup>c</sup>	39.3±1.5 <sup>c</sup>

Data are expressed as mean ± SEM,  $n=05$ , <sup>a</sup> $p < 0.05$ , <sup>b</sup> $p < 0.01$ , <sup>c</sup> $p < 0.001$ , significant as matched to normal control



TABLE 4. Antipyretic activity of *Pericampylus glaucus* by Brewer's yeast induced pyrexia

Treatment	Rectal temperature in °C after 17 h of s/c injection of Brewer's yeast						
	Normal	After 17 h	0 h	1 h	2 h	3 h	4 h
Normal	34.7±0.1	37.9±0.1	37.7±0.1	37.6±0.1	37.4±0.1	37.2±0.1	37.1±0.4
Ibuprofen 100 mg/kg (b.wt)	35.7±0.2	37.9±0.1	37.9±0.4	37.5±0.1	36.6±0.7	36.2±0.36	35.4 ±0.2 <sup>c</sup>
300 mg/kg (b.wt)	35.5±0.2	37.8±0.9	37.6±0.5	37.2±0.7	36.9±0.3	36.6±0.145 <sup>a</sup>	36.3±0.4 <sup>b</sup>
600 mg/kg (b.wt)	35.8±0.8	38.1±0.6	37.7±0.7	37.3±0.0	36.5±0.9	36.3±0.7 <sup>c</sup>	35.7±0.6 <sup>c</sup>

Values are expressed as mean ± SEM, n=5, <sup>a</sup>p <0.05, <sup>b</sup>p <0.01, <sup>c</sup>p <0.001, significant as matched to control

mg/kg (b.wt) was 73%. The results are summarised in Table 5.

#### DISCUSSION

The present research aimed to investigate analgesic, antipyretic and anti-inflammatory effects of ethanol extract of *Pericampylus glaucus* in mice. Preliminary phytochemical screening on *Pericampylus glaucus* showed the presence of alkaloids, tannins, saponins, flavonoid except anthraquinone and resin. Preliminary oral acute toxicity assessment of the ethanolic leaf extract *Pericampylus glaucus* in female Balb/c mice showed no mortality after 14 days of examination suggesting that the lethal dose (LD 50) of the extract is above 4000 mg/kg (b.wt). The ethanol extract of *Pericampylus glaucus* leaves was evaluated for analgesic effect against acetic acid-induced visceral pain and further supported by Eddy hot-plate test. Pain sensation was generated by intraperitoneal injection of acetic acid that caused contraction of abdominal muscle followed by extension of hind limbs and elongation of body part, which is mediated by local peritoneal receptor (Subedi et al. 2016). The acetic acid-induced writhing is a common technique used for screening of peripheral analgesic activity for plant-based drugs (Zulfiker et al. 2010). Administration of acetic acid causes release of endogenous substances that are supposed to excite the nerve endings and then results in pain (Subedi et al. 2016). The major contribution of prostaglandins to evoke pain response is mainly due to interaction with endogenous mediators that are responsible to stimulate sensitization of pain receptors (Gold & Gebhart 2010). The ethanol extract of *Pericampylus glaucus* at different doses was able to reduce writhing response significantly and this was possibly

mediated through prostaglandins inhibition mechanism. Prolonged reaction time of jumping with ethanol extract of *Pericampylus glaucus* was observed during all experimental observations indicating that extract might be acting spinally that might be explored additionally. However, it has been confirmed from previous studies that activation of  $\mu$ 2 opioid subtype receptor caused the formation of spinal analgesia (Bhowmick et al. 2014). Hence, it could be assumed that the central analgesic effects of *Pericampylus glaucus* extract is possibly to be mediated centrally. It is suggested that the ethanol extract exhibited central analgesic effects as proven by findings in the Eddy hot-plate method. Proteins present in Brewer's yeast are mainly linked to the developments of fever due to production of inflammatory cytokines in inflammatory reactions (Horai et al. 1998). Antipyretics such as Ibuprofen used in the management of fever decrease the levels of prostaglandins hence antipyretic messages in the brain (Aronoff & Neilson 2001). Giving similar results observed between the use of Ibuprofen and ethanolic extract, it is therefore, believed that antipyretic properties of ethanol extract is mostly mediated through interference of prostaglandin synthesis and inhibition of cytokines release. Various studies confirmed that plant-based drugs showing analgesic potentials also possess antipyretic effect in addition to anti-inflammatory activities (Kifayatullah et al. 2017), which are manifested by suppression of fever, pain and inflammation as a result of inhibition of inflammatory mediators. The plant-based biological benefits such as analgesic, antipyretic and anti-inflammatory potentials are mediated by secondary metabolites. Carrageenan-induced hind paw edema model is widely used to evaluate anti-inflammatory and anti-oedema effects of plant-based compounds. Development of edema after injection of carrageenan

TABLE 5. Effect of ethanolic extract of *Pericampylus glaucus* on inhibition of edema induced by carrageenan

