

## Sex-Dependent Variations in Anti-Nociceptive and Antipyretic Effects of Rhizome and Stem Extract of *Schumannianthus dichotomus* Roxb. in Male and Female Mice

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### ABSTRACT

*Schumannianthus dichotomus* (Roxb.) is a perennial shrub from Marantaceae family. In traditional medicine, rhizome of the plant is used in fever and stem is used in earache. We aimed to substantiate these therapeutic claims by examining their effects in mice model. Antinociceptive effect was evaluated by three pain models and antipyretic effect was tested by yeast induced hyperthermia experiment. Influence of mice sexes on these pharmacological effects was examined by performing experiments separately on male and female mice. Quantitative analyses of total phenols and flavonoids were performed. Antinociceptive effects showed striking sex dimorphism. In hind paw licking test, male mice showed significant reductions in licking in both phases for both rhizome and stem extracts while significant effect was observed only in late phase in female mice. In writhing test, antinociception is more profound in male than in female. In hot plate test, stem was more effective than rhizome in male mice while female mice produced little effect for both extracts. Antipyretic experiment also showed varied effect in male and female mice; both extracts showed significant decrease in body temperatures. Rhizome showed greater effect in female mice while stem was more effective in male mice. Total phenol and flavonoid in rhizome were found 103.08 mg GAE (gallic acid equivalent) and 9.07mg QE (quercetin equivalent) respectively while in stem, these were 43.39mg GAE and 20.93 QE. Antinociceptive and antipyretic effects of rhizome and stem extracts endorsed the traditional uses of *S. dichotomus*. Also, differential effects based on mouse sex indicate the prerequisite of both male and female mice model in therapeutic evaluations of plant extract.

**Keywords:** *Schumannianthus dichotomus*, swiss albino mice, sex dimorphism, antinociceptive, antipyretic

### INTRODUCTION

*Schumannianthus dichotomus* (Roxb.) Gagnep is a perennial shrub belongs to Marantaceae family (Mandal *et al.*, 2014). There are three species in genus *Schumannianthus* and the species are- *Schumannianthus virgatus*, *Schumannianthus monophyllus* and *Schumannianthus dichotomus*. Rhizome extract of *S. virgatus* was reported to possess hepatoprotective activity (Neethu *et al.*, 2017) while no reports on pharmacological investigations on *S. monophyllus* and *S. dichotomus* have been found. The plant- *S. dichotomus* is normally 3-5m high with a basal diameter of 2-5cm and grows mainly in clay or clay-

loam soil of swampy wetlands with shade ambience (Ahmed *et al.*, 2007). The species has widespread presence in countries of South and South-East Asia including India, Bangladesh, Myanmar, Vietnam, Malaysia and Indonesia (Ahmed *et al.*, 2007; Veldkamp & Turner, 2016). Traditional uses of plant parts of *S. dichotomus* against various ailments are well documented; rhizome is used against fever and skin diseases and stem is in the treatment of earache (Maneenoon *et al.*, 2015; Sajib & Uddin, 2015).

Fever and pain are the most common manifestations in diverse disease conditions. A wide variety of drugs are available in pain,

inflammation and fever but their uses are often accompanied by undesirable side effects. For example, common non-steroidal anti-inflammatory drugs cause gastric disturbances and long-term use of narcotic analgesics is penalized with addiction and dependency. Despite incredible advancement in synthetic chemistry ensuring prolific supply of promising drug candidates, plants are still an attractive source for new bioactive compounds against various diseases. Folkloric uses of plants against different diseases act as a pivot to explore those plants for new bioactive compounds and affirmative activities of crude plant extracts usher identification and isolation of many compounds. For instance, in recent years, several compounds from plant sources have been reported to possess antinociceptive (Mizokami *et al.*, 2012; Nieto *et al.*, 2013; Sun *et al.*, 2019) and antipyretic effect (Lee *et al.*, 2019; Muhammad *et al.*, 2019). A good number of plants native to Bangladesh have been traditionally used against pain and fever and their effects are underpinned by experimental findings in animal model (Ferdous *et al.*, 2020; Roy *et al.*, 2019; Subedi *et al.*, 2016).

Mice, rats and other vertebrates such as rabbits, guinea pigs, cats, dogs, monkeys, etc. play key role in mammalian genetic and biomedical research. Among these animal models, the mouse is deemed the most instrumental model organism because of high (~95%) DNA coding sequence conservation with human genomes (Lander *et al.*, 2001; Waterston *et al.*, 2002). Moreover, mutant and inbred mice often demonstrate syndromes that resemble to human inherited diseases due to metabolic and internal anatomical similarities. In 2017, around 61% experimental studies use mouse model in the field of life science researches in European countries (*Report from the Commission to the European Parliament and the council*, 2020). In designing experimental studies using mice model, many researchers prefer males assuming female animals would show greater variability due to their changing hormonal state during oestrous cycle; this cyclic hormonal fluctuations not only influence reproductive system but also other physiological systems; thus, the magnitude of responses due to an experimental factor may differ across the cycle (Beery & Zucker, 2011; Wald & Wu, 2010). There are numerous reports that reveals significant differences between male and female mice in nociception (Ballou *et al.*, 2000; Dina *et al.*, 2001; Karim *et al.*, 2020), body temperature (Sanchez-Alavez *et al.*, 2011; Wei *et al.*, 2018), energy intake (Nishikawa *et al.*, 2007), insulin secretion (Suzuki

*et al.*, 2017), liver function (Mohar *et al.*, 2014; Spruss *et al.*, 2012), neuroanatomy (An *et al.*, 2011), etc.

