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# ANTI-NOCICEPTIVE ACTIVITY AND POSSIBLE MECHANISMS OF ACTION OF AQUEOUS ROOT EXTRACT OF *STROPHANTHUS HISPIDUS* DC (APOCYNACEAE)

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**Keywords:**

Opioids, morphine, *Strophanthus hispidus*, anti-nociceptive, rodent, Apocynaceae

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**ABSTRACT: Background:** Decoction of the root of *Strophanthus hispidus* is highly valued in Africa herbal medicine for the treatment of skin diseases, leprosy, ulcers, malaria, dysentery, gonorrhea and in the management of inflammation. **Objective:** The present study aimed to evaluate the anti-nociceptive effect of the aqueous root extract of *Strophanthus hispidus* focusing on central involvement as well as exploring the possible mechanisms of action in Swiss albino mice of both sexes. **Methods:** The aqueous extract was administered in doses of 50, 100, and 200 mg $kg^{-1}$ via oral route to the animals used in the various models- acetic acid-induced writhing, formalin, Haffner's tail clip, hot plate and tail immersion tests. **Results:** In each of the models, the aqueous root extract of *Strophanthus hispidus* each possesses a significant ($p< 0.05$, $p< 0.01$, $p< 0.001$) anti-nociceptive effect in a dose-dependent manner. The anti-nociceptive activity of the extract may be mediated through central and peripheral mechanisms. The involvement of opioid and dopamine ($D_2$) receptors in anti-nociception were also established. The effect of the extract was comparable to that produced by peripheral analgesics, the NSAIDs (aspirin) and centrally acting analgesic opioids (morphine), both used as positive control in several models to access anti-nociception. **Conclusion:** These findings showed that *S. hispidus* possesses anti-nociceptive effect mediated both peripherally and centrally.

**INTRODUCTION:** Plants have been used as medicine for several thousands of years and they continue to be used by the majority (80%) of the world's population [1]. Many drugs contain herbal ingredients, and it has been said that 70–80% of the world's population relies on some form of non-conventional medicine and 25–40% of all prescription drugs contain active ingredients derived from plants in the United States alone [1].

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*S. hispidus* DC belongs to the family of plants known as Apocynaceae, they are popularly known as poison arrow vine, brown strophanthus and hairy strophanthus in western part of Africa including Nigeria [2]. A deciduous shrub of 5 m tall and up to 100 cm wide, having its stem bark dark grey in colour, with few lenticels, has been reported to have diverse medicinal uses; for example, in the Savannah Zone of West Africa, the latex and seeds of *S. hispidus* are used as arrow poison, while decoctions of root, stem bark or leaf are used externally to treat skin diseases, leprosy, ulcers, wound, malaria, dysentery, gonorrhea and Inflammatory disorders [3, 4]. In Nigeria and Ghana, the root decoction is ingested to treat rheumatic diseases, while in Togo; the root bark macerate is

employed for treating edema. The ethanolic root extract has previously been reported to have analgesic activity [5] using acetic acid and formalin tests only.

The present study aimed to evaluate the anti-nociceptive effect of the aqueous root extract of *Strophanthus hispidus* focusing on central involvement as well as exploring the possible mechanisms of action.

## MATERIALS AND METHODS:

### Animals:

Swiss albino mice (18-30 g) of both sexes obtained from National Agency for Food and Drugs Administration and Control, Yaba (NAFDAC), were used in this study and maintained under standard laboratory conditions, as approved by the United States National Institute of Health (NIH) guide for Care and Use of laboratory animals and recommendation of IASP [6]. The animals were acclimatized for one week, fed on rodent diet (Livestock Feeds PLC, Ibadan, Oyo-State, Nigeria) and had free access to drinking water. However, they were fasted for at least 12 h prior to experimentation.

### Drugs and Chemicals:

Acetic acid (BDH Chemicals Ltd., Poole, England), formalin, (Sigma-Aldrich chemical company, United Kingdom), acetylsalicylic acid ASA (Dispirin®; Reckitt & Coleman Ltd., Pakistan), morphine, naloxone, metoclopramide (Evans Medical Ltd., Leatherhead, England), methylcellulose (Koch-Light Laboratories Ltd., Suffolk, England).