Treatment	Inhibition (%)			
	1 h	2 h	3 h	4 h
Normal	0	0	0	0
Ibuprofen 100 mg/kg (b.wt)	28.43%	47.61%	62.5%	73%
300 mg/kg (b.wt)	12.75%	29.52%	34%	54.36%
600 mg/kg (b.wt)	8.82%	31.42%	44.64%	60.19%

occurs in two stages involving cyclooxygenases that last for 6 h (Halici et al. 2007). The first phase occurred within the first 2 h that is related to the release of bradykinin, serotonin and histamine by inflamed tissues whereas the second phase is mainly caused by the release of free radicals and prostaglandin by neutrophils that take place 3-5 h post injection of carrageenan (Ben Khedir et al. 2016). Reduction of hind paw oedema in a dose-dependent manner suggests that ethanol extract may inhibit prostaglandin formation. In brain, cyclooxygenase (COX)-2 is responsible to mediate prostaglandin synthesis that causes peripheral inflammation which then contributes to hyperalgesia and allodynia. In mast cells, prostaglandin D2, prostaglandin F2 $\alpha$  and prostaglandin E2 are metabolites of cyclooxygenase pathway that increase vascular permeability and vasodilatation hence edema (Hétu & Riendeau 2005). The inhibitory effect of ethanol extract on carrageenan-induced inflammation may therefore be due to the inhibition of cyclooxygenase and prostaglandin synthesis. Natural compounds such as flavonoids inhibit synthesis of prostaglandins in the late phase of inflammation (Rathee et al. 2009). Thus, the presence of flavonoids in the extract might suppress the formation of prostaglandins and bradykinin that contribute to anti-inflammatory effects.

#### CONCLUSION

The present study confirmed the analgesic, antipyretic and anti-inflammatory activities of ethanolic extract of *Pericampylus glaucus* used by local community against pain, fever and inflammation. However, further research is needed to establish the mechanisms involved in these effects and also to isolate active natural compounds responsible for the mentioned activities.

#### ACKNOWLEDGEMENTS

The authors are thankful to the Department of Pharmacy, Faculty of Life Sciences, Sarhad University of Science and Information Technology, Peshawar for providing financial support for publishing this research.

#### REFERENCES

- Aronoff, D.M. & Neilson, E.G. 2001. Antipyretics: Mechanisms of action and clinical use in fever suppression. *American Journal of Medicine* 4: 304-315.
- Ben Khedir, B.S., Mzid, M., Bardaa, S., Moalla, D., Sahnoun, Z. & Rebai, T. 2016. *In vivo* evaluation of the anti-inflammatory effect of *Pistacia lentiscus* fruit oil and its effects on oxidative stress. *Evidence-Based Complementary and Alternative Medicine* 2016: 6108203.
- Brahmbhatt, M., Patel, J., Patel, V. & Saluja, S. 2010. Analgesic and anti-inflammatory activity of leaves of *Rivea hypocrateriformis*. *Journal of Pharmacognosy and Phytotherapy* 1: 001-003.
- Bhowmick, R., Sarwar, M.S., RahmanDewan, S.M., Das, A., Das, B., NasirUddin, M.M. & Islam, M.S. 2014. *In vivo* analgesic, antipyretic, and anti-inflammatory potential in Swiss albino mice and *in vitro* thrombolytic activity of hydroalcoholic extract from *Litsea glutinosa* leaves. *Biological Research* 47: 47-56.
- Dray, A. 1995. Inflammatory mediators of pain. *British Journal of Anaesthesia* 75: 125-131.
- Ferrell, B., Grant, M., Padilla, G., Vemuri, S. & Rhiner, M. 1991. The experience of pain and perceptions of quality of life: Validation of a conceptual model. *American Heart Hospital Journal* 3: 9-24.
- Gold, M.S. & Gebhart, G.F. 2010. Nociceptor sensitization in pain pathogenesis. *Nature Medicine* 11: 1248-1257.
- Halici, Z., Dengiz, G.O., Odabasoglu, F., Suleyman, H., Cadirci, E. & Halici, M. 2007. Amiodarone has anti-inflammatory and anti-oxidative properties: An experimental study in rats with carrageenan-induced paw edema. *European Journal of Pharmaceutical Sciences* 566: 215-221.
- Hernández-Díaz, S. & Rodríguez, L.A.G. 2000. Association between nonsteroidal anti-inflammatory drugs and upper gastrointestinal tract bleeding/perforation: An overview of epidemiologic studies published in the 1990s. *JAMA Internal Medicine* 14: 2093-2099.
- Hétu, P.O. & Riendeau, D. 2005. Cyclo-oxygenase-2 contributes to constitutive prostanoid production in rat kidney and brain. *Biochemical Journal* 3: 561-566.
- Horai, R., Asano, M., Sudo, K., Kanuka, H., Suzuki, M. & Nishihara. 1998. Production of mice deficient in genes for interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , IL-1 $\alpha/\beta$ , and IL-1 receptor antagonist shows that IL-1 $\beta$  is crucial in turpentine-induced fever development and glucocorticoid secretion. *Journal of Experimental Medicine* 9: 1463-1475.
- Kifayatullah, M. & Sengupta, P. 2016. Effect of *Pericampylus glaucus* on plasma glucose concentration and lipid profile in streptozotocin-induced diabetic rats. *Bangladesh Journal of Pharmacology* 11: 200-205.
- Kifayatullah, M. & Ishrat, W. 2014. Evaluation of hydroethanolic extract of *Opuntia monacantha* Haw. for analgesic activity. *World Journal of Pharmaceutical Sciences* 2: 1006-1020.
- Kifayatullah, M. & Waheed, I. 2014. Evaluation of hydroethanolic extract of *Opuntia monacantha* Haw cladodes for antipyretic activity. *World Journal of Pharmaceutical Sciences* 3: 1021-1030.
- Kifayatullah, M., Mustapha, M.S., Sarker, M.M.R. & Amin, M. 2017. Effect of compounds identified in the active fraction of *Pericampylus glaucus* on blood glucose and lipid profiles in streptozotocin-induced diabetic rats. *Egyptian Pharmaceutical Journal* 16: 8-15.
- Kifayatullah, M., Sarker, M.M.R. & Mustapha, M.S. 2016. Phytochemical investigation of ethanolic extract of *Pericampylus glaucus* leaves from Malaysia by GC-MS analytical technique. *International Journal PharmTech Research* 12: 410-416.
- Kifayatullah, M., Sengupta, P., Mustafa, M.S., Das, S.K. & Sisugoswami, M. 2015a. Evaluation of ethanolic extract of *Pericampylus glaucus* (Lamk.) Merr for total phenolic, total flavonoids contents and *in vitro* anti-oxidant activity. *International Journal of Pharmacognosy and Phytochemical Research* 7: 677-683.
- Kifayatullah, M., Mustafa, M.S., Sengupta, P., Sarker, M.M.R., Das, A. & Das, S.K. 2015b. Evaluation of the acute and sub-acute toxicity of the ethanolic extract of *Pericampylus glaucus* (Lam.) Merr. in BALB/c mice. *Journal of Acute Disease* 4: 309-315.