In this work, firstly, we aimed to verify folkloric uses of *S. dichotomus* against pain and fever by providing scientific evidences on their claimed activities. Antinociceptive activity was examined by three experiments- formalin induced hind paw licking, acetic acid induced writhing and hot plate test, and activity against fever was evaluated by yeast induced hyperthermia test. All these experiments were conducted on Swiss albino mice model. Secondly, we investigated possible variations in putative antinociceptive and antipyretic effects of the plant extracts in male and female mice.

## MATERIALS AND METHODS

### Preparation of plant extracts

Whole plant of *Schumannianthus dichotomus* was collected from Sonapur, Noakhali, Bangladesh. An herbarium specimen (Accession No.: DACB-55914) was made and deposited at National Herbarium, Dhaka, Bangladesh. After collection, stem and rhizome were then cut into small pieces, air-dried at room temperature and finally grinded into powder. About 800g stem and rhizome material was taken in separate flat bottom glass containers and mixed with two times volume (w/v) of methanol (80%) at room temperature. Soaking with methanol was done for two weeks with occasional shaking and stirring. The mixtures were then filtered using filter cloth first followed by filtration through Whatman filter paper No. 1 and concentrated with rotary evaporator producing two different gummy concentrates. Percentage yield of greenish black colored stem concentrate was 1.36% (w/w) while rhizome extract was light brown coloured and yield was 1.86% (w/w).

### Drugs and chemicals

Standard drugs - diclofenac sodium (99.5%-100%), ketorolac (99.0%-99.8%) and paracetamol (99.9%-100%) were collected from Silva Pharmaceuticals Limited, Noakhali, Bangladesh. Methanol and formaldehyde solution (37%) were purchased from local vendor of Merck, Germany. Saline solution (0.9% NaCl) used to prepare Baker's yeast was a product of Beximco Pharmaceuticals Limited, Bangladesh.

### Grouping of animals and dose of samples

Prior approval regarding animal model was taken from Institutional Ethics Committee

(Approval ID. 17/2019). Both male and female Swiss albino mice (25-30g) were obtained from International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh. They were kept in polycarbonate cages with stainless steel grid as lid (40x 30x17cm) under naturally illuminated environment of 12/12h dark/light cycle. Temperature and humidity of the animal house was maintained at 23-25°C and 50-55% respectively. The animals got free access to formulated pellet feed and water during entire study period. Mice were acclimatized with the laboratory environment for one week before commencement of experiments. In all experiments, mice of each sex were divided into four groups having six mice per group and the experiments were performed on male and female mice separately. Groups were named as control (water, 10mL/kgBW), standard (based on experiment), SD200 (extract at dose of 200mg/kg BW) and SD400 (extract at dose of 400mg/kg BW). Plant samples were prepared by dissolving the crude extracts in distilled water.

#### Acute oral toxicity study

Acute oral toxicity study was performed according to Organization for Economic Cooperation and Development guidelines prior to antinociceptive and antipyretic experiments (OECD, 2001). Doses of plant extracts used were gradually increased from 5 to 2000mg/kg BW (50 and 300mg/kg BW as intermediate doses). The animals were observed for 14 days for any change of general behavior, food and water intake, salivation, frequency of defecation, any manifestation of toxicity, etc. During this examination period, no mortality and moribundity of animals were observed; hence, one-fifth and one-tenth of maximum administered dose i.e. 2000mg/kg BW were considered safe to conduct anti-nociceptive and antipyretic experiments.

