Pharmacological Evaluation of Methanolic Extract of Selected Medicinal Plants

Sajid Ali[1], Syed Awais Shah[1], Hamid Ullah[1], Jawad Akbar[1], Muhammad Sajjad Khan[1], Inzemam Ul Haq[1], Adil Muhammad[1]

1 Department of Chemical and Life Science, Qurtuba University of Science and Information Technology Peshawar/ DI Khan, Khyber Pakhtunkhwa Pakistan.

Abstract The pharmacognostic features of methanolic extracts from *Carthamus oxyacantha*, *Thevetia Peruviana and Otostegia limbata* were examined aimed at their potential as anti-inflammatory, antispasmodic, antipyretic and analgesic agents. The methanolic extract (200 & 400 mg/kg) were employed. Carrageenin-induced paw edema and Acetic acid-induced writhing were used for anti-inflammatory and Analgesic activities assessed in mice correspondingly. Anti-pyretic activity was evaluated through brewer's yeast-induced pyrexia while charcoal meal tests was used to evaluate anti-spasmodic activity in mice. The results revealed significant ($p < 0.05$) inhibition of acetic acid-induced writhing in mice in a dose-dependent manner, with *Otostegia limbata* demonstrating the highest inhibition at 45.45%. In anti-inflammatory activity, *Carthamus oxyacantha* and *Thevetia Peruviana* exhibited the highest reduction in inflammation from 1.3-1.0 cm after 4 hours. Anti-pyretic potential of selected plants were not satisfying compared to Paracetamol (standard drug), while it showed impressive results compared to control. *Otostegia limbata* showed the highest reduction in rectal temperature, lowering it from 38.39-38.11 ℃ at 4 hours. The antispasmodic response of tested medicinal plants was substantial, with *Otostegia limbata* showing the highest charcoal meal movement at 51.3%, followed by *Carthamus oxyacantha* at 35.7%. In conclusion, antispasmodic, analgesic, antipyretic and anti-inflammatory properties were observed in all selected plants. Additionally, pharmacognostic study of these plants opens new avenues for research and provides valuable insights for research scholars conducting molecular and phyto-chemical investigations on these plants.

Index-term: anti-inflammatory, analgesic, antipyretic, methanolic extract, anti-spasmodic.

I INTRODUCTION

Pharmacognosy, the study of medicinal derivatives from plants and other natural sources, involves the standardization, substantiation, and exploration of naturally derived drugs (Chanda, 2014). The term "pharmacognosy" was first used by Seydler and originates from the words 'pharmakon' and 'gignoso,' meaning to gain knowledge. This field delves into the biological, physical and chemical properties of raw materials in plants for preparation, drug development, and cultivation (Gokhele et al., 2008). Herbal medicines, derived from plants, have been utilized for various disease treatments. The chemical compounds occurring naturally and their derivatives have helped therapeutic purposes for several years. Modern techniques have advanced the analysis of natural components to determine new drugs, emphasizing bio-diversity, variability and chemical structure (Lahlou, 2013). Plants can synthesize numerous chemical compounds like cyanogen, alkaloids, quinines, glycosides, flavonoids, terpenes and tannins within cells and tissues, exerting specific effects on human body such as physiological effects. For instance, the antimicrobial drug from *Cephaelis ipecacuanha* contains phytochemicals, and the bark of *Cinchona* yields the malaria treatment alkaloid Quinine. Phytochemicals are distributed in various plant parts and differ among species and even within the same plant. Some, like aristolochic acid, are toxic, instigating cancer. Plants serve as rich bases of chemical constituents such as galantamine, caffeine, digoxin, salicylic acid, vincristine and morphine. Plants are rich in Phytochemicals, naturally occurring organic compounds with therapeutic uses (Hasler and Blumberg, 1999). Medicinal plants, containing phytochemicals in their cells and tissues, are now employed in treating chronic diseases like diabetes, heart diseases and cancer. These secondary metabolites possess anti-cancer, antioxidant, anti-allergic, anti-inflammatory and immunity-protecting properties (Bandaranayake, 2006). The practice of herbs is expanding globally due to their

minimal or no side effects, easy accessibility and cost-effectiveness. With approximately a quarter of a million herbs globally yet to be pharmaceutically researched, medicinal plants hold promising potential (Lajayer et al., 2017).

II METRIAL AND METHODS

Plants collection and preservation

The plants selected for this study were *Carthamus oxyacantha* M.Bieb, *Otostegia limbata* (Benth) Boiss. and *Thevetia Peruviana* (Pers.) K. Schum. The choice of these plants was based on their easy availability, medicinal value, and the fact that they are still not fully explored pharmacologically. Once the selection was made, healthy and fresh whole plants were collected. *Thevetia Peruviana* and *Carthamus oxyacantha* were obtained from district Mardan and *Otostegia limbata* was sourced from (Khairabad) district Nowshera. The identification of these plants was confirmed from existing literature. To ensure the best results and purity, the gathered plants' samples were washed with clean water to remove contaminants, dirt and solid particulates. Subsequently, the plants were shade-dried for 35 days to avoid the evaporation of bio-active compounds in plant due to heat and light. Drying served the purpose of eliminating water content, protecting the leaves from spoilage, and preventing fungal attacks. The dried leaves were then ground using a blender machine, resulting in a powder with particle sizes smaller than 1mm. Both dried and fresh particular plants were randomly sampled for moisture-content determination. The crushed materials (250g) were individually mixed with 10 volumes (v/w) of methanol in Erlenmeyer flasks, and left for a week at room temperature. After centrifugation, the supernatant was processed in rotary evaporator under low pressure at 40 ^{o}C to remove all the solvent, and pure plant extract was obtained in the rotary flask. This pure extract was transferred to china dish and for more processing, diluted in distilled water.

Experimental animals

Albino mice of Swiss origin, both female and male, weighing approximately 25-34 grams, were supplied by the Veterinary Research Institute, Peshawar (VRI). The experiment upheld appropriate nutritional and environmental conditions for the mice throughout. Prior to the commencement of the study, the mice were given standard animal water and feed.

Drugs and Chemicals

The testing drugs in this study comprised extracts from *Carthamus oxyacantha*, *Thevetia peruviana* and *Otostegia limbata*. As standard drugs, atropine sulfate, aspirin, diclofenac sodium, and paracetamol were utilized. The chemicals acetic acid, carrageenan, brewer's yeast, methanol, and charcoal were also employed in the experimental procedures.

Biological activity

Analgesic Activity

The acetic acid-induced writhing method was used to assessed the analgesic activity (Koster et al., 1959). Four groups were established containing two mice in each group. Normal saline was injected to Group 1 with dosage of 10 ml/kg and labelled as negative control. Group 2 (positive control) was injected a standard drug (aspirin) with dosage of 10 mg/kg. Groups 3 and 4 received plant extracts at doses of 200 and 400 mg/kg, correspondingly. Each mouse in every group was injected with acetic acid. The writhing was counted (caused by acetic acid) for a duration of 5 minutes, starting 15 minutes after the injection of the standard drug or plant extracts.

Anti-Inflammatory activity

The carrageenan-induced mice paw edema method was used to assessed the anti-inflammatory response proposed by Winter et al. (1962). Four groups, each consisting of 2 mice, were established. Group 1 received an injection of saline (10 ml/kg). Group 2 received treatment with a standard medication (Diclofenac sodium), whereas groups 3 and 4 were given doses of 200 mg/kg and 400 mg/kg of the plant extract, respectively. Carrageenan was applied to each group, and the paw volume of the mice was measured. Subsequently, plant extract was injected, and paw measurements were taken at intervals up to four hours and recorded. At the onset of carrageenan injection, the paw volume underwent

immediate measurement and was subsequently assessed hourly for up to 3 hours. The precise edema volume was ascertained by computing the variance between the initial and subsequent measurements. The percentage of inhibition was determined using the formula % inhibition = 100 (1 - Vt/Vc), where 'Vc' denotes the edema volume within the control group, and 'VT' signifies the edema volume within the groups subjected to extract application.

Anti-pyretic activity

The antipyretic potential of *Carthamus oxyacanthus*, *Otostegia limbata* and *Thevetia peruviana* was investigated using the method of inducing pyrexia in mice with brewer's yeast. Four groups of mice were established, each comprising two mice. The first group served as the negative control and was administered a normal saline solution (10 ml/kg). Group 2 was treated with the standard medication (Paracetamol), while groups 3 and 4 were given doses of the plant extract at 200 mg/kg and 400 mg/kg, respectively. Initially, the baseline temperature of all groups was recorded using a thermometer. Subsequently, brewer's yeast was injected subcutaneously into each group to induce an increase in the mice's temperature. The temperature was recorded after the 18th hour post yeast injection. Paracetamol was used as the standard drug. Following this, both the plant extracts and the standard drug were injected, and the temperature was measured at regular intervals - 1st, 2nd, 3rd, and 4th hour after injection (Barkatullah et al., 1977).

Anti-spasmodic activity

The antispasmodic effects of the extracts from *Carthamus oxyacanthus*, *Thevetia peruviana* and *Otostegia limbata* were assessed using the charcoal meal movement method. Four groups of Swiss albino mice, each consisting of two individuals, were established. Groups 1 and 2 were subjected to alternating injections of normal saline (10 ml/kg) and the standard drug (atropine sulfate), respectively. Meanwhile, groups 3 and 4 received alternating treatments of plant extracts at doses of 200 mg/kg and 400 mg/kg. Following this, each group was provided with a charcoal meal (1 ml/kg). After a 15-minute interval post charcoal meal administration, the intestine was isolated, and measurements were obtained for both the intestinal length (from the end of the duodenum to the caecum) and the extent of charcoal movement within the intestine (Mbagwu and Adeyemi, 2008).