### Plant Collection and Extraction:

Fresh roots of *S. hispidus* were purchased from Mushin local herbs market in Lagos, Nigeria. Identification and authentication were carried out by Mr. T.K. Odewo of the Department of Botany and Microbiology, University of Lagos, Lagos-Nigeria, where a voucher specimen was deposited (LUH 2618). The fresh root of *S. hispidus* was cut into tiny bits, dried at room temperature and pulverized in a mechanical grinder. The powdered material (500 g) was boiled in a measured volume of distilled water (1.5 L) on a hot plate for 1 h; and thereafter macerated for 72 h. The resulting mixture was filtered and evaporated to dryness on water bath at 40°C. The filtrate (0.86 L) was evaporated to dryness in an oven at 40° C. Percentage yield (w/w) of 7.75 % was obtained.

### Pharmacological Studies:

### Acetic acid induced writhing test:

Adult mice fasted overnight were divided into five groups of five animals each. Treatment was then carried out accordingly: distilled water (10 ml kg-1, *p.o*); *S. hispidus* (50, 100, and 200 mg kg-1, *p.o*); and ASA (100 mg kg-1, *p.o*). All treatments were through oral administration. Sixty minutes after treatment, acetic acid (0.6% v/v in saline, 10 ml/kg i.p) was administered. The number of writhes (characterized by contraction of the abdominal musculature and extension of the hind limbs) were counted for 30 minutes [7, 8, 9].

$$\text{Inhibition (\%)} = \frac{\text{Number of writhes (Control)} - \text{Number of writhes (Treatment)}}{\text{Number of writhes (Control)}} \times 100$$

### Formalin test:

Adult mice fasted overnight were divided into five groups of five animals each. Treatment was then carried out as scheduled: distilled water (10 ml kg-1, *p.o*); *S. hispidus* (50, 100 and 200 mg kg-1, *p.o*); and morphine (10 mg kg-1, *s.c*). Sixty minutes post-treatment for oral route or 30 minutes post-treatment for subcutaneous route, formalin (20 µL of 1 % solution) was injected *s.c* into the right hind paw. The time (s) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min. after formalin injection (first phase) and 15-30 min. after formalin injection (second phase) [9].

$$\text{Inhibition (\%)} = \frac{\text{Reaction Time (Control)} - \text{Reaction Time (Treatment)}}{\text{Reaction Time (Control)}} \times 100$$

### Hot plate test:

Mice used in this experiment were screened by placing the animals in turn on a hot plate (II-39, Ugo Basile, Italy) set at 55 ± 1 °C and animal which failed to lick the hind paw or jump (nociceptive responses) within 10s were discarded. Eligible animals were divided into five groups of five animals each. Treatment was then carried out

accordingly: distilled water (10 ml kg-1, *p.o*); *S. hispidus* (50, 100, and 200 mg kg-1, *p.o*); and morphine (10 mg kg-1, *s.c*). Sixty minutes after oral and thirty minutes after subcutaneous administration, the animal were placed on the hot plate and reaction times of each mouse were recorded for two and half hours at thirty minutes interval. A post treatment cut-off time of 30 seconds was used [10, 11].

$$\text{Inhibition (\%)} = \frac{\text{(Post- treatment Latency)} - \text{(Pre -treatment Latency)}}{\text{(Cut-off Time} - \text{Pretreatment Latency)}} \times 100$$

**Tail immersion test:**

Mice used in this experiment were screened by dipping the lower 5 cm portion of the tail into hot water bath (Ugo Basile, Italy) maintained at 55 °C ± 0.5 to induce pain and animal that failed to withdraw the tail in 10 seconds were discarded [12]. Eligible mice were divided into five groups of five animals each. Treatment was then carried out accordingly: distilled water (10 ml kg-1, *p.o*); *S. hispidus* (50, 100, and 200 mg kg-1, *p.o*); and ASA (100 mg kg-1, *p.o*). The time required for the animal to withdraw the tail clearly out of the water was taken as the reaction time. Reaction time was taken after oral administration of the extract at 30, 60, 90, 120, and 150 minutes. A post treatment cut-off time of 30 s was used [12].

$$\text{Inhibition (\%)} = \frac{\text{(Post- treatment Latency)} - \text{(Pre -treatment Latency)}}{\text{(Cut-off Time} - \text{Pre-treatment Latency)}} \times 100$$