#### Anti-nociceptive activity

##### Formalin induced hind paw licking test

In formalin induced licking test, 0.02mL of 2.5% formalin was used as algen and was injected subcutaneous (s.c.) into the plain surface of left hind paw of mouse (Dubuisson & Dennis, 1977; Wheeler-Aceto *et al.*, 1990). Control group received 10mL/kg BW distilled water while diclofenac (10 mg/kg BW) was administered in standard group. All test samples were administered (p.o.) oral administration 1h before this injection. Licking and biting of the injected paw were considered as nocifensive behaviours. Time

spent in licking and biting was recorded in two phases: at first 0-5min and last 15-30min after formalin injection. The results were expressed as percent inhibition of licking response (PIL):

$$\%PIL = \left( \frac{TSL_{(control)} - TSL_{(standard\ or\ extract)}}{TSL_{(control)}} \right) \times 100$$

TSL = Time spent licking

#### Acetic acid induced writhing test

In this experiment, induction of nociception such as abdominal constriction, caudal elongation along the abdominal wall, etc. was achieved by administration of 0.6% acetic acid (i.p.) intraperitoneal (Collier *et al.*, 1968; Siegmund *et al.*, 1957). Plant extracts, diclofenac (10mg/kg BW) and control (water, 10mL/kg BW) were administered (p.o.) 30min before administration of acetic acid. Number of writhes (squirring of the trunk and/or pelvis, elongation of the body, extension of the hind limbs) was recorded for 15min. Results were expressed as mean percent inhibition of writhing (PIW):

$$\%PIW = \left( \frac{NW_{(control)} - NW_{(standard\ or\ extract)}}{NW_{(control)}} \right) \times 100$$

NW=Number of Writhes

#### Hot plate test

In hot plate test, temperature of the apparatus was raised and maintained at 55±0.5°C (Woolfe & Macdonald, 1944). Prescreening of mice was done first by placing each mouse on the hot plate; animals those showed nociceptive responses (licking, jumping, flinching, etc.) within 10s were selected for final experiment. Water (10mL/kg BW) and ketorolac (10mg/kg BW) were used in control and standard group respectively. In the final experiment, each mouse was again placed on the hot plate and latency of mice to this thermal stimulus was recorded at 0, 0.5, 1 and 2h postprandial. Response time was recorded when the animal first licked its paw or started to jump. To avoid mouse tissue damage, 20s was chosen as the cut-off time. Results were expressed as mean percent maximal effect (% MPE):

$$\%MPE = \left( \frac{Post\ DL_{(t\ hr)} - Pre\ DL_{(0\ h)}}{Cut-off\ time - Pre\ DL_{(0\ h)}} \right) \times 100$$

DL=drug latency

#### Antipyretic activity

Antipyretic effect of the extract was determined by inducing hyperthermia in mice (Tomazetti *et al.*, 2005). Elevated body temperature of mice was achieved by injecting 20%

Baker's yeast suspended in 0.9% saline at a dose of 10mL/kg BW. Rectal temperature was measured using a digital thermometer with its probe lubricated with glycerin. After measuring the rectal temperature and yeast injection, mice were kept in fasting condition for 18h; animals those showed an elevated body temperature of 1.5°F was selected for the final experiment. The selected animals were then treated (p.o.) with water (10mL/kg BW, control group), paracetamol (150mg/kg BW, standard group) and plant extracts (200 and 400mg/kg BW). Rectal temperatures of all experimental animals were measured at intervals of 0, 0.5, 1, 2, 3 and 4h.

### Phytochemical analysis

Qualitative analyses of major phyto-constituents in methanol extract of rhizome and stem were carried out using standard procedures (Evans, 2009). Quantitative determination of phenolics, tannins and flavonoids were performed as described in Akter, 2019 (Akter *et al.*, 2019).

### Analysis of data

Statistical software GraphPad prism (version 8.4) was used for data analysis. Data were presented as mean  $\pm$ SEM. A probability level of 0.05 (adjusted p value) or less was accepted as significant; \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$  vs. control of the respective experiment.

## RESULT AND DISCUSSION

### Anti-nociceptive activity

#### Formalin induced hind paw licking test

The effect of plant extracts on formalin induced hind paw licking (Figure 1). Significant decrements in licking duration as an indication of antinociception were observed by both rhizome and stem extract at higher dose in male mice model. In female mice model, both extracts produced little effect in early phase of the experiment while significant effects were observed in late phase.

In early phase of the experiment, in male mice model, standard showed licking duration of 73s whereas control group exhibited 85.5s. Plant samples produced stronger effect than diclofenac in a dose dependent manner; rhizome and stem extract at 400mg/kg BW reduced the propensity to lick to 35.3s ( $p < 0.05$ ) and 29.9s ( $p < 0.01$ ) respectively while lower dose also superseded diclofenac and durations were 60.4s and 55.9s for rhizome and stem respectively. In the late phase, licking durations of control, diclofenac, both doses of rhizome extract and 200mg/kg BW stem extract

were increased indicating attenuation of antinociceptive effect over time; nonetheless, stem extract at 400mg/kg BW retained its activity. Percent inhibition of licking is the indication of antinociceptive potentiality of extracts compared with control group. In the early phase, diclofenac inhibited 14.62% whereas rhizome and stem at 200 mg/kg BW demonstrated 29.36% and 34.62% inhibition; higher extract doses showed greater effects producing 58.83% and 65.03% inhibition. In the late phase, inhibition by rhizome at 400 mg/kgBW dose was increased slightly to 61.29% while stem extract at the same dose produced substantial amelioration in antinociception and inhibited 80.52%.