III. RESULT AND DISCUSSION

My research was centered on exploring the antispasmodic, antipyretic, analgesic and anti-inflammatory capabilities of two distinct concentrations (200mg/kg and 400mg/kg) of the methanolic extract derived from *Otostegia limbata* (Benth) Boiss, *Thevetia Peruviana* (Pers.) K. Schum and *Carthamus oxyacantha* M. Bieb.

Analgesic activity

Carthamus oxyacantha M.Bieb

The figure (Fig.1) illustrates the analgesic effects of *Carthamus oxyacantha* on acetic acid-induced writhing in mice. After the administration of different doses of the *C. oxyacantha* extract, a dose-dependent decrease in acetic acid-induced writhing was observed. The methanolic extract, specifically at doses of 200 mg/kg and 400 mg/kg, resulted in reductions of 9.2% and 32.5%, respectively, in contrast to the 62.79% reduction seen with aspirin. The data suggests that the extract at a concentration of 400 mg/kg exhibited a noteworthy effect compared to the negative control.

Otostegia limbata (Benth) Boiss

The effects of the methanolic extract derived from *Otostegia limbata* on the writhing response in mice are depicted in (Fig.2). The findings indicated a decrease in the writhing response induced by acetic acid with increasing

concentrations of the *Otostegia limbata* methanolic extract. At doses of 200 mg/kg and 400 mg/kg, the methanolic extract, in conjunction with aspirin, displayed inhibition of the writhing response in mice by 27.27% and 45.45%, respectively, whereas aspirin achieved a 60.6% reduction. These findings suggest a robust analgesic potential in the plant.

Thevetia peruviana (Pers.) K. Schum

As depicted in (Fig.4.2), the intraperitoneal injection of various concentrations of *Thevetia peruviana* (200 and 400 mg/kg) led to a reduction in writhing induced by acetic acid, with the maximum response observed at 400 mg/kg, showing a 40% reduction. These outcomes were comparable to the standard drug (Aspirin), which demonstrated a 54% inhibition at a concentration of 10 mg/kg. The inferior dosage (200 mg/kg) exhibited a 36% inhibition. These findings suggest that plant possesses robust analgesic potential.

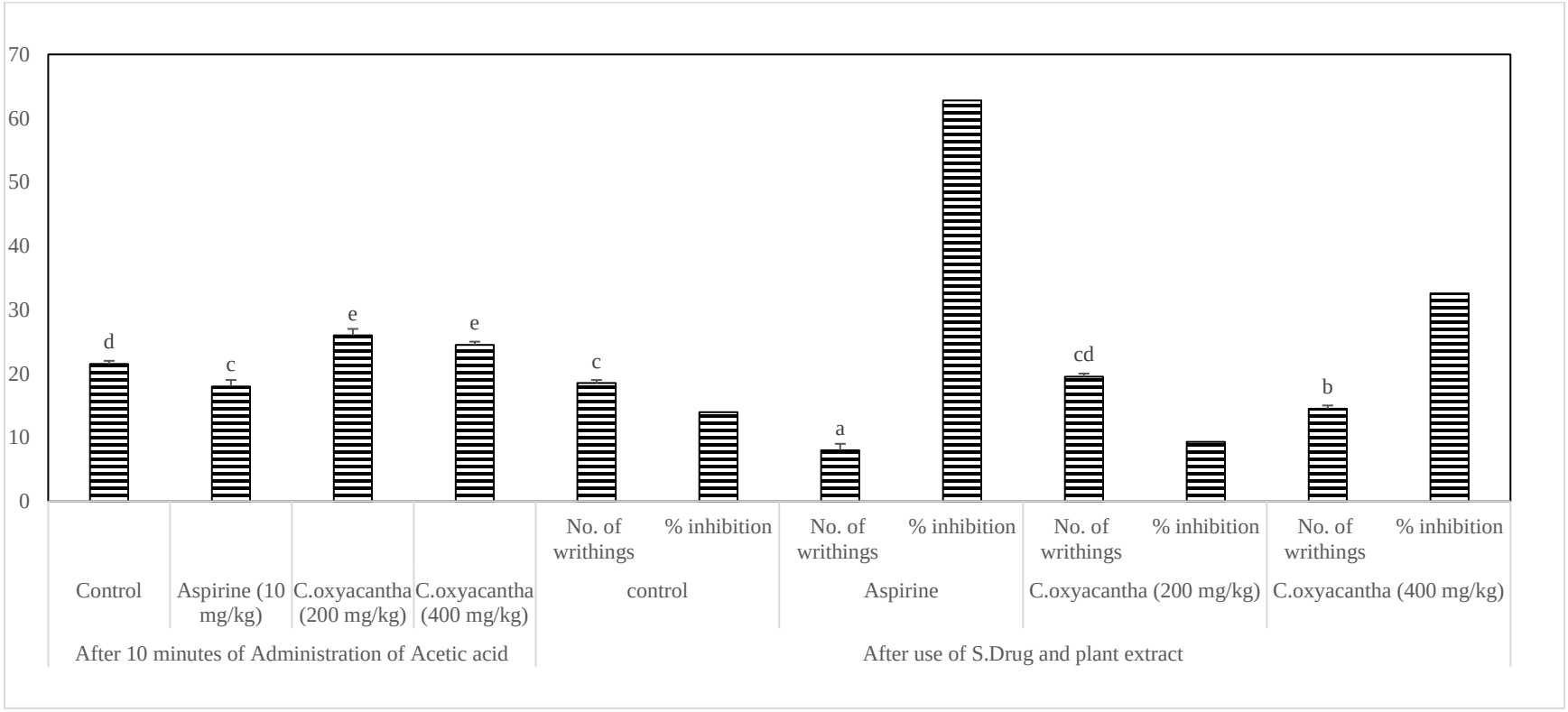


Figure 1: The influence of different concentrations of the methanolic extract obtained from Carthamus oxyacantha on acetic acid-induced writhing in mice is depicted. The data represents the mean of two replicates, presented with standard error bars. Significant differences ($p < 0.05$) are denoted by distinct alphabetical letters.

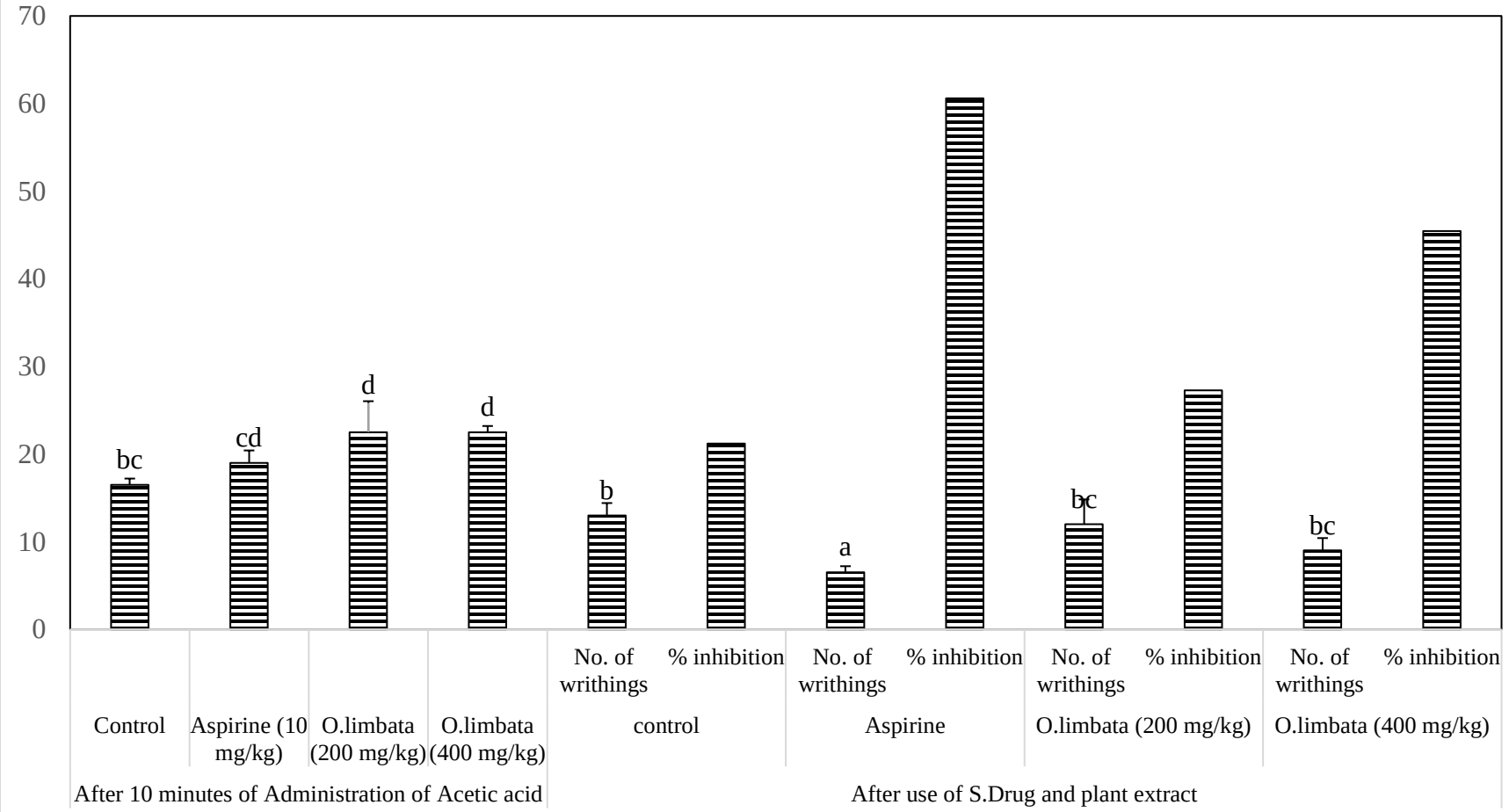


Figure 2: The impact of varying doses of methanolic extract derived from *Otostegia limbata* on acetic acid-induced writhing in mice is displayed. The data represents the mean of two replicates along with standard error bars, and significant differences ($p < 0.05$) are marked by distinct alphabetical letters.

