# Comparative analgesic evaluation of *Himalrandia tetrasperma* and *Wendlandia exserta* of family Rubiaceae after induction of pain in mice

**Muhammad Ajaib*[1], Saiqa Ishtiaq[2] and Muhammad Faheem Siddiqui[3]**
[1]Department of Botany, Mirpur University of Science & Technology (MUST), Mirpur (AJK), Pakistan
[2]Punjab University College of Pharmacy, University of the Punjab, Lahore, Pakistan
[3]Department of Botany, University of Karachi, Karachi, Pakistan

**Abstract**: *Himalrandia tetrasperma* and *Wendlandia exserta* medicinal plants belong to family Rubiaceae commonly known as coffee family were investigated by quantitative analysis of major bioactive compounds and analgesic effect. The analgesic potential was accessed using different parts of *H. tetrasperma* and *W. exserta* by induced acetic acid writhing and hot plate test method. Methanolic extracts of these two plants satisfactorily possesses analgesic activity. All the extracts showed good results as compared to standard drug, i.e. aspirin. Seeds of *H. tetrasperma* possess maximum, *i.e.* 86.73% inhibition at first phase where as aspirin possesses 52.73%. In second phase, leaves possess 99.8 % inhibition respectively. Hot plate analgesic activity of bark extract displayed maximum activity at 4.5h stage, *i.e.* 8.6±0.40. *W. exserta* methanolic extract of bark possesses 97.3% inhibition respectively in first phase and 99.8% in second phase. Bark extract displayed maximum activity at 4.5h stage, i.e., 9.7±0.5. Quantitative analysis of bark of *H. tetrasperma* possesses highest value of saponins, *i.e.* 30.21±0.8 where as flavonoids 17.50±1.2, phenolic compounds 23.25±0.5 and tannins 12.32±0.4. The leaf extracts of *H. tetrasperma* contains maximum value of phenols, i.e. 15.10±0.7 where as *W. exserta* bark possesses significant value of alkaloids, *i.e.* 16.41±0.4 and leaf extracts possesses flavonoids, i.e. 14.51±0.3, saponins 12.22±0.1 and phenolic compounds 11.31±0.4. The seeds of both plants possess significant value of tannins, *i.e.* 07.60±0.3.

**Keywords**: Analgesic, *Himalrandia tetrasperma*, *Wendlandia exserta*, Rubiaceae, flavonoids, saponins.

## INTRODUCTION

Historically human civilization used medicinal plants as a traditional type to provide relief from many ailments. A number of plants used possess excellent effects as compared to the ones obtained from allopathic medicines. It is widely known that numerous plant-derived compounds displayed excellent analgesic properties and with minimum side effects and can be used as substitute medicines (Verpoorte, 1999; Ajaib *et al.* 2015). Analgesics are the substances that greatly reduce pain sensation towards the harmful pain stimuli, i.e. thermal, chemicals and physical pressure (Tripathi, 2003). The analgesic activity in medicinal plants is depending on the bio-active compounds such as alkaloids, flavonoids, tannins and phenolic compounds and hence, many of the medicinally important plants are also used as spices, condiments and other food plants (Okwu, 1999, 2001; Hill 1952). Natural products play a key role in the field of new medications and preparations of new drugs, as a result of their low poisonous quality, availability and cost effectiveness (Chessbrough, 2000; Ajaib *et al.* 2013).

In recent times the drugs used as analgesic may be narcotics such as opioids or non-narcotics such as salicylates. All of these analgesic medicines produce several side effects beside treatment. Synthesis of new drugs are expensive, as the significant introduction of a new products calls numerous compounds to be manufacture which are tested and their cost of improvement was in millions of dollars. Alternatively, several herbal drugs have been extensively used by the local and rural people since a long time ago with little or no side effects. Hence, it is necessary to manufacture cheaper drugs by using all recourses including plants (Ikram, 1983). Medicinal plants are hence were one of the major natural resource for drug development in the modern biomedical sciences (Micozzi, 1995).

*Himalrandia tetrasperma* (Roxb.) Yamazaki and *Wendlandia exerta* (Roxb.) DC. of family Rubiaceae were used for quantitative estimation of different phytoconstituents and evaluation of analgesic activity.