In case of female mice model, in early phase of the experiment, the extracts did not produce noticeable change in licking duration compared to control group. Licking durations of control and standard were 69.1s and 38.8s respectively. Hence, diclofenac showed 43.85% inhibition in licking in early phase while both rhizome and stem extracts demonstrated little effect producing less than 10% inhibition. In late phase of the test, standard drug and extracts showed significant decrease ( $p < 0.001$ ) in licking. Rhizome and stem extracts of both doses showed greater effect than diclofenac (55.94%); rhizome at 200mg/kgBW and 400mg/kg BW demonstrated 69.5% and 69.16% inhibition while stem at the same doses produced 64.69% and 73.06% inhibition.

### Acetic acid induced writhing test

Inhibition of writhing responses upon administration of standard drug and plant extracts (Table I). The extracts demonstrated dose-dependent attenuation of nociception. Stem extract produced stronger effect than rhizome in both male and female mice and in all doses, male mice demonstrated better antinociceptive response than female mice.

Rhizome extract at 200mg/kg BW reduced writhes from 41.0 to 24.7 ( $p < 0.1$ , 39.75% inhibition) in male mice while little reduction in number of writhes by 3.2 (5.75%) was observed in female mice at the same dose. Stem extract at 200mg/kg BW was observed more potent in both male and female than rhizome extract; percentage inhibitions were 77.63% and 56.55% for male and female respectively. Increasing the dose at 400mg/kgBW, number of writhes in male mice were 12.3 ( $p < 0.001$ ) and 7.67 ( $p < 0.001$ ) for rhizome and stem respectively producing 70% and 81.29% inhibition.

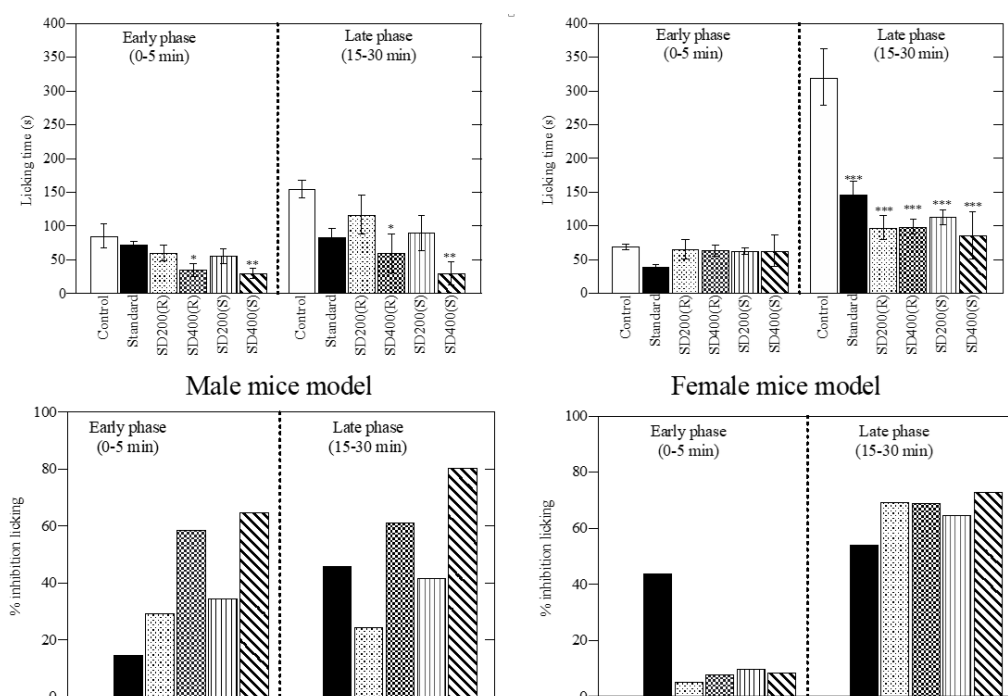


Figure 1. Effect of rhizome and stem extract of *Schumannianthus dichotomus* in formalin induced hind paw licking test in male and female mice model. Data were presented as Mean  $\pm$  SEM. One-way ANOVA with Dunnett multiple comparisons test was performed to determine statistical significance of test results; here, \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$  vs control.

Table I. Effect of methanol extract of rhizome and stem of *S. dichotomus* on the number of writhing responses in acetic acid induced writhing test.

