Evaluation of *Chenopodium album* Linn. Crude Methanolic Leaf Extract for Central Antinociceptive Activity in Albino Mice using the Hot Plate Test

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**ABSTRACT:** The aim of study was to evaluate the crude leaf extract of *Chenopodium album* for central antinociceptive activity using inbred NIH albino mice. No mortalities were observed during the 14 day period in any of the 4 groups of 5 mice per group orally given 1, 2, 3, and 5 g/kg bw respectively. It was therefore assumed that the LD₅₀ was higher than 5 g/kg bw. Eddy’s hot plate test for antinociception using 0, 50, 100 and 150 mg/kg bw and standard drug Aspirin (150 mg/kg bw) orally administered revealed that both Aspirin and the extract at 100 and at 150mg/kg bw exhibited significant (P < 0.05) dose-dependent antinociception compared with the negative control at the seven time intervals; 30, 60, 120, 180, 240, 300, 360 minutes. The maximum antinociception (71.47%) for the 150mg/kg bw group being at 30 minutes after administration and statistically not different from that of Aspirin (67.44%) at the same time interval. Between 30 and 180 minutes after oral administration of test substances, antinociception due to *C. album* extract (150 mg/kg bw) was statistically not different from that of Aspirin. Thereafter Aspirin remained more efficacious than the extract till the end of experiment at 360 minutes; a parallel shift from 240 minutes suggesting a similar mechanism of antinociception.

**KEYWORDS:** Hotplate Latency, Antinociception, *Chenopodium album*, Phytochemicals

1. **INTRODUCTION**

The hot plate test uses acute non-inflammatory neurological pain induced by the noxious thermal stimulus to study central nociception and antinociception (Ibironke and Ajiboye, 2007). Acute pain is intense, lasts for a relatively short time, and its presence is usually an indication of a severe injury (Linn, 1984). Acute pain is useful in protecting the tissues against noxious stimuli (Almeida et al., 2001). Nociception is the neural process of encoding and processing noxious stimuli, which can be developed by thermal, chemicals and physical pressure (Schaible, 2006). This activity is initiated by nociceptors which are also known as pain receptors. Nociceptors are embedded in the skin and internally in joints. They can detect mechanical, thermal or chemical changes above a set body threshold anywhere in the body where they are located. Once stimulated, a nociceptor sends the signal to the central nervous system through the spinal cord (Schaible, 2006).

Antinociception is the reduction in the sensitivity and detection of such pre-existing painful stimuli. It is also known as analgesia and it can be achieved by the use of analgesics. Analgesics are therefore, any substances which decrease pain sensation usually by increasing the pain threshold to external stimuli (Tripathi, 2004). Currently used analgesics include opioids such as morphine and heroin. In addition to relieving pain, these narcotic drugs cause drowsiness and dependence. On the other hand non-opioid analgesics, the Non-Steroidal Anti-inflammatory Drugs (NSAIDs), such as aspirin and paracetamol are non-narcotics but have other side effects such as gastric erosion, liver and kidney malfunction (Brunton et al., 2008; Zulfiker et al., 2010). Aspirin is readily available and is the most widely consumed NSAID (Brunton et al., 2008).

The World Health Organisation (WHO) encourages the use of herbal medicine as an essential component of primary health care in many developing countries since natural herbal products as alternative therapeutic options are cheap, abundant, and readily available.
available, and less toxic compared to conventional allopathic medicines (Kaur and Jaggi, 2010; Datta et al., 2014; Jami et al., 2014). Of the very many different plant species that form the diverse flora in Lesotho, over 2000 are used directly and indirectly as food and as medicinal remedies for various ailments (Maliehe and Oehrlein, 1997). Chenopodium album (seruo in Sesotho) is a herbaceous vegetable plant species of about 30 - 70cm in height, with a stout central stem and ascending lateral branches (Maliehe and Oehrlein, 1997; Sikarwar et al., 2013). It is one of the most widely distributed species of weeds across many climatic conditions in the world with average temperatures ranging from 5-30°C (Biswas et al., 2013). In Lesotho, Chenopodium album can be found in all the four ecological zones of the kingdom throughout the year; young leaves are consumed as a vegetable while the whole plant is used as a medicine in the rural communities to improve appetite, as a laxative, diuretic, tonic, treatment of biliousness, eye diseases and as an analgesic for abdominal pain (Maliehe and Oehrlein, 1997). The plant is rich in proteins, vitamins A and C, calcium, phosphorus, iron, potassium and phytoconstituents such as phenolic compounds (simple phenolics and polyphenolics, cinnamic acid amides), alkaloids, monoterpenoids and saponins (Sikarware et al., 2013; Agrawal et al., 2014).