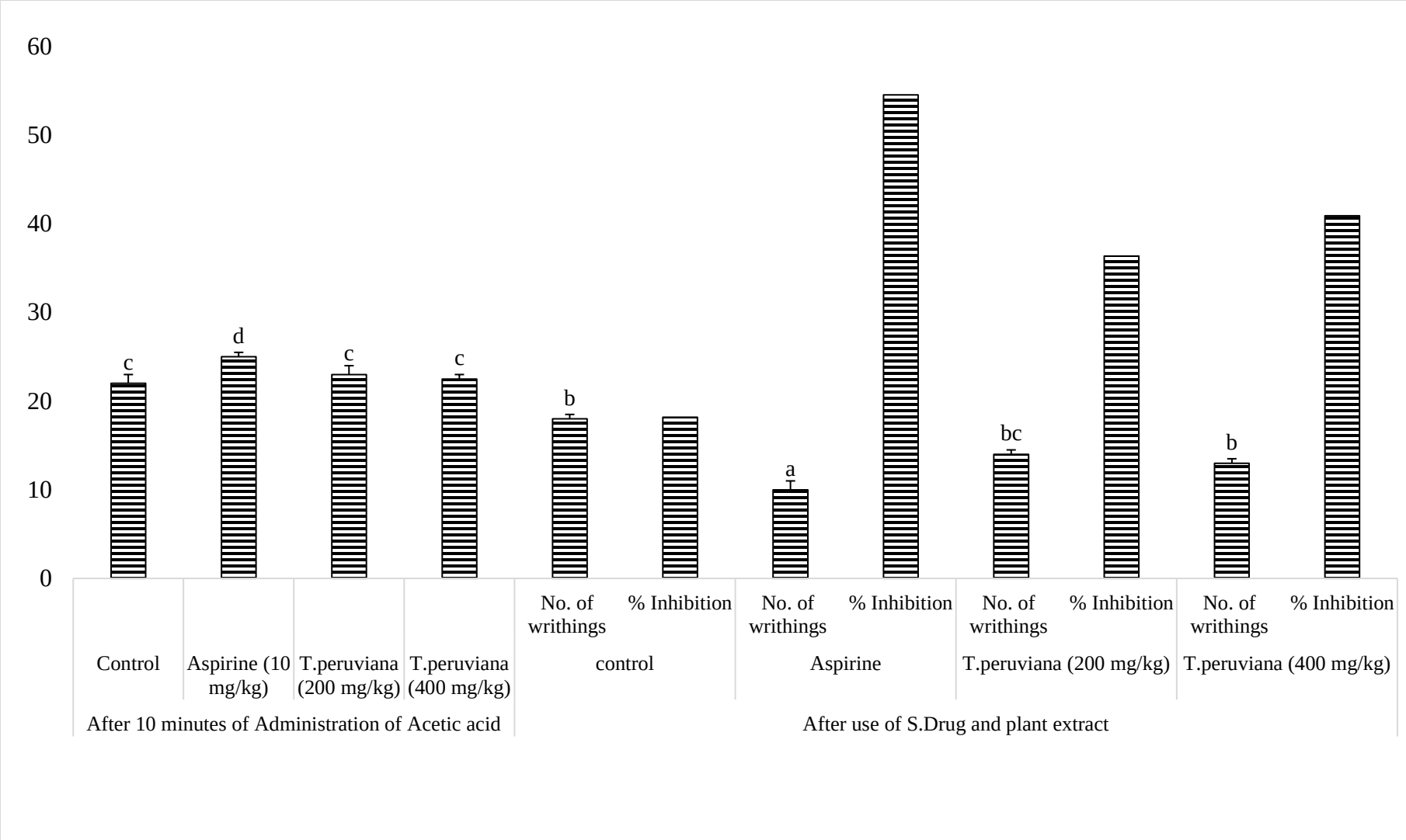


Figure 3: The impact of varying doses of the methanolic extract from *T. peruviana* on acetic acid-induced writhing in mice is depicted. The presented data shows the mean of two replicates, displayed with standard error bars. Notable differences ($p < 0.05$) are highlighted by distinct alphabetical letters.

Anti-inflammatory activity

Anti-inflammatory activity was studied for three different plants**.**

Carthamus oxyacantha

Localized edema was induced by the injection of carrageenan, with inflammation gradually improving to a maximum size post-carrageenan injection. The anti-inflammatory impact of *C. oxyacantha* on carrageenan-induced mice paw edema was assessed at the end of the 1st, 2nd, 3rd and 4th hours, as depicted in (Fig. 4). The results reveal a dose and time-dependent decrease in paw volume. The standard drug (Diclofenac sodium) exhibited the maximum reduction after the 4th hour, decreasing from 1.45-1.0 cm compared to the control. The 200 mg/kg dose of the plant extract also exhibited significant inhibition, reducing from 1.3-1.05 cm after the 4th hour. Similarly, the high dosage (400 mg/kg) of the plant extract demonstrated a comparable outcome to the standard drug, reducing from 1.35-1.0 cm after the 4th hour. The findings illustrated in (Fig. 4) suggest a promising anti-inflammatory response from the plants.

Otostegia limbata

The anti-inflammatory efficacy of *Otostegia limbata* at doses of 200 and 400 mg/kg is visualized in (Fig. 5), displaying the baseline paw edema volume. Post carrageenan injection, paw edema exhibited a gradual increase over time. The administration of the higher dosage (400 mg/kg) of *O. limbata* resulted in outcomes akin to the standard drug (Diclofenac sodium), reducing paw edema from 1.35-1.0 cm by the 4th hour. The standard drug exhibited the highest reduction in paw edema, decreasing inflammation from 1.35 to 0.90 cm after the 4th hour. The lower dose (200 mg/kg) also demonstrated promising results, reducing paw swelling from 1.35 to 1.1 cm after the 4th hour. Notably, all treatments involving the plant extract of *O. limbata* consistently reduced edema volume after the 1st, 2nd, 3rd and 4th hours of drug administration.

Thevetia peruviana

Following carrageenan injection, there was an observed increase in paw volume. Illustrated in (Fig. 6), the anti-inflammatory effects of varying doses (200 and 400 mg/kg) of *Thevetia peruviana* showcased a gradual decrease in paw edema volume over the 1st, 2nd, 3rd and 4th hours post drug administration. Both doses (200 and 400 mg/kg) of the plant extract displayed a decrease in carrageenan-induced paw volume that was dependent on both time and dose. The higher dose (400 mg/kg) exhibited inflammation reduction comparable to the standard drug (Diclofenac sodium), reducing from 1.45 to 1.05 cm after the 4th hour, while the standard drug decreased paw edema volume from 1.3 to 1.0 cm after the 4th hour. Notably, the lower dose of *T. peruviana* also displayed a significant reduction in inflammation from 1.3 to 1.10 cm after the 4th hour. These findings suggest a progressive reduction in paw edema volume due to the plant *T. peruviana's* anti-inflammatory properties.