*H. tetrasperma* an antidiabetic plant (Ajaib *et al.* 2016) commonly found in hilly areas and open river beds of Pakistan and AJK upto 2000m above sea level. It is a 1-2 m tall bush with leaves mostly in fascicles towards end of branches. Leaves are also elliptic, ovate-oblanceolate, acute-subacute and entire. Flowers are whitish with globose, purple fruit. *W. exserta* a small tree found frequently in hilly areas of Pakistan including Punjab (Muree foot hills), Kashmir (Kotli, AJK), Nepal, Sikkim and India. It is locally called Ukan in District Kotli Azad Kashmir. This evergreen tree having reddish brown bark. Leaves are opposite, broadly-ovate or

*Corresponding author: e-mail: majaibchaudhry@yahoo.com

**Table 1**: Quantitative analysis of phytochemical compounds in *H. tetrasperma*

| Plant | Alkaloids | Flavonoids | Phenols | Saponins | Tannins |
|---|---|---|---|---|---|
| Bark | 12.23±0.3 | 17.50±1.2 | 23.25±0.5 | 30.21±0.8 | 12.32±0.4 |
| Leaf | 15.10±0.7 | 14.30±0.4 | 19.21±0.7 | 11.33±0.6 | 06.03±0.01 |
| Seed | 11.50±0.5 | 13.30±0.3 | 09.33±0.09 | 21.22±0.7 | 05.07±0.3 |

**Table 2**: Quantitative analysis of phytochemical compounds in *W. exserta*

| Plant | Alkaloids | Flavonoids | Phenols | Saponins | Tannins |
|---|---|---|---|---|---|
| Bark | 16.41±0.5 | 14.30±1.2 | 10.33±0.5 | 10.23±0.4 | 06.21±0.02 |
| Leaf | 13.36±0.3 | 14.51±0.3 | 11.31±0.4 | 12.22±0.1 | 05.31±0.07 |
| Seed | 10.55±0.5 | 12.30±0.3 | 10.40±0.6 | 11.21±0.2 | 07.60±0.3 |

**Table 3**: Effect of methanolic extract of *H. tetrasperma* on acetic acid induced writhing in mice

| Treatment | Dose mg/kg orally | Mean No. of Writhes ± S.E.M | | Inhibition (%) | |
|---|---|---|---|---|---|
| | | First phase | Second phase | First phase | Second phase |
| Control | 0.5 ml Saline | 30± 1.86 | 28.4 ± 0.951 | - | - |
| Bark | 250mg/kg | 5.4± 4.21 | 2±1.36 | 82 | 92.95* |
| Leaf | 250mg/kg | 5.6± 3.17 | 0±0 | 81.33** | 99.8** |
| Seed | 250mg/kg | 4± 4.45 | 2.2±2.80 | 86.67 | 92.25** |
| Aspirin | 250mg/kg | 26.0±1.382 | 20± 1.186 | 52.73** | 28.57* |

Note: Values represent the Mean ± S.E.M. N = 5; Results are expressed at P≤ 0.05 and P≥ 0.05. Statistically significant from control and standard drug. * Significant, ** Highly

**Table 4**: Effect of methanolic extract of *W. exserta* on acetic acid induced writhing in mice

| Treatment | Dose mg/kg orally | Mean No. of Writhes ± S.E.M | | Inhibition (%) | |
|---|---|---|---|---|---|
| | | First phase | Second phase | First phase | Second phase |
| Control | 0.5 ml Saline | 30± 1.86 | 28.4 ± 0.951 | - | - |
| Bark | 250mg/kg | 0.8± 2.1 | 0±0 | 97.3 | 99.8* |
| Leaf | 250mg/kg | 5.2± 3.17 | 3±3.2 | 82.6** | 89.4** |
| Seed | 250mg/kg | 5.6± 3.2 | 3.6±3.0 | 82 | 87.3** |
| Aspirin | 250mg/kg | 26.0±1.382 | 20± 1.186 | 52.73** | 28.57* |