Group	Male		Female	
	Writhes response	PIW	Writhes response	PIW
Control	41.0 $\pm$ 4.75	-	55.7 $\pm$ 4.47	-
Standard	21.0 $\pm$ 2.31*	48.78	19.2 $\pm$ 5.10***	65.53
SD200(R)	24.7 $\pm$ 7.86*	39.75	52.5 $\pm$ 4.84	5.75
SD400(R)	12.3 $\pm$ 2.09***	70.00	35.3 $\pm$ 5.48*	36.62
SD200(S)	9.17 $\pm$ 1.74***	77.63	24.2 $\pm$ 4.79***	56.55
SD400(S)	7.67 $\pm$ 3.71***	81.29	15.8 $\pm$ 2.30***	71.63

'SD' stands for *Schumannianthus dichotomus*; (R) for rhizome and (S) for stem. Data were presented as mean $\pm$ SEM. All data were analyzed using ANOVA followed by Dunnett's test for multiple comparisons of means. Significantly different from control at \* $p < 0.05$ , \*\*\* $p < 0.001$ .

At the same dose, female mice showed 36.62% and 71.63% inhibition for rhizome and stem respectively. Standard drug- diclofenac produced 48.78% and 65.53% inhibition for male and female respectively.

### Hot plate test

In hot plate test, dose dependent significant increase in tolerance against thermal stimuli was observed in male mice for both rhizome and stem extract. However, stem extract was found more

potent than rhizome. On contrary, little antinociceptive activity was evident in female mice for both extracts (Figure 2).

In case of male mice, standard drug- ketorolac significantly ( $p < 0.001$ ) increased latency from 10.27s to 16.56s, 17.26s and 18.4s after 0.5h, 1h and 2h respectively. Stem at 400mg/kg BW raised the latency significantly ( $p < 0.01$ ) from 11.08 s to 15.81 s after 0.5h and the tolerance was further increased after 2h to 17.36s. At 200 mg/kgBW stem extract, latency was increased by 3.5s ( $p < 0.01$ )

within 0.5h but increased little afterwards. In case of rhizome extract, both SD200 and SD400 showed increased latency after 0.5h, then reduced it slightly after 1h that again augmented after 2h and increases in latency after 2h were 2.03s ( $p<0.05$ ) and 2.78s ( $p<0.01$ ) for 200mg/kg BW and 400 mg/kg BW respectively. For stem extract in female mice, significant change ( $p<0.05$ ) in latency was observed for both doses after 1h though latencies were briefly elevated from 4.32s to 5.01s for 200mg/kg BW and 4.2s to 4.85 s for 400mg/kg BW. Activity of similar extent was observed for rhizome extract and increased the latency by 0.52 s and 1.86 s after 2h for 200mg/kg BW and 400mg/kg BW respectively.

Quantitative assessment of antinociceptive potentials for rhizome and stem was done by calculating mean percent maximal effect from duration of latency at different time intervals (equation mentioned in section 2.4.2). For male mice, standard compound produced rapid and prominent effect (64.6%) immediately after 0.5h that subsequently increased as time elapsed showing 83.55% effect after 2h. Stem extract at 400 mg/kg BW dose demonstrated 70.35% effect after 2h whereas lower dose (200mg/kg BW) produced effect around 40% throughout the experiment. As rhizome extract exhibited little increase in latency compared to stem, hence produced maximal 25.7% and 31% effect at 200mg/kg BW and 400mg/kg BW respectively. In female mice, standard drug showed 31.13% effect whereas both rhizome and stem extracts exhibited minute effect. Rhizome showed 3.28% and 12.4% for 200 and 400mg/kg BW after 2h whereas for stem, the effects were 9.61% and 5.68% respectively after same time span.

#### Antipyretic activity

Reduction in mice body temperature from Baker's yeast induced fever (Table II). Both rhizome and stem extracts demonstrated significant antipyretic effect but varied in magnitude of reduction. Rhizome showed greater antipyretic effect in female mice while stem extract was more effective in male mice.

For rhizome extract, female mice at 200mg/kg BW showed greater antipyretic effect than higher dose (400mg/kg BW); the results were consistent from 0.5h to 4h. For instance, after 0.5h, decrements in temperature were 2.13°F ( $p<0.05$ ) and 1.05°F ( $p<0.05$ ) for 200mg/kg BW and 400mg/kg BW respectively while after 4h, the reductions were 2.95°F ( $p<0.05$ ) and 2.48°F

( $p<0.001$ ). On the other hand, for rhizome extract, the male mice experienced greater reductions at higher dose as time elapsed; for 2h, 3h and 4h, SD 400 reduced body temperatures by 1.90°F ( $p<0.05$ ), 2.30°F ( $p<0.05$ ) and 1.72°F ( $p<0.001$ ) whereas for 200mg/kg BW, these lowered by 1.83°F ( $p<0.01$ ), 1.65°F ( $p<0.01$ ) and 1.17°F ( $p<0.001$ ) respectively. The same result was observed for stem extract where body temperatures in male mice reduced at greater degree at 400mg/kg BW than at 200mg/kg BW. Maximum reduction by 3.15°F was observed after 2h for 400mg/kg BW while extract at 200mg/kg BW reduced it by 1.93°F ( $p<0.001$ ) after 4h. Female mice was found to follow similar trend as increased stem extract concentration showed better antipyretic effect except at 0.5h. Maximal effect was observed after 4h when temperature was reduced by 2.20°F ( $p<0.001$ ) at 400mg/kg BW; after the same time span, extract at 200mg/kg BW decreased body temperature by 1.68°F ( $p<0.001$ ).