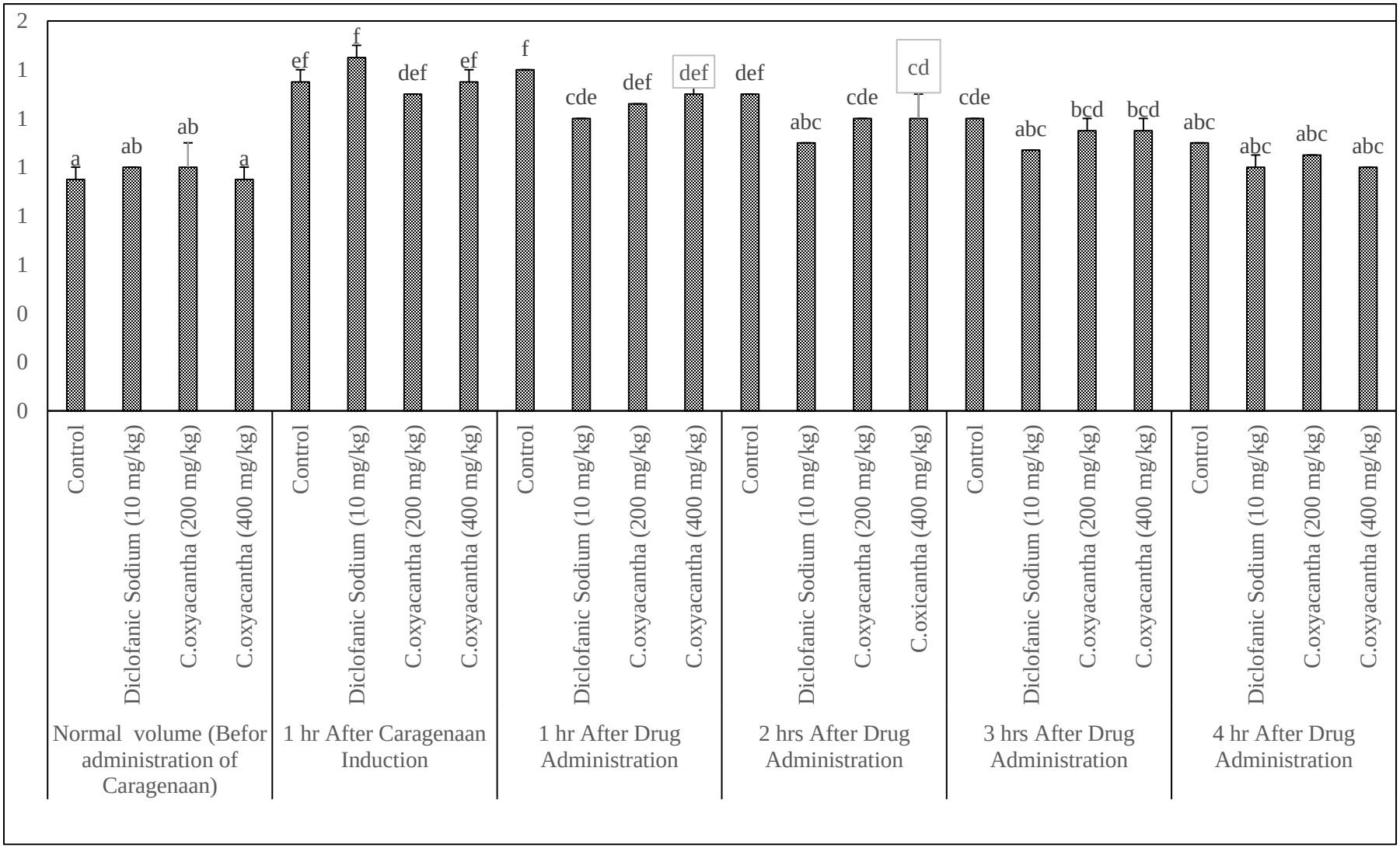


Figure 4: The impact of varying doses of methanolic extract from *Carthamus oxyacantha* on carrageenan-induced inflammation in mice is depicted. The data presented represent the mean of two replicates with standard error bars. Different alphabetical letters specify a significant difference ($p < 0.05$) among the groups.

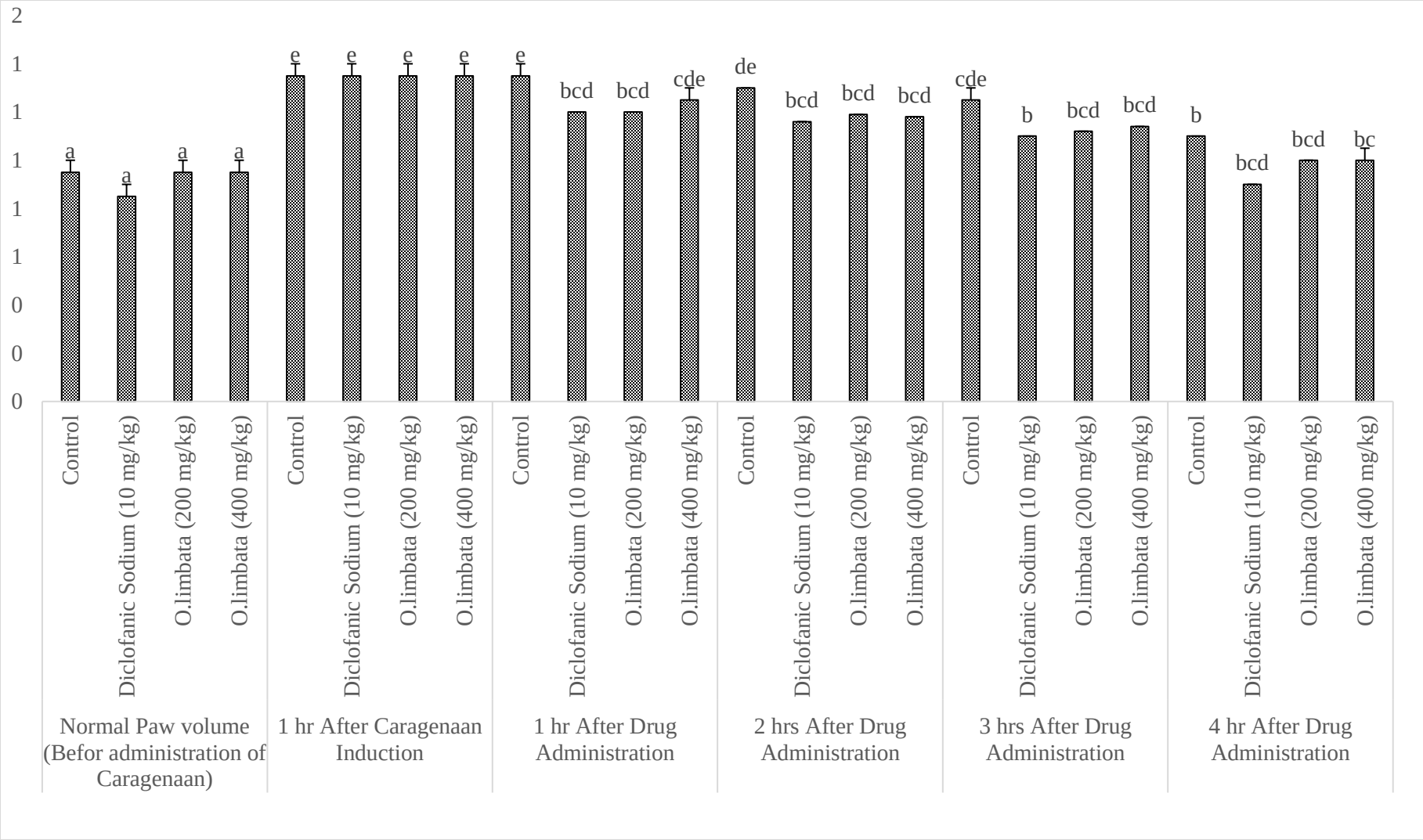
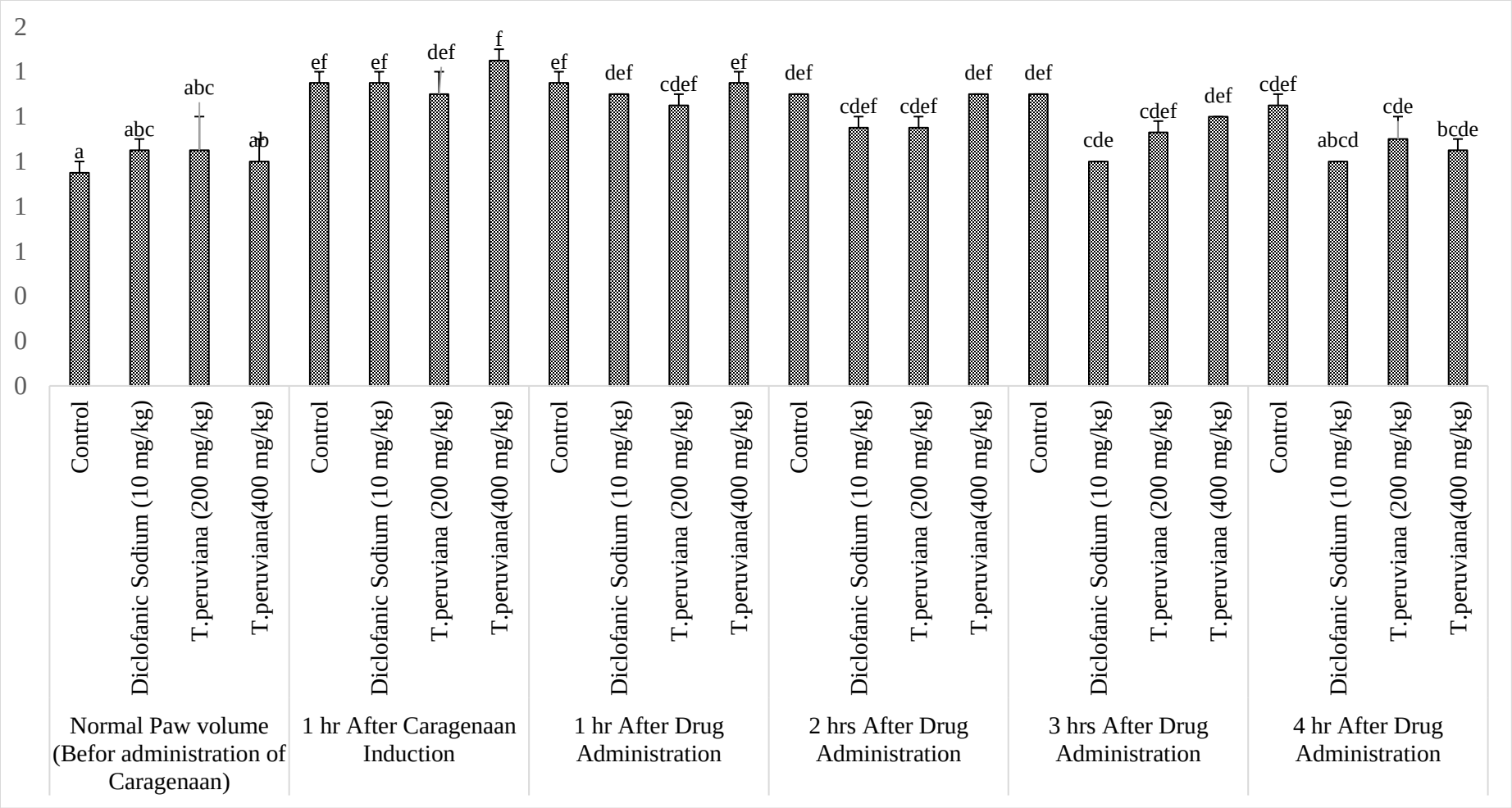


Figure 5: The influence of different doses of methanolic fraction from *O. limbata* on carrageenan-induced inflammation in mice is illustrated. The data, presented as the mean of two replicates, include standard error bars. Distinct alphabetical letters are indicative of a significant difference ($p < 0.05$) among the groups.