Chenopodium album is important in Southern African traditional medicine but we are not aware of studies that have been done on its toxicity and central analgesic effects. Studies that we have seen are only those on peripheral analgesic activity of C. album (Ahmad, et al., 2012; Dai et al., 2002; Begum et al., 2013). Therefore, in the present study, the crude methanolic extract prepared from leaves of C. album was assessed for toxicity and central antinociceptive activity in albino mice using the hot plate test (Ibironke and Ajiboye, 2007). This test measures the complex response to non-inflammatory, acute nociceptive stimulation and is one of the well-validated and widely used model for studying neurological pain and central antinociceptive activity (Ibironke and Ajiboye, 2007).

2. MATERIALS AND METHODS

2.1 Plant material

Chenopodium album was collected from the bush around the National University of Lesotho, Roma campus and from the adjacent bushes in Hata-Butle, east of the National University of Lesotho Roma campus and was authenticated by the curator of the herbarium at the Department of Biology. Leaves were separated from the rest of the plant and used in the study.

2.2 Preparation of crude extract of C. album leaves

Leaves of C. album were dried at 35°C in a fanned oven (Labcon) until they were brittle, then they were ground to powder in a pulveriser (Kenwood). The powdered leaf material (500 grams) was soaked in 95% methanol (v/v) in distilled water (1.5 Litres) for three days followed by vacuum filtration of the extract through a Whatman #1 filter paper (Kent, England). The solvent was then evaporated from the extract under vacuum using a rotary evaporator (Gallenkamp) and the remaining water was removed by drying the extract in a fanned oven (Labcon) set at 30°C.

2.3 Animals

Inbred NIH albino mice between 8-10 weeks weighing 27-30 grams, bred and kept in the animal house of the Department of Biology were used for the study. The animals were allowed free access to food (Meadow sheep pellets) and water ad libitum (Asita et al., 2008).

2.4 Toxicity assay

Five groups of five mice per group, (25-30grams each) were set up and the mice were fasted for 12 hours prior to the morning of the oral administration of test substances as follows: Group 1 was the negative control group and the mice were given 5mL/kg bw of 6% Tween-80 (v/v solution in distilled water). Mice in groups 2, 3, 4 and 5, were given C. album extract at 1, 2, 3 and 5 g/kg bw respectively (Yadav et al., 2011). The mice were weighed prior to administration and acute toxicity was determined by monitoring them for deaths and slow movement for 24 hours after the administration of test substances. Mice were then re-weighed and monitored daily for the next 14 days for any delayed toxicity (Morales et al., 2014; Obidah et al., 2014). No mouse died even in the 5 g/kg bw group therefore the extracts were considered non toxic when administered orally at the dosage range used.

For the determination of the antinociceptive activity of the C. album crude leaf extract, the following safe oral doses, which are well below the highest concentration used in the toxicity tests, based on Datta et al., 2014 were chosen as follows: 50, 100 and 150 mg/kg bw.