**Haffner's tail clip test:**

Mice used in this experiment were screened by applying a metal artery clip (RS-7440-35, Roboz surgical store, Gaithersburg, United State) to the root of the tail to induce pain and animal that failed to attempt dislodging the clip in 10 seconds were discarded. Eligible mice were divided into five groups of five animals each. The pre-treatment reaction times of all mice in attempt to dislodge the clip were determined after which the animals were treated as follows: distilled water (10 ml kg-1, *p.o*); *S. hispidus* (50, 100, and 200 mg kg-1, *p.o*); and morphine (10 mg kg-1, *s.c*). Reaction time of each mouse was determined 60 minutes post treatment for two and half hours. A post-treatment cut-off time of 30 seconds was used [13, 9].

$$\text{\% Inhibition} = \frac{\text{(Post-treatment Latency)} - \text{(Pre-treatment Latency)}}{\text{(Cut-off Time} - \text{Pre-treatment Latency)}} \times 100$$

**Investigation of possible mechanism of antinociceptive effect:**

**Involvement of opioidergic system:**

Male Swiss albino mice fasted overnight were divided into groups of five animals each. Animals were pre-treated with naloxone (5 mg/kg, s.c., opioid receptor antagonist), 15 min later, vehicle (10 ml/kg, p.o.), *S. hipidus* (200 mg/kg, p.o.) or morphine (10 mg/kg, *s.c*.). Forty five minutes post treatment; mice were subjected to formalin test [14, 15].

**Involvement of dopaminergic system:**

Male Swiss albino mice fasted overnight were divided into 4 groups of five animals each. Animals were pretreated with metoclopramide (1.5 mg/kg; a dopamine $D_2$ receptor antagonist), 15 min later, vehicle (10 ml/kg, p.o.), *S. hipidus* (200 mg/kg, p.o.) or morphine (10 mg/kg, *s.c*.). Forty five minutes post treatment; mice were subjected to formalin test [14].

**Statistical analysis:**

Results obtained were expressed as mean ± SEM. The data were analyzed using one way ANOVA followed by Tukey's multiple comparison test or by two- way ANOVA followed by Bonferroni posttest using GraphPad Prism 5 (GraphPad Software Inc., CA, USA). Results were considered significant when $p<0.05$, $p<0.01$ and $p<0.001$.

**RESULTS:**

**Acetic acid-induced writhing test:**

Intraperitoneal injection of acetic acid elicited the writhing syndrome in control mice with 121 ± 11.18 writhes counted in 30 minutes. *S. hispidus* produced a significant dose-dependent ($p< 0.001$) reduction in the number of writhes: 63 ±11.27 (47.93%), 39.2 ±4.02 (67.60%) and 32.6 ±2.42 (73.06%) for 50, 100, and 200 mg/kg dose of the extract respectively compared with the control. The peak inhibitory effect (73.06%) was produced at 200 mg/kg. This effect compared effectively with the reference drug (74.05%) **Fig. 1**.

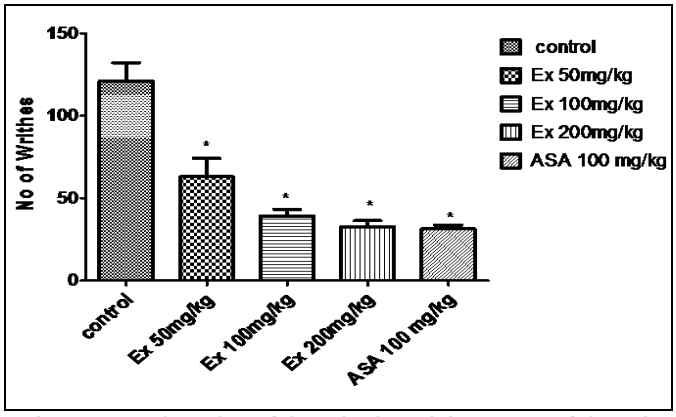


**FIG.1: EFFECT OF AQUEOUS ROOT EXTRACT OF *STROPHANTHUS HISPIDUS* ON ACETIC ACID INDUCED WRITHES TEST.**

ASA: Acetylsalicylic acid (Aspirin)

$^{*}p$< 0.001 statistically significant compared to control (one way ANOVA followed by Tukey's multiple comparison test).