2
1
1
1
1
0
0
0
a
abc
abc
ab
ef
ef
def
f
ef
def
cdef
ef
def
cdef
cdef
def
def
cde
cdef
def
cdef
abcd
cde
bcde
Control
Diclofanic Sodium (10 mg/kg)
T.peruviana (200 mg/kg)
T.peruviana(400 mg/kg)
Normal Paw volume (Befor administration of Caragenaan)
1 hr After Caragenaan Induction
1 hr After Drug Administration
2 hrs After Drug Administration
3 hrs After Drug Administration
4 hr After Drug Administration

Antipyretic activity

Carthamus oxyacantha

The assessment of the antipyretic effect was conducted in mice 18 hours post the administration of brewer's yeast. Illustrated in (Fig. 7), a noticeable increase in temperature was observed after 18 hours of yeast administration, indicating induced pyrexia in mice. The results revealed that two distinct concentrations (200 and 400 mg/kg) of *C. oxyacantha* led to a reduction in rectal temperature in mice in a dose and time-dependent manner. Paracetamol demonstrated a significant temperature decrease, returning to normal levels from 38.65 to 37.05℃ after the 4th hour. Administration of the higher dose (400 mg/kg) of the methanolic extract of *C. oxyacantha* significantly reduced temperature from 38.5 to 37.45℃ after the 4th hour. The lower dose (200 mg/kg) also resulted in a reduction in rectal temperature, although to a lesser extent, decreasing from 38.5 to 38.1℃ after the 4th hour compared to the negative control. As evident in (Fig. 7), both plant extracts exhibited a significant reduction in rectal temperature.

Otostegia limbata

The effects of different doses of methanolic extracts on rectal temperature in mice are depicted in (Fig. 8). Following 18 hours of yeast injection, the mice showed an increase in rectal temperature. Both concentrations (200 and 400 mg/kg) of *O. limbata* extracts demonstrated a dose and time-dependent reduction in the mice's rectal temperature. The higher dosage (400 mg/kg) notably decreased body temperature from 38.39 to 38.11℃ after the 4th hour of administration. The outcomes from the 200 mg/kg dosage also displayed positive effects compared to the control. Notably, the antipyretic effect of the standard drug (paracetamol) was significantly potent, evident even at the 1st hour, consistently lowering rectal temperature from 38.8 to 36.9℃.

Thevetia peruviana

After 18 hours of brewer's yeast injection, mice exhibited induced pyrexia, resulting in a progressive increase in rectal temperature. (Fig. 9) demonstrates the antipyretic potential of *Thevetia peruviana*. The various doses (200 and 400 mg/kg) of *T. peruviana* displayed a gradual reduction in pyrexia in mice over the 1st, 2nd, 3rd and 4th hours post drug administration. This decrease in temperature was both dose and time-dependent. The higher dose (400 mg/kg) notably reduced rectal temperature from 38.33 to 37.8℃ after the 4th hour, compared to the standard drug. Similarly, the lower dose (200 mg/kg) significantly decreased rectal temperature from 38.33 to 38.09℃ after the 4th hour. Paracetamol exhibited highly significant antipyretic effects, lowering rectal temperature from 38.75 to 37.5℃ after the 4th hour. While the antipyretic effect of *T. peruviana* was not as potent as paracetamol, it remained significant compared to the negative control, as indicated in (Fig. 9)

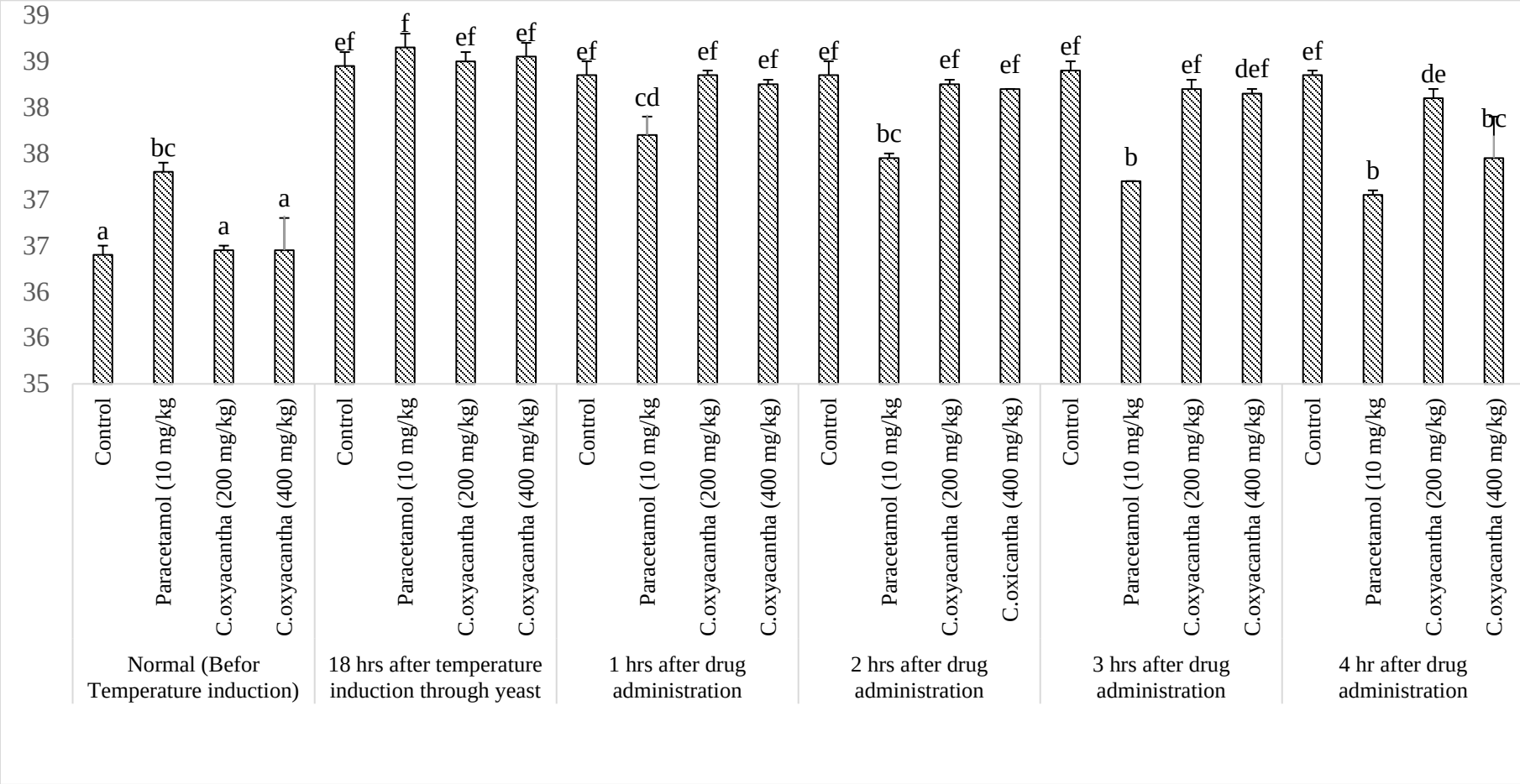


Figure 7: The impact of various doses of methanolic extract from *Carthamus oxyacantha* on Brewer's yeast-induced pyrexia in mice is presented. The data, represented as the mean of two replicates, include standard error bars. Different alphabetical letters indicate a significant difference ($p < 0.05$) among the groups.