2.5 Eddy’s hotplate nociception test

Central analgesic activity of the C. album extract was evaluated using the hot plate nociception test (Bhattacharya et al., 2014; Abdullahi et al., 2013) in which the mice were fasted for 12 hours with provision of clean water ad libitum prior to the experiment (Omeh and Ezega, 2010). In this assay,
test substances and controls were administered orally (Yadav et al., 2011; Sajeesh and Parimelazhagan, 2014). Briefly, five groups of mice of both sexes, each group containing five mice (replicates) were set up as follows; (Groups 1, 2 and 3 were orally given 50, 100 and 150 mg/kg bw of extract respectively. Group 4 was the negative control group, orally given 5 mL/kg bw of 6% Tween-80 v/v solution in distilled water. Group 5 was the positive control group also orally administered with Aspirin at 150 mg/kg bw (Shanmugasundaram and Venkataraman, 2005; Chyad, 2017; Yadav et al., 2011); Rajalakshimi et al., 2015). Each mouse served as its own control and the “initial reaction time” was determined before treatment (Sajeesh and Parimelazhagan, 2014). The hot plate latency for each mouse was determined by placing the mouse in a plexiglass cylinder of diameter 20 cm and height 25 cm on the heated surface of a hot plate maintained at 55 ± 0.5°C (Jami et al., 2014). The time between placement of the mouse on the hot-plate and the occurrence of pain sensation in the form of either a hind paw lick or a jump off the surface (whichever appeared first) was recorded as the hot-plate latency (Jami et al., 2014). A cut-off time of 10 seconds (regarded as complete analgesia) was observed in order to avoid thermal injury to the animals which meant that mice with a basal latency of more than 10 seconds were not included in the study (Barua et al., 2010). The base line latency of nociceptive response was the reaction time before oral treatment (that is, reaction time at time 0). The hot plate latencies for the mice were determined at 30 minutes intervals up to 60 minutes, thereafter at 60 minute intervals up to 360 minutes after oral administration of the test substances (Yadav et al., 2011). Percentage (% analgesia) was calculated as follows:

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\text{Percentage (% analgesia)} = \frac{(R_1 - R_0)}{R_0} \times 100
\]

where R1 = reaction time at any given interval after oral administration of plant extract or drug (Aspirin) R0 = reaction time before oral administration of plant extract or drug (Aspirin) (Shanmugasundaram and Venkataraman, 2005; Shojaei et al., 2015; Dash et al, 2015)

2.6 Data analysis

For each group, the mean ± SD of the reaction times of the mice were determined according to the method of Zimudzi et al., (2013) and the mean reaction time for each group (n= 5) was compared with the negative control group using t-test. Data for each time interval for the five groups (Tween 80, Aspirin (150 mg/kg bw), C. album (50, 100 and 150 mg/kg bw) were statistically analysed by the Tukey’s multiple comparisons test using one-way analysis of variance (ANOVA) to determine if the mean reaction times of the five groups of mice at each time interval were significantly different from those of the negative control group and from each other. Levels of significance were set at p<0.05. The statistical analyses were carried out using SPSS 16.0.

3. RESULTS

The yield of the crude leaf extract from the powdered leaf material after extraction with 95% methanol (v/v) in distilled water was 8.06%.

3.1 Toxicity test for C. album crude extract

Expected signs of toxicity were mortality and slow movement of mice (Lalitha et al., 2010). No mortality was observed during the first 24 hours of observation and the next 14 days of monitoring for delayed toxicity even at the maximum administered extract dose of 5000 mg/kg bw (Group 5). The LD₅₀ was therefore estimated to be higher than 5000 mg/kg bw.