**Table 5**: Effect of Methanolic extracts of *H. tetrasperma* on Hot plate Analgesiometer in mice

| Variation response time with ± SEM (Time in sec at 55 ± 1ºC) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Groups | 0h | 0.5h | 1h | 1.5 | 2h | 2.5 | 3h | 3.5h | 4h | 4.5h |
| Control | 2.37±0.37 | 3.11±.31 | 3.19±0.33 | 3.01±0.32 | 2.97±0.32 | 3.07±0.31 | 3.05±0.3 | 2.93±0.1 | 3.17±0.2 | 2.94±0.4 |
| Bark(250 mg/kg) | 0.93±0.2 | 4.10±0.7 | 6.33±1.14 | 5.87±0.89 | 4.66±8.7 | 3.88±0.30 | 5.11±0.5 | 7.5±0.36 | 6.9±1.03 | 8.6±1.05 |
| Leaf(250 mg/kg) | 0.93±0.2 | 4.11±0.5 | 4.65±2.1 | 5.1±1.9 | 5.34±2.2 | 6.45±1.4 | 6.6±1.5 | 7.2±1.06 | 7.9±1.03 | 8.0±1.05 |
| Seed(250 mg/kg) | 0.93±0.2 | 2.11±0.6 | 3.26±0.73 | 4.2±0.49 | 5.12±1.13 | 5.67±1.4 | 6.12±0.69 | 6.76±0.69 | 7.77±0.49 | 7.9±0.4 |
| Aspirin(300 mg/kg) | 12.8±0.16 | 28.6±0.2* | 15.2±0.3 | 20.0±0.3* | 20.2±0.2* | 19.79±0.6 | 17.03±0.3 | 20.4±0.4* | 21.2±0.4 | 21.5±0.42 |

**Table 6**: Effect of Methanolic extracts of *W. exserta* on Hot plate Analgesiometer in mice

| Variation response time with ± SEM (Time in sec at 55 ± 1ºC) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Groups | 0h | 0.5h | 1h | 1.5 | 2h | 2.5 | 3h | 3.5h | 4h | 4.5h |
| Control | 2.37±0.37 | 3.11±.31 | 3.19±0.33 | 3.01±0.32 | 2.97±0.32 | 3.07±0.31 | 3.05±0.3 | 2.93±0.1 | 3.17±0.2 | 2.94±0.4 |
| Bark(250 mg/kg) | 0.93±0.2 | 2.11±0.2 | 3.26±2.01 | 4.22±0.12 | 5.12±0.29 | 5.57±1.7 | 6.12±0.25 | 6.76±0.5 | 7.1±0.36 | 9.7±0.5 |
| Leaf(250 mg/kg) | 0.93±0.2 | 3.11±0.7 | 4.39±1.3 | 5.12±0.9 | 5.98±3.2 | 6.11±1.4 | 6.52±1.5 | 5.21±0.6 | 7.1±1.03 | 7.0±1.05 |
| Seed(250 mg/kg) | 0.93±0.2 | 2.11±0.6 | 4.1±2.3 | 5.87±0.9 | 4.23±2.03 | 5.11±1.0 | 7.50±0.4 | 6.99±1.2 | 7.1±0.2 | 7.3±0.5 |
| Aspirin (300mg/kg) | 12.8±0.16 | 28.6±0.2** | 15.2±0.3 | 20.0±0.3* | 20.2±0.2* | 19.79±0.6 | 17.03±0.3 | 20.4±0.4** | 21.2±0.4 | 21.5±0.42 |

**Note:** Values represent the Mean ± S.E.M. N = 5; Results are expressed at P≤ 0.05 and P≥ 0.05. Statistically significant from control and standard drug. * Significant, ** Highly significant

lanceolate and coriaceous. Flowers white in terminal pyramidal racemes about 25cm long, white, minute and sessile (Nazimuddin and Qaiser, 1989).