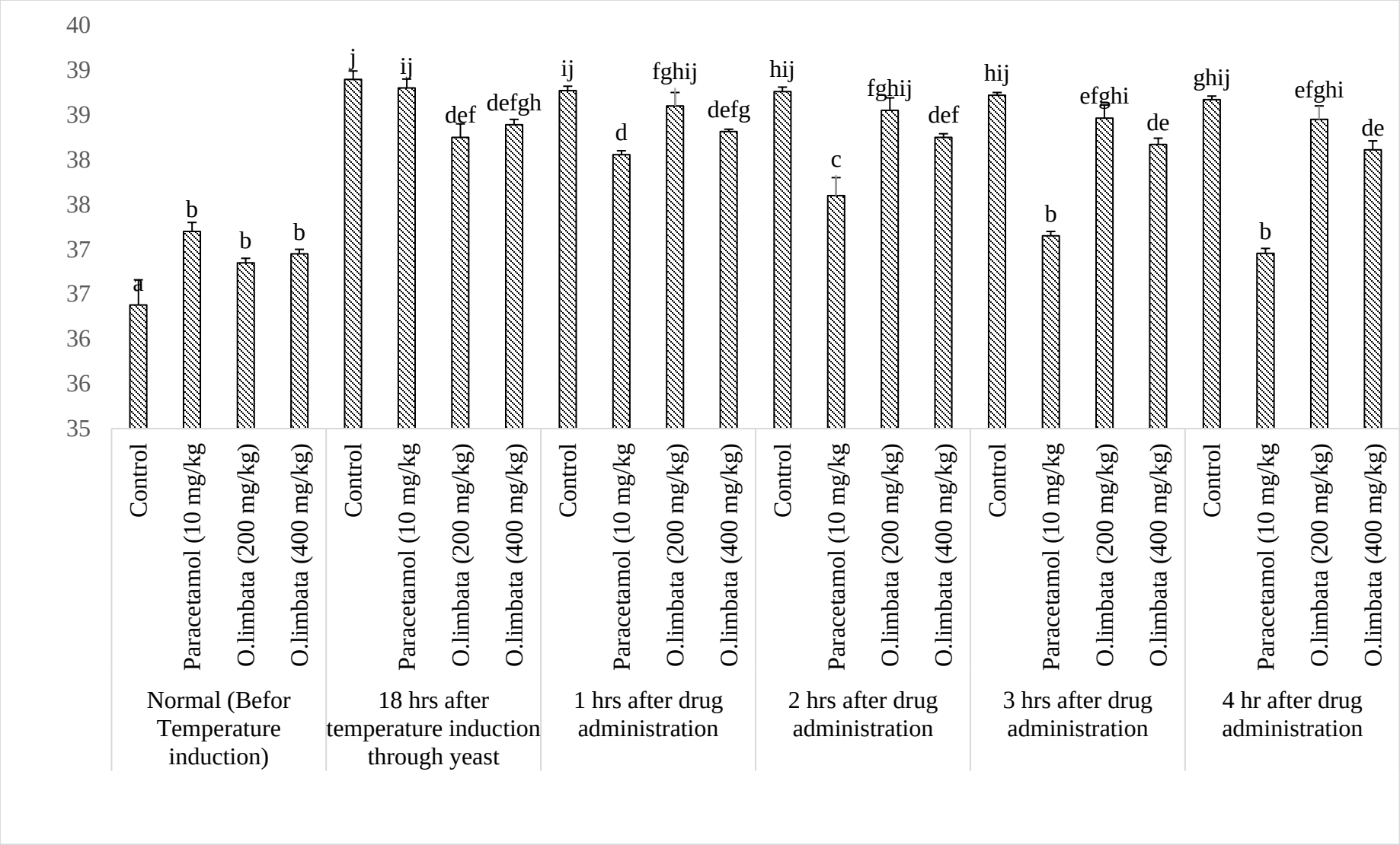


Figure 8: The influence of different doses of methanolic extract from *O. limbata* on Brewer's yeast-induced pyrexia in mice is depicted. The data represents the mean of two replicates, accompanied by standard error bars. Distinct alphabetical letters are used to signify a significant difference ($p < 0.05$) among the groups.

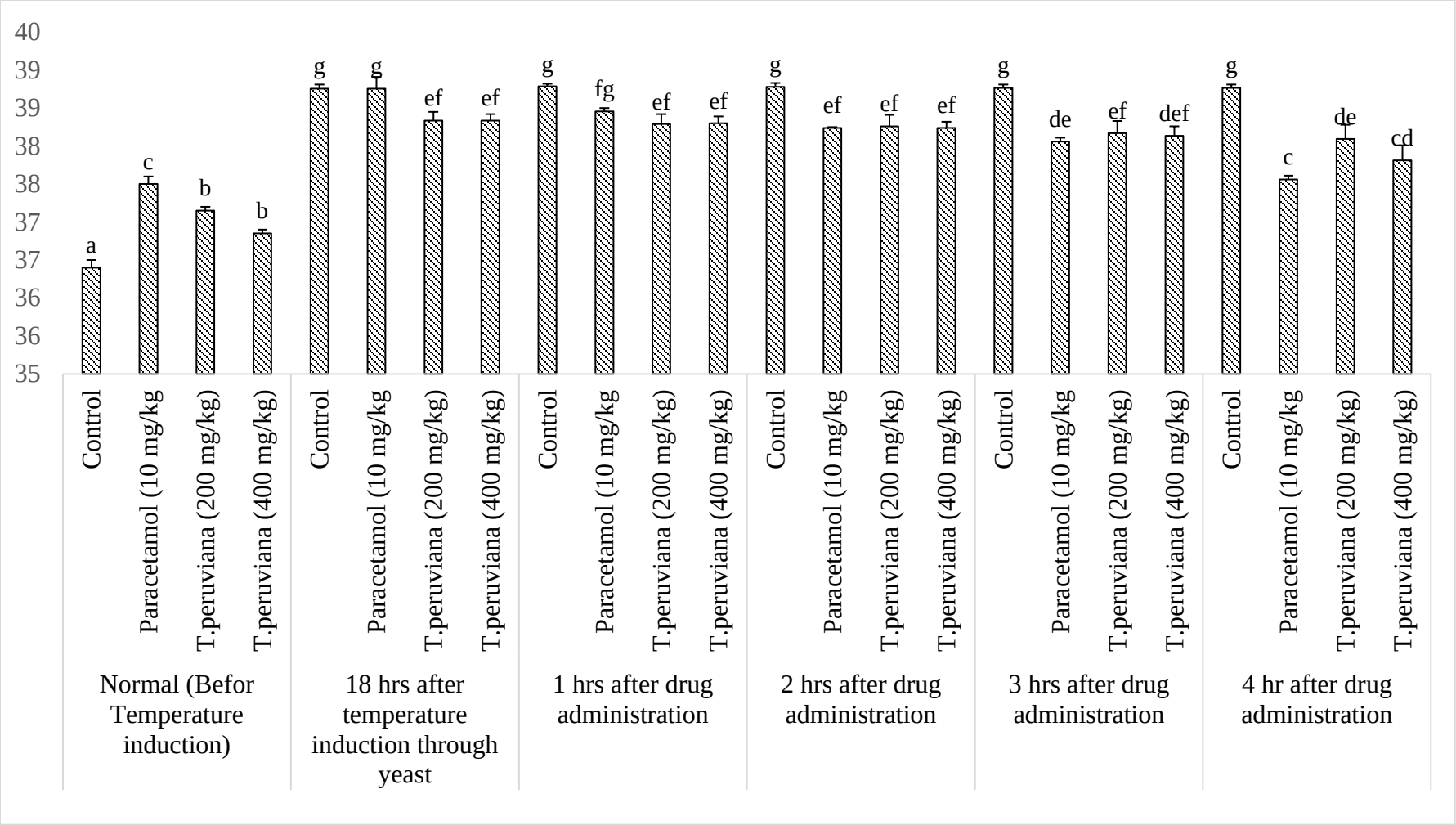


Figure 9: The data depicted as the mean of two replicates along with standard error bars, illustrates the impact of varying doses of methanolic extract derived from *T. peruviana* on Brewer's yeast-induced pyrexia in mice. Notable differences ($p < 0.05$) among the groups are denoted by distinct alphabetical letters.

Antispasmodic activity

Carthamus oxyacantha

The charcoal meal test evaluated the antispasmodic effectiveness of different doses of *Carthamus oxyacantha*. Illustrated in (Fig. 10), the outcomes revealed a dose-dependent inhibitory impact of these plant extracts. At the measured doses of 200 and 400 mg/kg, *C. oxyacantha* displayed a percentage increase in intestinal motility of 30.14% and 35.74%, respectively. In contrast, the percentage results for the control and standard drug (Atropine sulfate) were 14.7% and 52.34%, respectively. While the antispasmodic effect of *C. oxyacantha* did not exceed that of the standard drug, it remained significant when compared to the negative control.

Otostegia limbata

The illustration in (Fig. 11) demonstrates that the methanolic extract obtained from *Otostegia limbata* enhances intestinal motility in a manner dependent on the dosage. The standard drug (Atropine sulfate) exhibited the highest percentage of distance covered by charcoal (64.7%). Comparatively, the control registered 16.03%, while both doses (200 and 400 mg/kg) of the plant extract showcased significant antispasmodic potential. The percentage increase in intestinal motility for both doses (200 and 400 mg/kg) was 42.8% and 51.37%, respectively. These findings from (Fig. 11) distinctly highlight a robust antispasmodic response to the *O. limbata* plant extract.

Thevetia peruviana

The figure (Fig. 12) showcases the antispasmodic effects of different doses of the methanolic extract derived from *Thevetia peruviana*. Atropine sulfate displayed the highest charcoal meal transit at 57.3%. Comparatively, the control group showed 18.5% charcoal movement, while in the *T. peruviana* group, the highest charcoal movement was observed for the (400 mg/kg) dose at 32.8%, followed by (200 mg/kg) at 30.08%. The outcomes highlighted in (Fig. 12) clearly demonstrate that both doses of the plant extract present a significant antispasmodic response in comparison to the positive control.