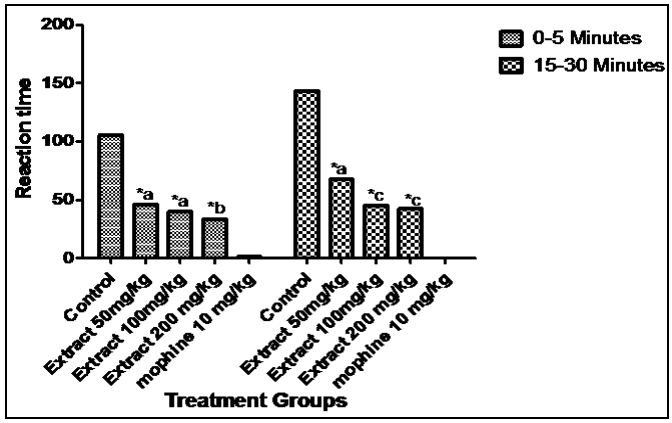


**FIG.2: EFFECT OF AQUEOUS ROOT EXTRACT OF *STROPHANTHUS HISPIDUS* ON FORMALIN TEST.**

$^{*}p$<0.001 statistically significant compared to control, $^{a}p$<0.001, $^{c}p$< 0.05 statistically significant compared to morphine (one way ANOVA followed by Tukey's multiple comparison test).

## Formalin test:

In the first phase, injection of formalin into the sub-plantar tissue of the right hind paw of control mice produced nociceptive response of biting and licking of the paw with a duration of 105.54 ±4.03 seconds. *S. hispidus* produced a significant ($p$<0.001) dose dependent inhibition of nociceptive reaction with peak effect inhibitory effect (68.09 %) produced at the highest dose (200 mg/kg). This effect was less than and statistically significant ($p$<0.05) from that produced by morphine (98.67 % inhibition). In the second phase, the duration of nociceptive reaction in the control group was 143.47±14.55 seconds. *S. hispidus* significantly ($p$<0.001) inhibited the biting and licking response in dose dependent manner with peak inhibitory effect (70.27 %) produced at the same dose. (**Fig.2**).

## Haffner's tail clip test:

Application of the metal artery clip unto the tail of animals in the control group elicited reactions towards clip removal with the post-treatment latency being 1.94±0.31s, 1.89±0.34s, 1.79±0.33s and 1.29±0.16s measured at 60, 90, 120 and 150 minutes respectively with a pre-treatment latency of 2.08±0.58s. The extract of *S. hispidus* produced a significant ($p$< 0.01, $p$< 0.001) dose dependent increase in reaction latency time with peak inhibitory effect of 87.84 % at 120 minutes post-treatment by the highest dose (200 mg/kg). This effect was comparable and statistically significant ($p$< 0.05, $p$< 0.001) to that produced by 10 mg/ kg morphine (100 %). At all the doses of the extract (50, 100 and 200 mg/kg), the highest inhibitory effects were produced at 120 minutes post-treatment latency, and that of the standard at 90 minutes post-treatment latency (**Table 1**).

**TABLE 1: EFFECT OF AQUEOUS ROOT EXTRACT OF *STROPHANTHUS HISPIDUS* ON TAIL CLIP TEST.**

| | | Reaction time (secs) | | | | |
|---|---|---|---|---|---|---|
| | | Pre treatment | Post treatments | | | |
| Treatments | Dose (mg/kg) | | 60 minutes | 90 minutes | 120 minutes | 150 minutes |
| Control (DW) | 10 ml/kg | 2.08±0.58 | 1.94±0.31 | 1.89±0.34 | 1.79±0.33 | 1.29±0.16 |
| *Strophanthus hispidus* | 50 | 2.41±0.33 | 7.24±1.35$^{b}$ | 14.65±1.29$^{b}$ | 16.54±1.08$^{b}$ | 12.94±0.39$^{b}$ |
| | | Inhibition% | 8.33 | 21.25 | 24.54 | 18.28 |
| | | 2.03±0.42 | 15.48±3.38$^{b}$ | 26.59±6.74$^{b}$ | 26.68±6.19$^{c}$ | 15.52±1.78$^{c}$ |
| | 100 | Inhibition% | 23.20 | 42.37 | 42.52 | 23.27 |
| | | 2.18±0.22 | 23.07±9.05$^{*}$ | 42.30±3.28$^{a}$ | 52.97±4.58$^{*,c}$ | 45.17±6.91$^{a}$ |
| | 200 | Inhibition% | 36.13 | 69.39 | 87.84 | 74.35 |
| | | 2.21±0.44 | 57.08±1.64$^{*}$ | 60.00±0.00$^{*}$ | 56.8±1.33$^{*}$ | 53.12±1.32$^{*}$ |
| Morphine | 10 | Inhibition% | 94.95 | 100 | 94.96 | 88.10 |