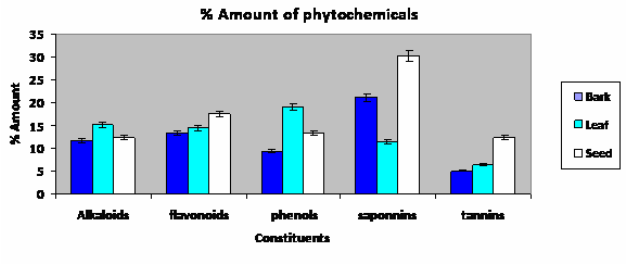


**Fig. 1**: Quantitative analysis of pytochemiclas in bark, leaves and seeds of *H.tetrasperma*

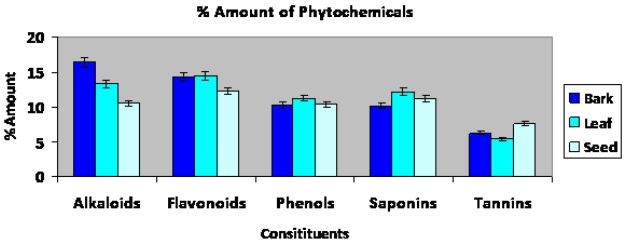


**Fig. 2**: Quantitative analysis of phtochmicals in bark, leaves and seeds of *W.exertia*

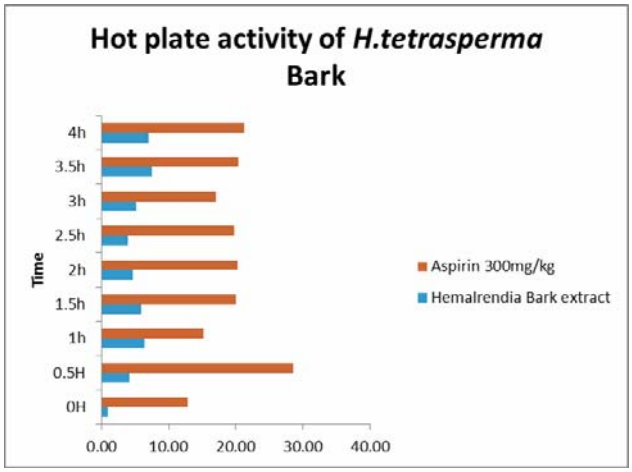


**Fig. 3**: Hot plate activity of bark methanolic extract of *H.tetrasperma*

## MATERIALS AND METHODS

### *Plant material*

Both medicinal plants belonging to family Rubiaceae were collected from District Kotli Azad Jammu & Kashmir. The plant specimen was identified from Herbarium SAH, GC University, Lahore by Prof. Dr. Zaheer-ud-din Khan with the voucher no. SAH. 2945 and 2946 for *H. tetra sperma* and *W. exserta* respectively.