#### Phytochemical analysis

Qualitative phytochemical screening of methanolic extract of rhizome and stem showed the presence of phenols, tannins, flavonoids, alkaloids, carbohydrates and phytosterols. Proteins were observed only in stem while rhizome showed the presence of terpenes. Glycosides, saponins and gums were not observed in both rhizome and stem. Total phenolic contents in rhizome and stem were found 103.08 mg GAE and 43.39 mg GAE per g of extract respectively while flavonoid contents were 9.07 gm QE and 20.93 QE per g of extract.

Qualitative phytochemical screening of methanol extract of *S. dichotomus* demonstrated that rhizome and stem extracts contain similar phytochemicals. Quantitative analyses showed that total phenol content was present in greater amount in rhizome than in stem while flavonoid content was higher in stem. Numerous researches on different plants reported diverse pharmacological effects including antinociceptive, anti-inflammatory and antipyretic effects of phenols (Boussouf *et al.*, 2017; Gomathi & Manian, 2015; Nguyen *et al.*, 2017) and flavonoids (Chen *et al.*, 2016; Owoyele *et al.*, 2008; Wang *et al.*, 2014). As rhizome and stem extract of *S. dichotomus* possess similar phytoconstituents; hence, both extracts are anticipated to show analogous effects. And, different contents of total phenols and flavonoids in rhizome and stem correspond to their varied degree of antinociceptive and antipyretic effects.

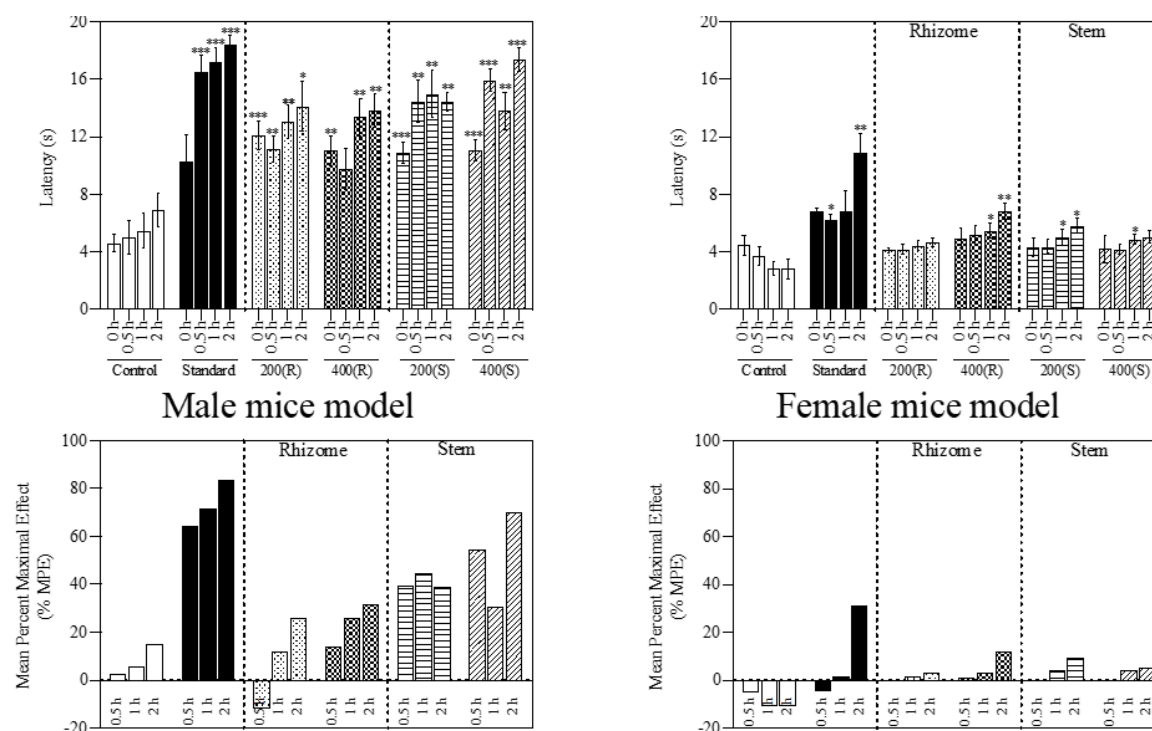


Figure 2: Effect of rhizome and stem extract of *Schumannianthus dichotomus* against thermally induced pain in male and female mice model. Data were presented as Mean  $\pm$  SEM. Two-way ANOVA with Tukey multiple comparisons test was performed to determine statistical significance of test results; here, \* $p$ <0.05, \*\* $p$ <0.01 and \*\*\* $p$ <0.001 vs control.