DW: Distilled Water

Mean ±S.E M: Standard Error of Mean

$^{*}p$<0.001, $^{a}p$<0.01 statistically significant compared to control, $^{b}p$<0.001, $^{c}p$< 0.05 statistically significant compared to morphine (2way ANOVA followed by bonferroni posttests)

**Tail immersion test:**

Extract of *S. hispidus* produced a significant ($p< 0.05$, $p< 0.01$, $p< 0.001$) inhibition of nociception when compared to the control and standard drug. The post treatment latency observed in the control were: 1.45 ±0.13 s, 1.48 ±0.09 s, 1.42 ±0.17 s, 1.23 ±0.09 s measured at 60 minutes, 90 minutes, 120 minutes and 150 minutes respectively with pre-treatment latency of 1.31±0.14 s. The anti-nociceptive effect of the extract was dose dependent with peak effect (59.43 %) at 120 minutes post-treatment at 200 mg/kg. At the same time, the effect of morphine was also pronounced (67.89 %). The effect of the extract was comparable to that produced by the standard drug (**Table 2**).

**TABLE 2: EFFECT OF AQUEOUS ROOT EXTRACT OF *STROPHANTHUS HISPIDUS* ON TAIL IMMERSION TEST IN MICE.**

| | | Reaction time (secs) | | | | |
|---|---|---|---|---|---|---|
| | | Pre treatment | Post treatments | | | |
| Treatments | Dose (mg/kg) | | 60 minutes | 90 minutes | 120 minutes | 150 minutes |
| Control (DW) | 10 ml/kg | 1.31±0.14 | 1.45±0.13 | 1.48±0.09 | 1.42±0.17 | 1.23±0.09 |
| | | 1.82±0.19 | 7.49±0.62$^{c}$ | 10.46±0.38$^{c}$ | 12.88±0.68$^{c}$ | 10.43±0.36$^{c}$ |
| *Strophanthus hispidus* | 50 | Inhibition % | 9.75 | 14.85 | 19.0 | 14.80 |
| | | 1.43±0.13 | 9.47±0.37$^{d}$ | 15.77±1.81$^{c}$ | 20.19±1.34$^{c}$ | 15.59±0.74$^{*,d}$ |
| | 100 | Inhibition % | 13.73 | 24.48 | 32.03 | 24.18 |
| | | 1.28±0.13 | 16.47±1.10$^{a}$ | 19.35±0.24$^{b,d}$ | 36.42±0.64$^{b}$ | 23.67±0.85$^{b}$ |
| | 200 | Inhibition % | 28.87 | 30.77 | 59.84 | 38.13 |
| | | 3.66 ±0.45 | 37.92±2.29$^{b}$ | 41.67±2.31$^{b}$ | 41.91±2.26$^{b}$ | 36.61±2.17$^{b}$ |
| Morphine | 10 | Inhibition % | 60.8 | 67.46 | 67.89 | 58.48 |

DW: Distilled Water

Mean ±S.E M: Standard Error of Mean

$^{*}p<0.05$, $^{a}p<0.01$, $^{b}p<0.001$ statistically significant compared to control, $^{c}p<0.001$, $^{d}p<0.01$ statistically significant compared to morphine (2way ANOVA followed by bonferroni posttests

**Hot plate test:**

The placement of the mice on the hot plate maintained at 55°C ±1 in the control group elicited reactions towards the pain with the post-treatment latency being 2.46±0.08s, 2.44±0.08 s, 2.30±0.05 s and 2.17±0.04 s measured at 60, 90, 120 and 150 minutes respectively with a pre-treatment latency of 2.52±0.01 s. The *S. hispidus* produced a significant ($p< 0.01$, $p< 0.001$) dose dependent increased in reaction latency time with peak effect (51.0 %) observed at 120 minutes post-treatment by the highest dose (200 mg/kg). At all the doses of the extract (50, 100, 200 mg/kg), the highest inhibition was observed at 120 minutes post-treatment latency and that of standard at 90 minutes post latency (**Table 3).**