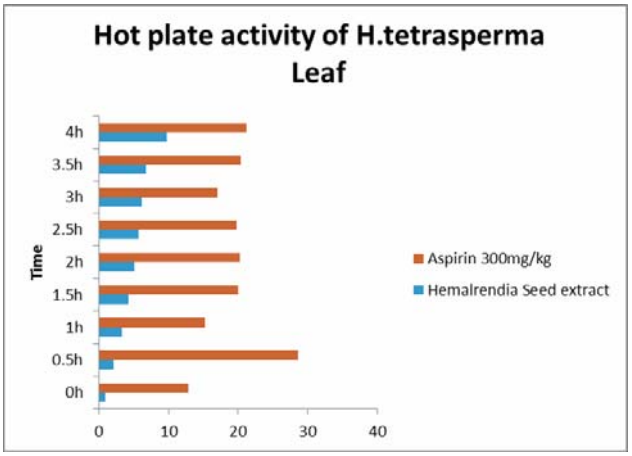


**Fig. 4**: Hot plate activity of leaf methanolic extract of *H.tetrasperma*

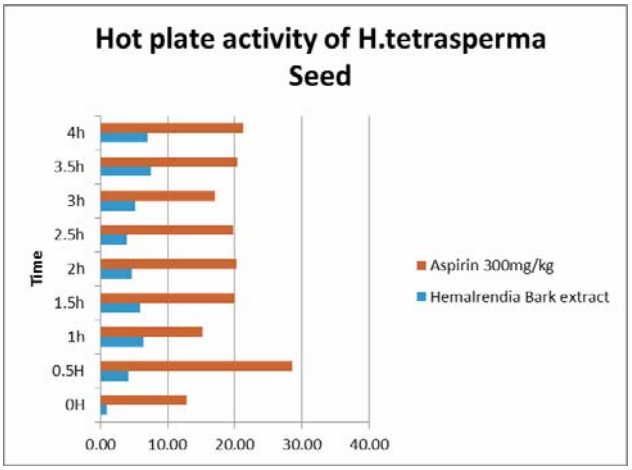


**Fig. 5**: Hot plate activity of seed methanolic extract of *H.tetrasperma*

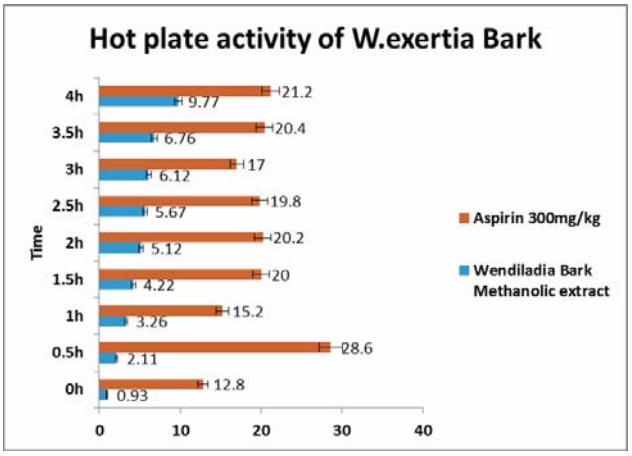


**Fig. 6**: Hot plate activity of bark methanolic extract of *W. exerta*

### *Preparation of methanolic extract*

The powdered plant material (250g) was taken in a flask and was soaked in about 1L of 85% methanol kept for a

period of 7days with shaking and stirring occasionally. The plant material filtered with white cotton material and then again filtered using Whatman filter paper. The filtrate (methanol crude extract) was desiccated using a rotary evaporator.

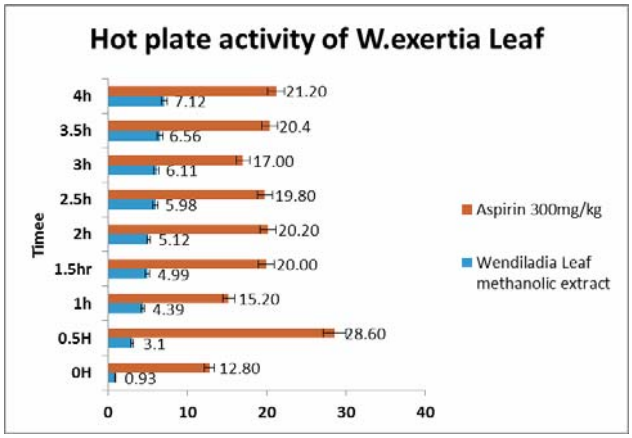


**Fig. 7**: Hot plate activity of leaf methanolic extract of *W. exerta*

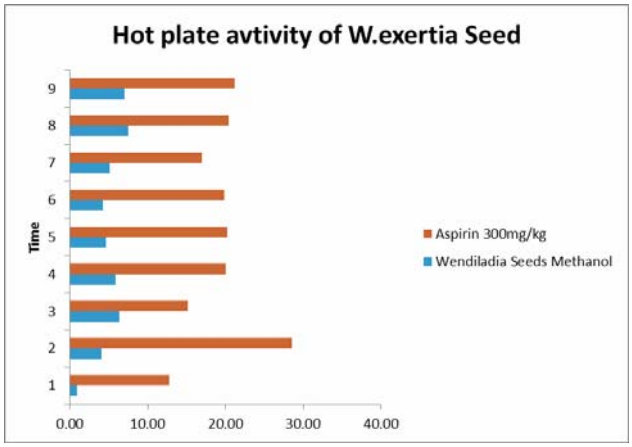


**Fig. 8**: Hot plate activity of seed methanolic extract of *W. exerta*

***Animals***

In this study Swiss albino male mice (23-28g) were used which were obtained from Tolenton market Lahore and were kept under ideal temperature and light. All animal were kept in animal house at controlled temperature 22±1 C under 12h dark and light cycles. All the animals had free access to food and water *ad libitum*. The protocol followed to carry out the project was approved by Animal Ethical committee of Punjab University College of Pharmacy. (AEC/UCP/1042/4313).