Effects against nociception and fever differed not only between extracts but also between sexes of animal. In hind paw licking test, the licking time was recorded in two phases. Significant decrements in licking duration as an indication of antinociception were observed by both rhizome and stem extracts in male mice model. In female mice model, both extracts produced little effect in early phase of the experiment while significant effects were observed in late phase. Generation of pain in licking test is a biphasic event and mediates through independent afferent pathway. Early phase generally involves C-fiber activation producing neurogenic pain and late phase is mainly mediated by various cytokines in the peripheral tissues (Davidson & Carlton, 1998; Linhart *et al.*, 2003). Centrally acting analgesics and paracetamol like drugs can inhibit equally in both phases; on the other hand, (non-steroidal anti-inflammatory drug) NSAIDs such as naproxen and indomethacin are more effective in late phase (Hunskar & Hole, 1987). Induction, manifestation and magnitude of nocifensive behaviours show distinct sex dimorphism in mice owing to

differences in underlying pathways. In case of early phase, injuries to peripheral nerves to induce pain revealed that microglia accounts for pain sensation in male mice whereas in female mice, one of the immunological components i.e. T cell controls pain (Sorge *et al.*, 2015). In the context of pro-inflammatory cytokine mediators such as prostaglandins and leukotrienes, these mediators are conversely regulated by mice sexes. Both mediators are synthesized from same precursor i.e. arachidonic acid but through two different pathways; for leukotrienes, it is lipoxygenase (LOX) pathway while cyclooxygenase (COX) pathway is responsible for production of prostaglandins. In case of female mice, leukotrienes are generated in greater extent than prostaglandins; on the contrary, male mice shows preferential activation of cyclooxygenase (COX) pathway generating prostaglandins (Pace *et al.*, 2017). Selective inhibition of either COX or LOX pathway will display preferential attenuation of pain in any particular sex while indiscriminate inhibition between two pathways will result similar antinociceptive effects in both male and female mice.



Table II. Reduction in body temperatures (°F) in male and female mice in yeast induced febrile condition after administration of rhizome and stem extracts

Time	Groups	Rhizome		Stem	
		Male	Female	Male	Female
0.5h	Control	-0.50±0.14	-0.53±0.09	-0.50±0.14	-0.53±0.09
	Standard	2.58±1.62***	2.33±0.72*	2.58±1.62***	2.33±0.72*
	SD200	1.98±0.56*	2.13±0.54*	0.73±0.37	2.17±0.24
	SD400	1.68±0.54*	1.05±0.33*	2.02±0.77	1.55±0.75
1h	Control	-0.28±0.21	-1.10±0.22	-0.28±0.21	-1.10±0.22
	Standard	2.52±0.26***	2.67±0.44***	2.52±0.26***	2.67±0.44***
	SD200	2.35±0.42**	2.02±0.57**	0.68±0.43	0.90±0.46*
	SD400	1.70±0.42*	1.48±0.37**	2.70±0.93	1.47±0.77
2h	Control	-0.50±0.31	-1.03±0.50	-0.50±0.31	-1.03±0.50
	Standard	2.32±0.43**	2.47±0.55**	2.32±0.43**	2.47±0.55**
	SD200	1.83±0.40**	2.25±0.61**	1.20±0.33*	0.90±0.46
	SD400	1.90±0.56*	2.05±0.31**	3.15±1.05	1.70±0.76
3h	Control	-0.65±0.31	-1.70±0.23	-0.65±0.31	-1.70±0.23
	Standard	3.68±0.24***	1.43±0.28***	3.68±0.24***	1.43±0.28***
	SD200	1.65±0.44**	2.77±0.59**	0.85±0.40	1.45±0.44**
	SD400	2.30±0.67*	2.38±0.30***	2.55±1.19	2.15±0.72**
4h	Control	-4.12±0.54	-1.52±0.37	-4.12±0.54	-1.52±0.37
	Standard	3.42±0.35***	1.38±0.35***	3.42±0.35***	1.38±0.35***
	SD200	1.17±0.38***	2.95±0.98*	1.93±0.53***	1.68±0.39***
	SD400	1.72±0.71***	2.48±0.38**	2.61±0.61***	2.20±0.43***

SD200 and SD400 represent extract concentrations at 200 mg/kgBW and 400 mg/kgBW respectively. Data were presented as Mean ± SEM. Minus (-) sign indicates elevation in body temperature. Two-way ANOVA with Tukey multiple comparisons test was performed to determine statistical significance of the test results; here, \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$  vs control.