**TABLE 3: EFFECT OF AQUEOUS ROOT EXTRACT OF *STROPHANTHUS HISPIDUS* ON HOT PLATE TEST.**

| | | Reaction time (secs) | | | | |
|---|---|---|---|---|---|---|
| | | Pre treatment | Post treatments | | | |
| Treatments | Dose (mg/kg) | | 60 minutes | 90 minutes | 120 minutes | 150 minutes |
| Control (DW) | 10 ml/kg | 2.52±0.01 | 2.46±0.08 | 2.44±0.08 | 2.30±0.05 | 2.17±0.04 |
| *Strophanthus hispidus* | 50 | 2.90±0.31 | 6.68±0.62$^{b}$ | 13.47±1.34$^{a,b}$ | 14.33±1.70$^{a,b}$ | 13.68±1.34$^{a,b}$ |
| | | Inhibition% | 6.62 | 18.51 | 20.0 | 18.88 |
| | 100 | 3.51±0.22 | 9.38±0.66$^{b}$ | 15.99±1.64$^{a,b}$ | 24.22±0.72$^{a,b}$ | 14.55±1.76$^{b}$ |
| | | Inhibition% | 10.39 | 22.1 | 36.66 | 19.50 |
| | | 3.32±0.25 | 11.55±0.53 | 16.66±1.58$^{a,b}$ | 31.94±1.85$^{a,b}$ | 17.11±1.72$^{a,b}$ |
| | 200 | Inhibition% | 14.52 | 23.54 | 51.0 | 24.24 |
| | | 3.89±0.43 | 60.00±0.00$^{*}$ | 55.96±1.69$^{*}$ | 52.49±1.22$^{*}$ | 43.51±3.26$^{*}$ |
| Morphine | 10 | Inhibition% | 100 | 92.80 | 86.62 | 70.61 |

DW: Distilled Water

Mean ±S.E M: Standard Error of Mean

$^{*}p<0.001$, $^{a}p<0.01$ statistically significant compared to control, $^{b}p< 0.001$ statistically significant compared to morphine (2way ANOVA followed by bonferroni posttests).

**Opioid Receptor Mediation:**

In the investigation of the opioid receptor mediation with subcutaneous administration of naloxone as an antagonist to block opioid receptor using tail clip induced pain in mice, naloxone significantly ($p<0.001$) reduced the reaction time.

Thus, the anti-nociceptive effect produced by *S. hispidus* (200 mg/kg) was significantly reduced (**Table 4**).

**TABLE 4: INVESTIGATION OF INVOLVEMENT OF OPIOID RECEPTOR MEDIATION USING TAIL CLIP TEST.**

| | | Reaction time (secs) | | | | |
|---|---|---|---|---|---|---|
| | | Pre treatment | Post treatments | | | |
| **Treatments** | **Dose (mg/kg)** | | **60 minutes** | **90 minutes** | **120 minutes** | **150 minutes** |
| Control (DW) | 10 ml/kg | 1.69±0.41 | 1.85±0.28 | 1.31±0.16 | 1.21±0.75 | 1.11±0.08 |
| Naloxone + | 5 mg/kg + | 1.32±0.18 | 3.07±0.40* | 1.57±0.12* | 1.33±0.14 | 1.33±0.13 |
| *Strophanthus hispidus* | 200 mg/kg | Prevention % | 2.98 | 0.35 | 0.01 | 0.01 |
| Naloxone + | 5mg/kg + | 2.06±0.63 | 2.46±0.66 | 2.27±0.58* | 2.34±0.72* | 2.18±0.60 |
| Morphine | 10 mg/kg | Prevention % | 0.69 | 0.36 | 0.48 | 0.20 |

DW: Distilled Water
±S.E M: Standard Error of Mean
*$p<0.001$ statistically significant compared to control (2Way ANOVA followed by Bonferroni posttests).

**Dopamine receptor mediation:**

In the investigation of the dopamine ($D_2$) receptor mediation with oral administration of metoclopramide as an antagonist to block dopamine ($D_2$) receptor using tail clip induced pain in mice, metoclopramide significantly ($p<0.01$) impaired (1.16 %) the reaction time induced by 200 mg/kg dose of *S. hispidus* at 60 minutes post treatment and there was an increased impairment of the action of the extract at 90 (1.06 %), 120 (1.13 %) and 150 minutes (1.21 %) (**Table 5).**