***Analgesic activity***

Analgesic activity of *H. tetrasperma* and *W. exserta* was studied using 2 analgesic activity procedures.

***Acetic acid induced writhing***

The methanolic extracts of *H. tetrasperma* and *W. exserta* were examined for analgesic activity using acetic acid-induced writhing method following Ahmed *et al*. (2004). Four groups of male mice were made each group contain five animals. Group I used as control and was administered vehicle (1% Tween 80in water, 10mL/kg body weight). Diclofenac sodium as standard drug was administered to Group II mice at the doses of 10mg/kg/day while Group III and IV received the sample extract at dose (250mg/kg/day) intraperitoneal 30minutes before intraperitoneal administration of acetic acid solution (0.6%) at a dose of 10mL/kg/day body weight. A period of 5 minutes was given to each animal to ensure bio-availability of acetic acid, following which period, the number of writhing was counted for 0-15 and 15-30 minutes after dose. The formula for calculating the percentage inhibition was:

Average writhes in the control group minus average writhes in the test group divided by average writhes in the control group multiplied by 100.

***Hot plate test***

To determine analgesic activity using hot plate test, mice were placed on a hot plate (54ºC±2) and their reaction to heat was noticed. The animals were abruptly removed when raised and lick front paws.

***Quantitative tests for secondary metabolites***

To determine the quantity of the secondary metabolites standard methods were used. Alkaloids, flavonoids and saponins were determined by using the reported method of Harborne (1998) where as phenols and tannins were measured following Pearson (1976).

## RESULTS

The present study carried out on the *H. tetrasperma* and *W. exserta* showed the presence of medicinal active constituents including analgesic compounds. Quantitative analysis of phytochemicals of *H. tetrasperma* was given in table 1 which revealed that bark contained highest value of saponins (30.21±0.8), and flavonoids (17.50± 1.2), phenolic compounds (23.25±0.5) and tannins (12.32 ±0.4), whereas the leaf contain the maximum value of phenols (19.21±0.7), alkaloids (15.10±0.7), flavonoids (14.30±0.4), saponins (11.33±0.6) and tannins (06.03± 0.01). Seed showed maximum value of saponins (21.22± 0.7), flavonoids (13.30±0.3), alkaloids (11.50±0.5), phenols (09.33±0.09) and tannins (05.07±0.3) (fig. 1).

Quantitative analysis of *W. exserta* (table 2) bark contained maximum value of alkaloids (16.41±0.5), leaves contain highest value of flavonoids (14.51±0.3) saponin (10.23±0.4), phenolic compounds (10.33±0.5) and tannins (06.21±0.02) whereas the leaf contain the maximum value of flavonoids (14.51±0.3), alkaloids (13.36±0.3), saponins (12.22±0.1), phenols (11.31±0.4) and tannins (05.31±0.07). Seed contain maximum amount of flavonoids (12.30±0.3), saponins (11.21±0.2), alkaloids

(10.55±0.5), phenols (10.40±0.6) and tannins (07.60±0.3) (fig. 2).

Acetic acid induced writhing in mice and Hot plate analgesic activity is evaluated in selected plant *H. tetrasperma* and *W. exserta*. Methanolic extracts were only chosen for the activity. All the extracts showed excellent results as compare with the standard drug aspirin. Leaf, seed and bark of *H. tetrasperma* showed 81.33, 86.73 and 82% inhibition respectively at first phase while the aspirin showed 52.73%. At the second phase, leaf, seed and bark showed 99.8, 92.27 and 92.95% while the aspirin showed 28.57% inhibition respectively (table 3).

*W. exserta* methanolic extract also displayed excellent results. Leaf, seeds and bark extracts have 82.6, 82 and 97.3% inhibition respectively while the aspirin showed 52.73% in first phase, while in the second phase the leaf, seed and bark showed 89.4, 87.3 and 99.8% respectively (table 4).