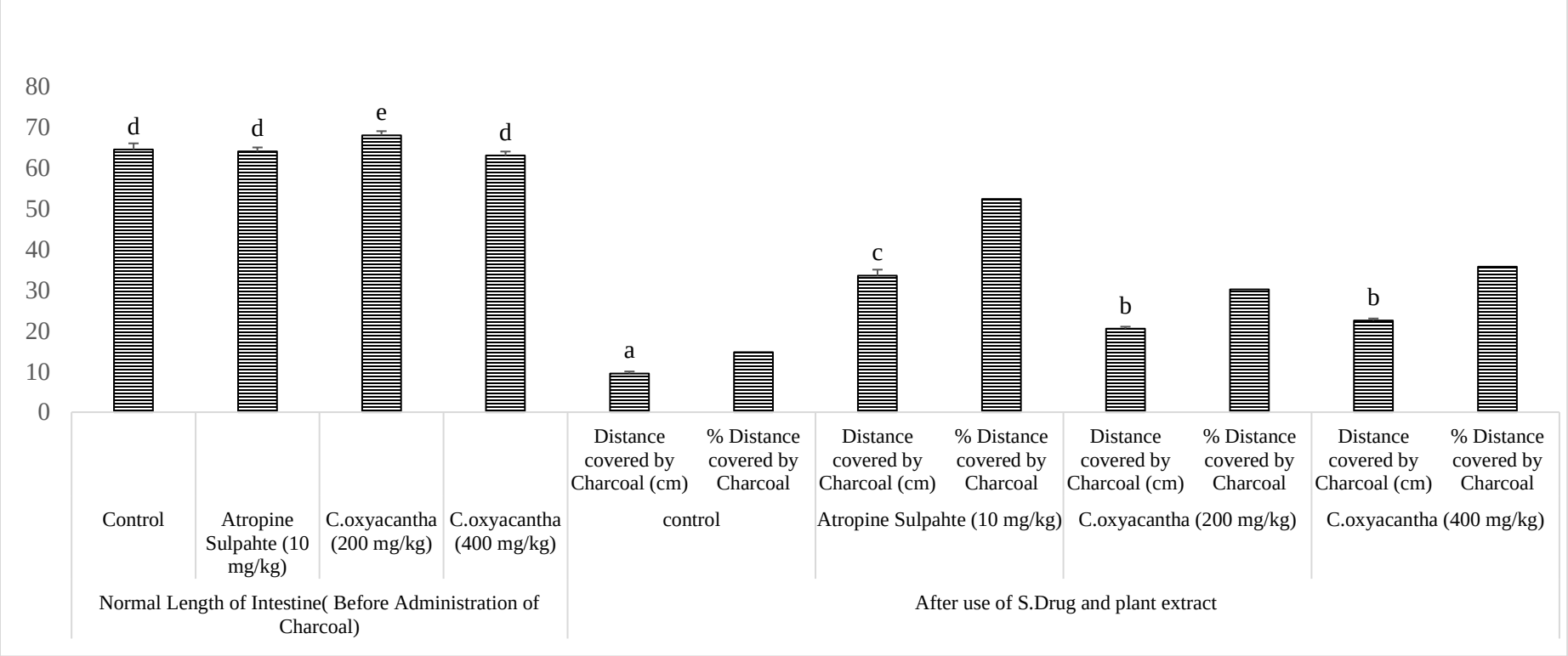


Figure 10: The impact of various concentrations of methanolic extract from *C. oxyacantha* on Brewer's yeast-induced pyrexia in mice is presented. The data, representing the mean of two replicates with standard error bars, indicate significant differences ($p < 0.05$) among the groups, as denoted by different alphabetical letters.

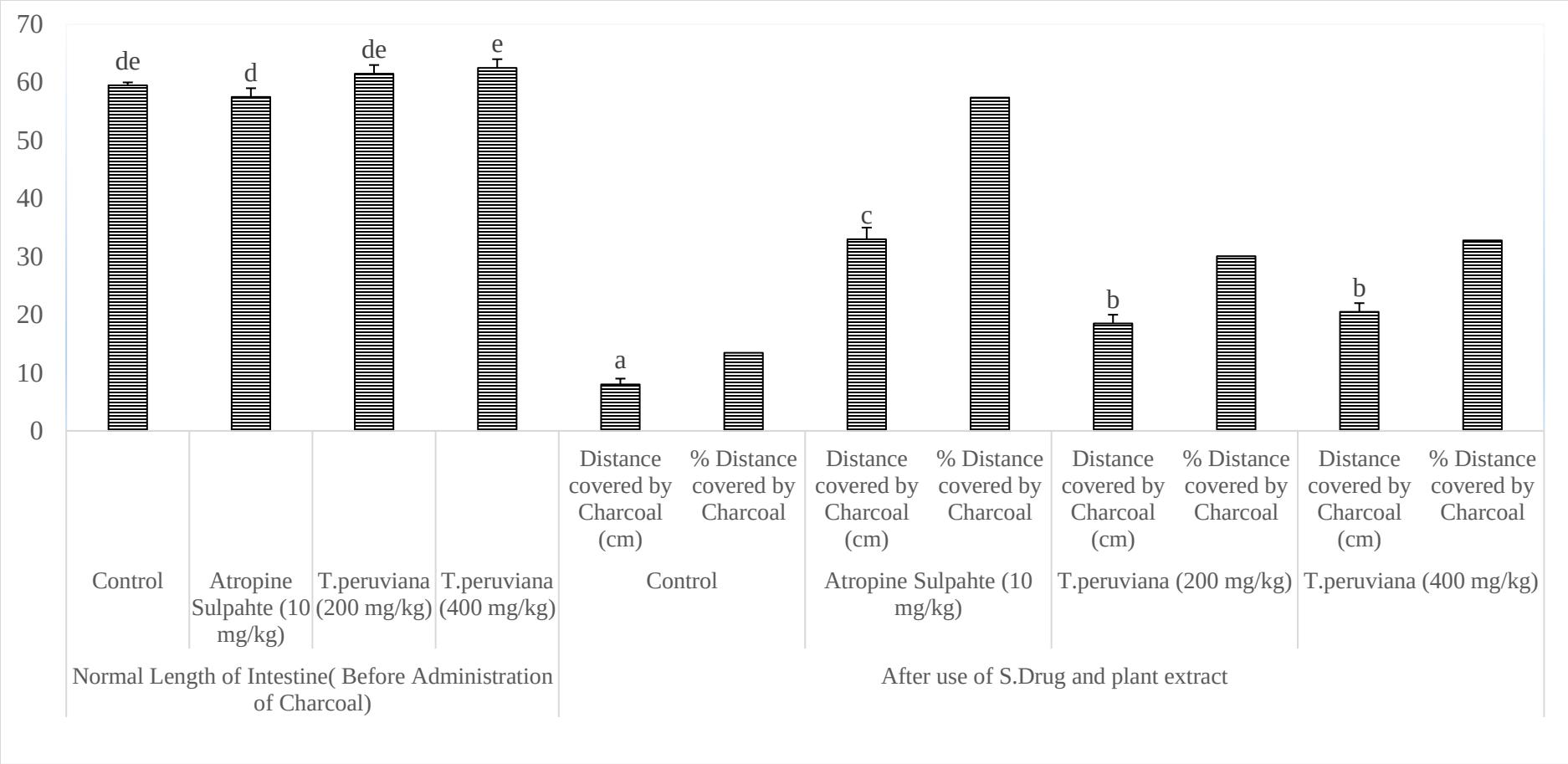


Figure 11: The impact of various concentrations of methanolic extract from *O. limbata* on Brewer's yeast-induced pyrexia in mice is presented. The data, representing the mean of two replicates with standard error bars, indicate significant differences ($p < 0.05$) among the groups, as denoted by different alphabetical letters.

IV DISCUSSION

The study aimed to assess the analgesic potential of methanolic extracts derived from *Carthamus oxyacantha* (Pers.) K. Schum, *Otostegia limbata* (Benth) Boiss and *Thevetia peruviana* (Pers.) K. Schum using the acetic acid-induced writhing method (Koster et al., 1959). The analgesic effects of these selected plants displayed a significant dose-dependent impact compared to the control group. *O. limbata* notably exhibited the highest reduction in writhing at 45% with a dosage of 400mg/kg. Both doses of plant extracts showed substantial inhibition of writhing compared to the negative control. The presence of chemical compounds like flavonoids, phenols, and alkaloids in the plants, particularly alkaloids, played a role in their analgesic potential (Sultana et al., 2014). *C. oxyacantha* demonstrated notable analgesic effects in formalin and hot plate tests, significantly suppressing acetic acid-induced writhing in mice (Bukhari et al., 2013). Similar investigations by Ali et al. (2012) on Spilanthes calva and Ishola et al. (2011) on Cnestis ferruginea supported our findings, demonstrating significant analgesic effects. Ghule et al. (2011) also discovered significant analgesic effects of *Cuscuta campestris* at higher doses, aligning with our results. Additionally, the study aimed to explore the anti-inflammatory response of methanolic extracts from *C. oxyacantha*, *O. limbata* and *T. peruviana* using a carrageenan-induced paw edema model in mice. The study delineated two phases in this method: the initial stage involving the release of histamine and serotonin after 1 hour of carrageenan injection, followed by the second stage, where prostaglandins are excreted after 3 hours. *Carthamus oxyacantha* and *Thevetia peruviana* demonstrated the most significant reduction in paw edema volume, decreasing inflammation from 1.3 to 1.0 cm after the 4th hour. Otostegia limbata also notably reduced inflammation compared to the negative control. Diclofenac sodium exhibited the highest reduction. All treated plants exhibited a significant anti-inflammatory response compared to the negative control. Flavonoids and quinochalcones phytochemicals present in the plants were suggested as potential contributors to their anti-inflammatory activity (Zhou et al., 2014). Several researchers supported the anti-inflammatory potential of *Carthamus oxyacantha*. Zhou et al. (2014) reported multiple biological activities, including anti-inflammatory, antioxidant, anticoagulant, antiaging, and analgesic properties among 104 compounds isolated from *Carthamus oxyacantha*. Toubane et al. (2017) found significant anti-inflammatory and antioxidant activities in the ethanolic extract of *Carthamus Caeruleus* L. Similarly, Vilela et al. (2010) reported the anti-inflammatory potential of Sonchus oleraceus, which reduced paw volume. *Otostegia limbata* was recognized for its anti-ulcer, antispasmodic, and anti-inflammatory activities. The study concluded that the tested plants, rich in alkaloids and flavonoids, possess potent anti-inflammatory responses (Sadaf et al., 2016). Furthermore, the research investigated the antipyretic response of methanolic extracts from *Carthamus oxyacantha*, *Otostegia limbata* and *Thevetia peruviana*. All plant extracts displayed a significant decrease in pyrexia. Paracetamol exhibited the maximum reduction in temperature after the 4th hour. *Otostegia limbata* exhibited the most promising reduction in temperature, followed by *Carthamus oxyacantha* and *Thevetia peruviana*. All plant extracts reduced rectal temperature in a time and dose-dependent manner. The presence of phytochemicals like alkaloids and flavonoids in the plant extracts was considered responsible for reducing pyrexia. The increase in pyrexia by yeast in mice, referred to as pathogenic fever, is attributed to prostaglandin production (Moltz, 1993). The inhibition of prostaglandin synthesis could be a potential mechanism for the antipyretic action, similar to paracetamol. Many researchers have reported the antipyretic response of various plant

species, supporting the traditional use of these plants for pain and fever management. The research also delved into the antispasmodic potential of methanolic extracts from *Carthamus oxyacantha*, *Otostegia limbata* and *Thevetia peruviana*. The findings indicated a significant increase in intestinal motility for all plants, dose-dependently. *Otostegia limbata* displayed the highest charcoal movement at 51.3%, followed by *Carthamus oxyacantha* at 35.7% and *Thevetia peruviana* at 32.8%. All selected plants induced a dose-dependent contraction in the small intestine of mice. Several researchers, including Wiya et al. (2018) and Shamkuwar and Pawar (2013), reported the antispasmodic potential.