3.2 Antinociceptive activity of Chenopodium album crude leaf extract

In Table 1 is presented the results of comparison of the seven time intervals of the five groups of mice at each time interval within the 360 minute period of monitoring, the response times of the mice to nociception increased with increasing dosage of extract, i.e. were dose dependent (Ashidi et al., 2015). The longer the response time (i.e., hot plate latency) the more analgesic the extract or drug is (Abdullali et al., 2013). The results of the Tukey’s multiple comparisons analysis using ANOVA showed that the mean response times to the thermal stimulus of the Aspirin treated group and C. album (150 mg/kg bw) extract treated groups were significantly different (p<0.05) from that of the negative control group at all the seven time intervals from 30 to 360 minutes. With reference to antinociception (as % analgesia) the test substances, Aspirin (150 mg/kg bw) and C. album crude leaf extract at 100 mg/kg bw and at 150 mg/kg bw produced significant (P<0.05) antinociception (as % analgesia) at all the seven time intervals when compared with the negative control. However, 50 mg/kg bw C. album extract treated group produced significant (P<0.05) antinociception (as % analgesia) only at 120,180 and at 240 minutes after administration of the crude extract. Table 1 also shows that between 30 and 180 minutes after oral administration of test substances at the dose of 150 mg/kg bw C. album extract, antinociception (as % analgesia)
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analgesia) was statistically (P<0.05) not different from that induced by Aspirin. The highest antinociceptive response (71.47%) in mice treated with C. album extract was observed with 150 mg/kg bw, at 30 minutes after administration of the crude extract; statistically (P<0.05) not different from that with Aspirin (67.44%) at the same time interval.

In Figure 1 is presented the curves of the hot plate latencies for the three concentrations of C. album leaf extract and the reference drug Aspirin against time. Treatment with C. album leaf extract increased the response time of mice to thermal stimulus in a dose dependent manner with the group treated with 150 mg/kg bw showing the longest response times at any given time interval.

In Figure 2 is represented the calculated percent (%) analgesia based on the observed hot plate latencies for each of the three concentrations of C. album leaf extract with time. Beyond the 180th minute of administration the reference drug Aspirin remained more efficacious than the C. album extract (at all concentrations), as reflected by the steep slope of the gradient up to 240 minutes. After the 240th minute, the % analgesia curves for both C. album extract (150mg/kg bw) and Aspirin remained nearly-parallel, with the C. album curve shifted to the left of the Aspirin curve till the end of experiment at 360 minutes; this observation suggested a similar mechanism of antinociception. However, 50 mg/kg bw C. album extract produced the lowest antinociceptive responses compared to Aspirin at all the seven time intervals.

3 DISCUSSION

In the present study, the crude methanolic extract of leaves of Chenopodium album, used for the treatment of several ailments in Lesotho traditional medical practice, was assessed for toxicity and central antinociceptive activity by the hot plate assay using mice.

This study demonstrated that systemic administration of C. album crude extract at doses that did not produce any toxicity resulted in consistent dose-dependent antinociceptive effects using the hot plate test in mice. The oral administration of a maximum dose of 5 g/kg bw of C. album leaf extract did not produce any signs of acute toxicity or delayed toxicity in any of the mice during the observation period. This suggested that the median lethal oral dose (LD50) of the extract was greater than 5 g/kg bw. The fact that the LD50 of the extract was above 5g/kg bw was an indication that the extract could be considered nontoxic, when given orally (Kennedy et al., 1986; Obidah et al., 2014). The doses used in the hot plate test were therefore; 150 mg/kg bw, 100 and 50 mg/kg bw. The chosen doses were in agreement with those of Kataria et al. 2012.

The hot plate test produces two kinds of behavioural response; paw licking and jumping, both of which are said to be supraspinally integrated responses (Chapman et al., 1985). Centrally acting analgesic agents can increase reaction time in the hot plate test through their action at the spinal cord level (Mondal et al., 2014); Battacharya et al., 2014, Rajashekar and Naraismamurthy, 2017. Experimental data represented in Table 1 and in Figures 1 and 2, indicate that C. album crude leaf extract exhibited central analgesic activity by increasing the latency to discomfort in the hot-plate test in a dose-dependent manner. Since the thermally induced nociception in the hot plate test is considered to be a selective method used to screen for centrally acting opiate analgesic drugs (Abbott, 1982), the effect of the C. album extract on this pain model indicated that it might be centrally acting.