Greater antinociceptive effect in the late phase of licking test indicates that the plant extracts mainly act on inflammatory pathways. Also, higher effect in male mice than female could be an important evidence that phytochemicals in *S. dichotomus* inhibit COX in greater degree over LOX pathway. Preferential effect on pro-inflammatory COX pathway by *S. dichotomus* extract was further substantiated by the findings in acetic acid induced writhing study that showed stronger effect in male mice than female. In writhing test, acetic acid induces release of prostaglandins that results acute inflammation in abdominal cavity; the event in turn stimulates nociceptive sensory nerve endings in the peritoneal area (Sutradhar *et al.*, 2007). Prostaglandins are synthesized in tissues by two cyclooxygenases- COX-1 and COX-2; the former is constitutive in nature while the later one is induced by inflammatory stimuli. Ballou *et al* reported variable writhing responses in male and female mice due to differences in COX-1 and COX-2 activities (Ballou *et al.*, 2000). In hot plate test, rhizome and stem extracts of *S. dichotomus* showed

potent antinociceptive effect in male mice while in female mice, little maximal effects were observed for both extracts. Fatehi *et al.* reported that inhibition of nitric oxide synthesis by administration of enzyme inhibitor such as N<sup>G</sup>-nitro-L-arginine methyl ester had resulted diminished perception of pain in hot plate test in male mice but not in female (Fatehi-Hassanabad *et al.*, 2005). It was postulated that thermal stimulus from hot plate stir L-arginine-nitric oxide signalling pathway more prominently in male than in female with possible involvement of sex hormones. Similar experiments in both sexes of rats also demonstrated that inhibitors of nitric oxide synthetase (NOS) antagonized pain only in male and is dependent on sex hormones (Dina *et al.*, 2001). Thus, any phytoconstituent that hinder NOS pathway will render antinociceptive effects preferentially in male mice and rhizome and stem extract must contain bioactive compounds that have some effects on NOS pathway; thus, pain perception is selectively observed in male over female. Thermal stimuli also affect opioid receptors



manifested as spinal analgesia (Pathan & Williams, 2012). For opioids, pain is actuated by three types of receptors-  $\mu$ ,  $\kappa$  and  $\delta$ . Activity of these receptors significantly differ in male and female mice; opioids with  $\mu$  and  $\kappa$  receptors agonist activity are found more effective in male mice in response to various chemical and thermal fillips while  $\delta$  receptor has no preferences over sex (Cicero *et al.*, 1996; Kepler *et al.*, 1991).

Methanol extracts of *S. dichotomus* showed significant antipyretic effect. Both extracts and paracetamol produced a sharp decrease of elevated rectal temperature. Reduction of body temperature by paracetamol involves inhibition of prostaglandin synthesis by blocking cyclooxygenase pathway. Production of heat in mammals is an important physiological event to regulate body temperature in low ambient temperature condition. This thermogenesis process also plays a pivotal role to evoke fever to combat infections. In response to viral and bacterial invasions, synthesis of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is increased specially in brain vasculature (Eskilsson *et al.*, 2017; Matsumura *et al.*, 1998). PGE<sub>2</sub> then binds with different EP receptors, such as EP<sub>1</sub>, EP<sub>3</sub> and EP<sub>4</sub>, located in the preoptic region of hypothalamus resulting elevation of body temperature (Stitt, 1973). This hyperthermic internal environment is unfavorable to pathogen viability facilitating host defence system to surmount infections. Among these EP receptors, EP<sub>3</sub> is the principle binding site of PGE<sub>2</sub> for its pyrogenic action. Genetic deletion of EP<sub>3</sub> receptors in mice demonstrated complete diminution of febrile response to PGE<sub>2</sub> (Ushikubi *et al.*, 1998) or endotoxin whereas knock-out mice lacking EP<sub>1</sub> receptors showed attenuated induction of fever (Oka *et al.*, 2003). Despite the general mechanism of thermogenesis in human, rabbit, rat, mouse, etc., various factors influence the overall effect of this physiological process. Among these factors, sex of the animal is one of the foremost factors that manifests distinct variations in animal's body temperature. Young female mice of C57B1/6, C57BL strains are found to show higher core temperature than male; the opposite phenomenon is observed in C3H strain (McLaren, 1961; Sanchez-Alavez *et al.*, 2011). Such variations in body temperatures between male and female mice are attributed to the subtle neuroanatomical differences in preoptic area of hypothalamus (Kim *et al.*, 2019; Wei *et al.*, 2018) and different rates of PGE<sub>2</sub> production (Gregory *et al.*, 2000). Body temperature can also be fluctuated between light

and dark part of the day (Sanchez-Alavez *et al.*, 2011). Even holding of a mouse by its tail and scruff of the neck during experiment showed rapid reduction of body temperature in some strains irrespective of their sexes (McLaren, 1961). Phytochemical(s) in methanol extract of *S. dichotomus* must interfere in the COX activity similar to antinociceptive action resulting normalization of body temperature.

The results of the present study confirmed the folkloric use of *Schumannianthus dichotomus* in pain and fever that warrant comprehensive biochemical and chemical analyses to identify potential bioactive compounds responsible for these pharmacological effects. Also, differential antinociceptive and antipyretic effects based on animal sexes strongly advocates use of both male and female mice in pharmacological investigations of plants.

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