- Muhammad, K., Mohd, S., Muhammad, A., Sardar, H. & Izhar, U. 2016. A review on Malaysian medicinal plants having anti-hyperglycemic activity. *Journal of Coastal Life Medicine* 4: 898-901.
- Munford, R.S. & Pugin, J. 2001. Normal responses to injury prevent systemic inflammation and can be immunosuppressive. *Am. J. Respir. Crit. Care Med.* 2: 316-321.
- Ong, H., Chua, S. & Milow, P. 2011. Ethno-medicinal plants used by the Temuan villagers in Kampung Jeram Kedah, Negeri Sembilan, Malaysia. *Studies on Ethno-Medicine* 5: 95-100.
- Rathee, P., Chaudhary, H., Rathee, S., Rathee, D., Kumar, V. & Kohli, K. 2009. Mechanism of action of flavonoids as anti-inflammatory agents: A review. *Current Drug Targets-Inflammation & Allergy* 8: 229-235.
- Ricciotti, E. & FitzGerald, G.A. 2011. Prostaglandins and inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology* 5: 986-1000.
- Rock, K.L. & Kono, H. 2008. The inflammatory response to cell death. *Annual Review of Pathology: Mechanisms of Disease* 3: 99-126.
- Spacer, C.B. & Breder, C.D. 1994. The neurologic basics of fever. *New England Journal of Medicine* 330: 1880-1886.
- Subedi, N.K., Rahman, S. & Akbar, M.A. 2016. Analgesic and antipyretic activities of methanol extract and its fraction from the root of *Schoenoplectus grossus*. *Evidence-Based Complementary and Alternative Medicine* 12: 1-8.
- Yan, M.H., Cheng, P., Jiang, Z.Y., Ma, Y.B., Zhang, X.M., Zhang, F.X., Yang, L.M., Zheng, Y.T. & Chen, J.J. 2008. Periglaucines A-D, anti-HBV and-HIV-1 alkaloids from *Pericampylus glaucus*. *Journal of Natural Products* 5: 760-763.
- Zhao, W. & Cui, C. 2009. Triterpenoidal constituents of *Pericampylus glaucus* and their antitumor activity *in vitro*. *Chinese Journal of Chemistry* 19: 195-199.
- Zulfiker, A., Rahman, M.M., Hossain, M.K., Hamid, K., Mazumder, M. & Rana, M.S. 2010. *In vivo* analgesic activity of ethanolic extracts of two medicinal plants-*Scoparia dulcis* L. and *Ficus racemosa* Linn. *Biology and Medicine* 2: 42-48.

Department of Pharmacy  
Faculty of Life Sciences  
Sarhad University of Science and Information Technology,  
Peshawar  
Pakistan

\*Corresponding author; email: kifayatpharma86@yahoo.com

Received: 7 July 2018

Accepted: 29 November 2018