The C. album leaf extract (at all concentrations) inhibited nociception starting at 30 minutes after administration of extract till 360 minutes when the experiment ended; though inhibition of nociception began to slow down gradually after one hour of administration of the extracts. Maximum antinociception occurred between 30 to 60 minutes after administration of the agent for all treatments (Aspirin and C. album). This was in agreement with findings from other authors (Ismail and Mirza, 2017; Ashidi et al., 2015, Jami et al., 2014). Ismail and Mirza, 2017 reported that, based on hot plate latencies, the aqueous leaf extracts of Lactuca sativa (lettuce) 1g/kg bw administered orally to rats and the reference drug Aspirin (10mg/kg bw) exhibited significant maximum analgesic activity compared to the negative control (saline) at one hour (of the 2 hour experiment) after administration after which it began to decline. Significant analgesic activity based on hot plate latencies (17.6 sec for the leaf extract and 17.8 sec for Aspirin) in comparison to the negative control (saline) 5.4 sec was observed at one hour after which it began to decline. Ashidi et al., 2015 reported that oral administration of the ethanolic extract of Elytraria marginata (Acanthaceae) prepared from the whole plant (50,100 and 150 mg/kg bw) and the reference drug Ibuprofen (100 mg/kg bw) to rats significantly reduced pain induced by the hot plate in all experimental groups when compared to the negative control (water). The effect of the extract at 150 mg/kg bw (72.46% inhibition of pain) was statistically comparable to that of

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Ibuprofen (100 mg/kg bw); 67.15% inhibition of pain after one hour at which the experiment was terminated. Jami et al., 2014 reported that in an experiment using the hot plate test, oral administration of the ethanolic extract of *Terminalia chebula* fruit to mice at doses of 250 mg/kg and 500 mg/kg induced a significant increase in reaction time compared to the negative control (water) group at 30 and 60 minutes (of the 180 minute experiment) after extract and drug administration which was statistically comparable to the standard drug Ketorolac (8.54±0.228 and 9.48±0.309 respectively). The maximum reaction time was observed at 30 minutes for both 250 and 500mg/kg body weight (11.13±1.388 and 9.217±1.122 respectively) compared with the negative control (8.117±0.897 and 7.97±1.010 respectively).

As indicated by results of the Tukey’s multiple comparisons analysis, the efficacy of the maximum concentration of 150 mg/kg bw *C. album* extract was not different from that of Aspirin (150 mg/kg bw) from 30 minutes to 180 minutes after administration of the test substances. Similarly, Chyad, 2017 using the hot plate test on mice orally treated with the ethanolic extract of *Lepidium draba* leaves (dose range 100-500 mg/kg bw) observed maximum hot plate latencies at two hours (end of experiment) after administration of the highest doses 400 (15.98 sec from 7.01 sec) and 500 mg/kg bw (16.12 sec from 6.98 sec) and Aspirin 100 mg/kg bw (18.01 sec from 7.03 sec) were significantly higher than those of the negative control (saline; 8.77 sec) at p<0.05. Only hot plate latencies were used by this author.