V. CONCLUSION

In our comprehensive pharmacological evaluation, we thoroughly investigated the effects of two distinct concentrations (200mg/kg and 400mg/kg) of methanolic extracts derived from *Carthamus oxyacantha* M.Bieb, *Otostegia limbata* (Benth) Boiss and *Thevetia peruviana* (Pers.) K. Schum. Our study encompassed multiple assessments, including acetic acid-induced writhing in mice, carrageenan-induced paw edema, brewer's yeast-induced pyrexia, and charcoal meal movement in mice. Our findings strongly support the significant analgesic, anti-inflammatory, antipyretic, and antispasmodic potential of these plant extracts, showcasing varying effectiveness based on dosage. This validates the traditional usage of these plants for alleviating pain, inflammation, fever, and muscle spasms. The results of this research advocate further exploration to isolate and identify bioactive compounds, potentially laying the groundwork for the development of new therapeutics with enhanced efficacy against diseases and fewer side effects.

ACKNOWLEDGEMENT

We are very thankful for Department of Chemical and Life Sciences Qurtuba University of Science and Information Technology Peshawar / DI Khan for providing of opportunities for research Work.

Reference

Chanda, S. 2014. Importance of pharmacognostic study of medicinal plants: An overview. Journal of Pharmacognosy and Phytochemistry, 2(5).

Gokhale, S., Kohajda, T., and Schlink, U. 2008. Source apportionment of human personal exposure to volatile organic compounds in homes, offices and outdoors by chemical mass balance and genti algorithm receptor models. Sci. Tot Environ. 407: 122-138.

Hasler, C. M., & Blumberg, J. B. 1999. Phytochemicals: biochemistry and physiology. Introduction. The Journal of nutrition, 129(3), 756S.Lahlou, M. 2013. The Success of Natural Products in Drug Discovery, 2013(June), 17–31.

Lajayer, B. A., Ghorbanpour, M., & Nikabadi, S. 2017. Heavy metals in contaminated environment: destiny of secondary metabolite biosynthesis, oxidative status and phytoextraction in medicinal plants. Ecotoxicology and Environmental Safety, 145, 377-390.

Bandaranayake W.M., 2006. "quality control, screening, toxicity and regulation of herbal drugs", modern phytomedicines, pp 25-55.

Barkatullah, I. M., Ali, N., Muhammad, N., & Ehsan, M. 2012. In-vitro pharmacological study and preliminary phytochemical profile of *Viola canescens* Wall. *Ex Roxb. Afr J Pharm Pharmacol*, *6*(15), 1142-1146.

Mbagwu, H. O. C., & Adeyemi, O. O. 2008) Anti-diarrhoeal activity of the aqueous extract of *Mezoneuron benthamianum* Baill (Caesalpiniaceae). *Journal of* Koster, R. 1959. Acetic acid for analgesic screening. In *Fed proc* (Vol. 18, p. 412).

Ethnopharmacology, *116*(1), 16-20.

Sultana, S., Nandi, J. K., Rahman, S., Jahan, R., & Rahmatullah, M. 2014. Preliminary antihyperglycemic and analgesic activity studies with *Angiopteris evecta* leaves in Swiss albino mice. *World J Pharm Pharmaceut Sci*, *3*(10), 1-12.

Owoyele, V.B., Oloriegbe, Y.Y., Balogun, E.A. and Soladoye, A.O. 2005. Analgesic and anti-inflammatory properties of *Nelsoniacanescens* leaf extract. *Journal of ethnopharmacology*, *99*(1), pp.153-156.

Qadir, M. I., Abbas, K., Hamayun, R., & Ali, M. 2014. Analgesic, anti-inflammatory and anti-pyretic activities of aqueous ethanolic extract of *Tamarix aphylla* L. (Saltcedar) in mice. *Pakistan Journal of Pharmaceutical Sciences*, *27*(6), 1985–1988.

Ali, S., R. Habib, R. Islam, S. Islam, M. Rahman and N. Hasan. 2012. Analgesic and Antioxidant Activities of the Methanolic Extract of *Spilanthes calva* (D.C) Leaves in Male Rats Glo. *Journal of Pharma.* 6: 12-18.

Ishola, I. O., A. J. Akindele and O. O. Adeyemi. 2011. Analgesic and anti- inflammatory activities of *Cnestis ferruginea* Vahl ex DC (Connaraceae) methanolic root extract. *J.* 135(1): 55-62.

Ghule, R. S., R. Venkatnarayanan, S. P. Thakare , H. Jain and P. R. Ghule. 2011. Analgeic activity of *Cuscuta campestris yuncker* a parasitic plant grown on *Nerium indicum* Mill. *Journal of advanced Pharmacy Education & Research* 1:45-51

Zhou, X., Tang, L., Xu, Y., Zhou, G., & Wang, Z. 2014. Towards a better understanding of medicinal uses of *Carthamus tinctorius* L. in traditional Chinese medicine: a phytochemical and pharmacological review. *Journal of ethnopharmacology*, *151*(1),

Wiya, C., Saenphet, K., Saenphet, S., & Buncharoen, W. 2018. In vitro analysis of antispasmodic activity of ethanolic stem extracts of *Uvaria rufa* Blume and *Anomianthus dulcis* (Dunal) J. Sinclair on excised rat's ileum. *Comparative Clinical Pathology*, *27*(2), 295-299. 27-43.

Toubane, A., Rezzoug, S. A., Besombes, C., & Daoud, K. 2017. Optimization of Accelerated Solvent Extraction of *Carthamus Caeruleus* L. Evaluation of antioxidant and anti-inflammatory activity of extracts. *Industrial Crops and Products*, *97*, 620–631.

Sadaf, H. M., Bibi, Y., Riaz, I., Sultan, M. A., Bibi, F., Bibi, M., & Sabir, S. 2016. Pharmacological aptitude and profiling of active constituent from *Otostegia limbata*-Comprehensive review. *Asian Pacific Journal of Tropical Disease*, *6*(11), 918-924.

Moltz, H. 1993. Fever: causes and consequences. *Neuroscience & Biobehavioral Reviews*, *17*(3), 237-269.

Rawlins, M., & Postgrad, R. 1973. Mechanism of salicylate-induced antipyresis. In *Pharmacology Thermoregulatory Proceeding Satellite Symposium* (pp. 311-324).

Garg, V. K., & Khosa, R. L. 2008. Analgesic and anti-pyretic activity of aqueous extract of *Cynodon dactylon*. *Pharmacologyonline*, *3*, 12-8.

Ali, S., R. Habib, R. Islam, S. Islam, M. Rahman and N. Hasan. 2012. Analgesic and Antioxidant Activities of the Methanolic Extract of *Spilanthes calva* (D.C) Leaves in Male Rats Glo. *Journal of Pharma.* 6: 12-18.

Firestone, D. 2006. *Physical and chemical characteristics of oils, fats and waxes* (No. L- 0671). Aocs Press.

Shamkuwar, P. B., & Pawar, D. P. 2013. Antidiarrhoeal and antispasmodic effect of *Berberis aristata*. *International Journal of Pharmacognosy and Phytochemical Research*, *5*(1), 24–26.

Desirea, O., C. Rivere, R. Razafindrazak, L. Goossense, S. Moreau, J. Guillon, S. Uverg, Ratsimamang, P. Andriamadio, N. Moore, A. Randriantsoa, and A. Raharisololalaod. 2010. Antispasmodic and antioxidant activities of fractions and bioactive constituent davidigenin isolated from *Mascarenhasia arborescens*. *J. Ethnopharm*. 130: 320-328.

Azam, S., S. Bashir and B. Ahmad. 2011. Antispasmodic action of crude methanolic extract and a new compound isolated from the aerial parts of *Myrsine africana*. *BMC Compl. Med*., 11: 55.

Authors

First Authors- Sajid Ali M. Phil Botany Department of Chemical and Life sciences Qurtuba University Peshawar.

Second Author- Syed Awais Shah Ph.D. Botany Department of Chemical and Life Sciences Qurtuba University Peshawar.

Third Author- Hamid Ullah M. Phil Botany Department of Chemical and Life Sciences Qurtuba University Peshawar.

Fourth Author - Jawad Akbar M. Phil Botany Department of Chemical and Life Sciences Qurtuba University Peshawar.

Fifth Author- Muhammad Sajjad Khan M. Phil Botany Department of Chemical and Life Sciences Qurtuba University Peshawar.

Sixth Author- Inzemam Ul Haq M. Phil Zoology Department of Chemical and Life Sciences Qurtuba University Peshawar.

Seventh Author- Adil Muhammad Ph.D. Botany Department of Chemical and Life Sciences Qurtuba University Peshawar.