Hot plate analgesic activity of the selected plant *H. tetrasperma* is carried out by the methanolic extracts. The mice licked the front paws at that point the reading is noted. Observation is collected at 0hour, 0.5h, 1h, 1.5h, 2h, 2.5h, 3h, 3.5, 4h and 4.5. Bark extract display maximum activity at 4.5h stage i.e., 8.6±0.40 (table 5).

Hot plate analgesic activity of the selected plant *W. exserta* is carried out only by the methanolic extracts. The mice licked the front paws at that point the reading is noted. Observation is collected at 0hour, 0.5h, 1h, 1.5h, 2h, 2.5h, 3h, 3.5, 4h and 4.5. Bark extract displayed maximum activity at 4.5h stage, i.e. 9.7±0.5 (table 6).

## DISCUSSION

This study revealed the analgesic activity of methanolic extracts of *H. tetrasperma* and *W. exserta* by acetic acid-induced writhing test in male mice and Hot plate analgesic activity. Both the selected plant species contains important secondary metabolites such as flavonoids, tannins, alkaloids and phenolic compounds in leaves, bark and seeds.

Analgesic activity of methanolic extracts of *H. tetrasperma* and *W. exserta* was examined using writhing induced by acetic acid and hot plate analgesic models. Acetic acid writhing test is used to evaluate the drugs having peripheral analgesic effect. The IP injection of acetic acid produce pain by the releasing of endogenous mediators, such as serotonin histamine, prostaglandins (PGs), bradykinins and substance P, endings. Peritoneal pain receptors are involved in the abdominal contractions. Decrease in abdominal constrictions and stretching of hind limbs of mice induced by the intraperitoneal injection of acetic acid indicates peripheral analgesia as per reported methods (Gené *et al.* 1989; Bentley *et al.* 1983).

All the extracts plants, i.e. *H. tetrasperma* and *W. exserta* displayed good results as compared with the standard drug aspirin. Bark, leaf and seed of *H. tetrasperma* showed 82, 81.33 and 86.73% inhibition respectively at first phase while the aspirin showed 52.73%. At the second phase, Bark, leaf and seed showed 92.95, 99.8 and 92.27% inhibition respectively. *W. exserta* methanolic extract also displayed good results (fig. 3-8). Bark, leaves and seeds extracts have 97.3, 82.6 and 82% inhibition respectively in first phase, while in the second phase the bark, leaf and seed showed 99.8, 89.4 and 87.3 respectively. At second phase both plants showed maximum results. Similar findings were also noticed by Ali *et al.* (2012) while working on leaf extract (ethanolic) of *Typhonium trilobatum* to determine analgesic activity.

On the other hand hot plate model is used to evaluate the drugs having centrally mediated antinociceptive response. A series of complex processes such as opiate, dopaminergic descending noradrenergic and serotonergic systems are well working when pain is controlled centrally as reported by Wigdor and Wilcox (1987).

Hot plate analgesic activity of the selected plants *H. tetrasperma* and *W. exserta* also showed good results i.e Bark extract of *H. tetrasperma* showed maximum activity at 4.5h stage, *i.e.* 8.6±0.40 and Bark extract of *W. exserta* showed maximum activity at 4.5h stage, *i.e.* 9.7±0.5. Same results were listed by Pandey *et al*. (2014) and Yusufoglu (2014) that they worked on *Vernonia anthel mintica* and *Pulicaria arabica* respectively by using hot plate and writhing method for analgesic effect.

The secondary metabolites are present in *H. tetrasperma* and *W. exertia.* In selected parts, *i.e.* leaf, bark and seed, the alkaloids, flavonoids, tannins, saponins, and phenols are present in good amount. This result is similar with the results of Nisa *et al.* (2013) that worked on phytochemical analysis of *Rumex dentatus* L.

## CONCLUSION

*H. tetrasperma* and *W. exertia* are medicinal plants that contain active compounds and have potential to reduce the pain in comparison with available commercial analgesic drugs. However, these plants can be used for further investigation for isolation, identification and characterization of different active constituents and their mode of action and therapeutic range.

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