The fact that the response times to nociception as shown in Figure 1 (and hence % analgesia in Figure 2) peaked during the same time period (from 30 minutes to 60 minutes) for the *C. album* extract (at all the concentrations) and for Aspirin indicated similar rates of absorption from the stomach and small intestine (GI tract) and build-up of the pharmacologically active phytochemicals in the blood plasma of mice (Brunton et al., 2008). Figure 2 also shows that beyond 180 minutes, antinociception (as % analgesia) with *C. album* extract (150 mg/kg bw) declined at a faster rate than with Aspirin (150 mg/kg bw); this trend continued up to 240 minutes, meaning that the active constituent of Aspirin in plasma (salicylate) was inactivated by the liver or used up more slowly than those in *C. album* extract; the serum half-life of active compound of Aspirin being longer than that of the active compounds in *C. album* crude extract (Brunton et al., 2008). Higuchi et al., (1986) reported that salicylic acid (the active compound in Aspirin) may produce its analgesic action via a central mechanism. A crude extract may contain active compounds in small amounts and these may act either synergistically or individually if very potent; on the other hand, the plant extract might have few active compounds in large amounts that act individually or synergistically to exert its therapeutic efficacy (Barton and Ollis, 1985). After the 240th minute, the % analgesia curves for both *C. album* extract (150 mg/kg bw) and Aspirin remained almost parallel; with the *C. album* curve shifted to below the Aspirin curve till the end of the experiment at 360 minutes, Aspirin remaining more efficacious indicating that the active compound in Aspirin was metabolized more slowly to its inactive form (Brunton et al., 2008) compared to *C. album* active constituents; but no difference in mode of antinociception between them. The observed antinociceptive responses of mice after treatment with *C. album* extract and Aspirin in the hot plate test suggested that the extract acted in a similar manner to Aspirin which is also known to exhibit central analgesic effects (Bromm et al., 1991; Zimudzi et al., 2013; Higuchi et al., 1986). Considering that *C. album* was a crude extract while Aspirin was a pure drug (active compound acetylsalicylic acid), *C. album* compared well with Aspirin in antinociception. It is reported that *C. album* is hepatoprotective (Pal et al., 2011) and anti-ulcerogenic (Nigam and Paarak, 2011; Biswas, 2013). The main side-effect of NSAIDs is their ability to produce gastric lesions (Brunton et al., 2008). *Chenopodium album* has also been found to possess more effective hepatoprotective activity against Paracetamol intoxication in rats because of its flavonoid bearing capacity (Pal et al., 2011).

Results of the study by Zimudzi et al., (2013) using the hot plate assay showed that intraperitoneal administration of *Dicoma niccolifera* extract (1000, 500, 250 and 125 mg/kg bw) increased the pain threshold in mice in a similar way to Aspirin (200mg/kg bw), which indicated that the activity may be through a centrally mediated analgesic mechanism. Some of the phytochemicals (flavonoids, tannin, alkaloids, terpenoids, anthraquinones) known to exhibit central analgesic activity were identified in *D. niccolifera* by these authors. In agreement with the findings of Zimudzi et al., 2013, the *C. album* leaf extract used in this study increased reaction times of mice to the nociceptive thermal stimulus in a dose-dependent manner. The findings of our study using the hot plate test also indicated that the central analgesic activity of the *C. album* leaf extract may be linked partly to the reported presence of simple phenols and polyphenols, terpenoids, alkaloids (Sikarvar et al., 2013; Agrawal et al., 2014; Kumar and Kumar 2015) all of which are known to have antinociceptive effects. Aromatic compounds are
reported to constitute up to 60.1% of the phytochemical composition of *C. album* leaf extractives (Agrawal et al., 2014). The presence of phenolics, such as flavonoids, coumarins and tannins, steroids, alkaloids, terpenoids such as triterpenoids like terpenoid saponins in plant extracts is reported to enhance the centrally mediated response to pain in a dose-dependent manner (Abdullahi et al., 2013; Jamii et al., 2014, Abdullahi et al., 2016; Bagheri et al., 2017; Sajeeesh and Parimalazhagan, 2014; Barua et al., 2010; Shojaii et al., 2015). In our study, the pharmacologically active phytochemicals in the *C. album* extract might have acted on the central opioid receptors or promoted release of endogenous opioid peptides to inhibit central nociception (Le Bars et al., 2001).

The present study showed that the antinociceptive efficacy of *C. album* extract at 150 mg/kg bw p.o. was statistically not different from that of Aspirin from 30 minutes to 180 minutes; after which the mechanism of action remained similar though antinociceptive activity became lower. Studies by other authors indicated that *C. album* extract exhibited peripheral analgesic and anti-inflammatory activity (TrivellatoGrassi et al., 2013), significant reduction of the ulcer index (Nigam and Paarakh, 2011), preservation of the gastric mucosa (not observed with many NSAIDs) and hepatoprotective effects (Pal et al., 2011). The observations listed above suggest that there is a potential to develop extracts of *C. album* as non-toxic natural analgesics (Ulrich-Merzenich et al., 2010).

4. CONCLUSION

The toxicity tests showed that *Chenopodium album* leaf extract was not toxic at oral doses of up to 5000 mg/kg bw during the 14 day observation period. The crude leaf extract of *C. album* possessed significant dose-dependent central antinociceptive effects at the dosage range of 50–150 mg/kg bw in mice as it increased the pain threshold in a similar manner to Aspirin. At 150 mg/kg bw, the efficacy of *C. album* extract was statistically not different from that of Aspirin (150 mg/kg bw) for the first 180 minutes.

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# Evaluation of *Chenopodium album* Linn. Crude Methanolic Leaf Extract for Central Antinociceptive Activity in Albino Mice using the Hot Plate Test

## TABLES AND FIGURES

**Table 1.** Mean reaction times of mice given different doses of *Chenopodium album* leaf extracts (n = 5, ± S.D)

| Time intervals (Minutes) | Tween-80 (5 mL/kg bw) | Aspirin (150 mg/kg bw) | Doses of extract (mg/kg body weight) | ANOVA |
|-------------------------|------------------------|------------------------|-------------------------------------|-------|
|                         |                        |                        | 50                                  | 100   | 150   | Time Intervals F Values |
| 0                       | 1.97 ± 0.18a           | 2.05 ± 0.27a           | 2.12 ± 0.15a                        | 2.31 ± 0.55 a | 1.87 ± 0.17 a | 1.54 |
| 30                      | 2.34 ± 0.44a           | 6.28 ± 0.73bc (67.44*) | 2.57 ± 0.33ad (17.51)               | 5.03 ± 0.75e (53.96*) | 6.55 ± 0.48b (71.47*) | 61.5# |
| 60                      | 2.38 ± 0.35a           | 6.75 ± 0.87b (69.69*)  | 2.98 ± 0.63a (28.76)                | 5.55 ± 0.56b (58.32*) | 6.38 ± 0.79b (70.70*) | 45.41# |
| 120                     | 2.18 ± 0.19a           | 6.01 ± 0.24b (65.98*)  | 3.00 ± 0.33c (29.38*)               | 4.63 ± 0.61d (50.06*) | 5.57 ± 0.53b (66.44*) | 78.42# |
| 180                     | 2.22 ± 0.18a           | 5.71 ± 0.56b (64.14*)  | 2.84 ± 0.15a (25.46*)               | 4.63 ± 0.98c (50.06*) | 5.19 ± 0.44b,c (66.04*) | 37.80# |
| 240                     | 2.13 ± 0.15a           | 3.96 ± 0.63b (56.41*)  | 2.97 ± 0.38a,c (28.52*)             | 4.65 ± 0.85b (50.19*) | 3.53 ± 0.40c,d (47.05*) | 20.85# |
| 300                     | 2.13 ± 0.09a           | 4.47 ± 0.67b (54.27*)  | 2.51 ± 0.50a,d (15.47)              | 2.74 ± 0.66a,d (15.67*) | 3.19 ± 0.57c,d (41.48*) | 14.00# |
| 360                     | 2.18 ± 0.03a           | 3.72 ± 0.38b (44.94*)  | 2.50 ± 0.34a,c (15.06)              | 2.26 ± 0.27a,c (-2.48*) | 2.71 ± 0.44a,c (31.02*) | 18.01# |

Numbers in parentheses are percentages of pain inhibition; * = Percent (%) inhibition of pain was significant compared to the negative control; # = Mean response time of control and four treatments, at the given time interval, where n = 5 in each case, is statistically significant (F (4,20) = 2.866, P<0.05); †= Means with the same letter do not differ statistically at the level of 0.05 for each time interval; ‡= Mean response time significantly higher than that of the control group in the t-test
**Figure 1.** The mean response time (s) ± S.D. of mice to hot plate treatment at different time intervals following treatment with different doses of *C. album* leaf extract.

**Figure 2:** Percentage analgesia in mice in the Eddy’s hot plate test following treatment with different doses of *C. album* leaf extract, vehicle (Tween-80) and reference drug (Aspirin) at different time intervals.