**TABLE 5: INVESTIGATION OF INVOLVEMENT OF DOPAMINE RECEPTOR MEDIATION USING TAIL CLIP TEST.**

| | | Reaction time (secs) | | | | |
|---|---|---|---|---|---|---|
| | | Pre treatment | Post treatments | | | |
| **Treatments** | **Dose (mg/kg)** | | **60 minutes** | **90 minutes** | **120 minutes** | **150 minutes** |
| Control (DW) | 10 ml/kg | 1.69±0.41 | 1.85±0.28 | 1.31±0.16 | 1.21±0.75 | 1.11±0.08 |
| *Strophanthus hispidus* | 200 mg/kg | 1.12±0.05 | 1.81±0.20 | 2.32±0.63 | 1.68±0.13 | 1.15±0.11 |
| | | Prevention % | 1.17 | 2.04 | 0.95 | 0.48 |
| Metoclopramide in 1ml methylcellulose + | 1.5 mg/kg + 200 mg/kg | 1.37±0.10 | 2.05±0.45 | 1.99±0.28 | 2.03±0.23* | 2.08±0.52 |
| *Strophanthus hispidus* | | Prevention % | 1.16 | 1.06 | 1.13 | 1.21 |

DW: Distilled Water
Mean ±S.E M: Standard Error of Mean
*$p<0.01$ statistically significant compared to control (2Way ANOVA followed by Bonferroni posttests).

**DISCUSSION:** In the evaluation of medicinal plants and herbal products for analgesic activity and in the search for newer, more efficacious and better tolerated pure analgesic compounds, various pharmacological methods of pain evaluation have been used. Apart from helping to determine presence or absence of analgesic activity, the various models help to elucidate the mechanism of action of active medicinal plants, herbal products, and pure compounds. In this study, mouse writhing, formalin, tail clip, hot plate and tail immersion tests have been used to screen for analgesic activity of *S. hispidus* extract. The intraperitoneal injection of acetic acid elicited writhing (a syndrome characterized by a wave of abdominal musculature contraction followed by extension of the hind limb), the response which is a sensitive procedure to establish peripherally acting analgesic thought to involve local peritoneal receptors at the surface of the cell lining the peritoneal cavity [16, 17, 18]. Significant protection was observed in the crude extract treated groups of animals and they compared effectively with the standard drug (ASA) (**Fig.1**). The agent reducing the number of writhing

will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [19]. *S. hispidus* has been shown to effectively inhibit prostaglandin synthesis [4]. The significant pain reduction of the *S. hispidus* might be due to the presence of analgesic principles acting with the prostaglandin pathways.

Subcutaneous injection of 1% formalin into the mice right hind paw produces biphasic nociceptive response: the first transient phase is caused by the direct effect of formalin on sensory C-fibers, and the second prolonged phase is associated with the development of the injury induced spinal sensation, responsible for facilitated pain processing, a central sensitization of the dorsal horn neuron occurred during inflammation pain [20, 21, 22]. Drugs which act mainly centrally, like narcotic analgesics e.g. morphine, inhibit both phases of the formalin test while peripherally acting drugs like ASA inhibit the late phase only [23, 24, 9].

Results of the present study showed that the extract of *S. hispidus* inhibit both the early and the late phases of formalin-induced pain (**Fig.2**), thus suggesting its central and peripheral anti-nociceptive actions. Asides this, the extract of *S. hispidus* produced greater inhibition at the late phase than the early phase and this effect was comparable to that produced by the standard drug (morphine).

Findings in the formalin test suggest that *S. hispidus* extract acts through peripheral mechanism of action, as established in the mouse writhing test, demonstrating possible effectiveness in the treatment of chronic inflammatory pain by inhibition of associated inflammatory processes, basically release and/or action of inflammatory mediators. This assertion is supported by the report of Agbaje and Fageyinbo (2011) that *S. hispidus* possesses anti-inflammatory activity via inhibition of histamine, serotonin, substance P and prostaglandin synthesis.

The tail clip and hot plate tests were used in this study to investigate the involvement of central mechanisms in the analgesic activity of *S. hispidus* extracts, as centrally acting analgesic drugs like morphine elevate the pain threshold of rodents towards heat and pressure [7]. In these models, increase in the pain reaction time (latency period) indicates the level of analgesia induced by the drug or extract [25]. *S.hispidus* crude extract produced a significant ($p<0.001$), dose dependent increase in pain threshold in the mice (**Table 1**). The effect produced in the tail clip model was comparable to the reference drug (morphine) (**Table 4**) but the effect produced in the hot plate model was much lesser than that of the reference drug (**Table 3).** These effects peaked at 120 minutes in both models. This shows the involvement of both κ-opioid and μ-opioid receptors. Based on the effectiveness of *S. hispidus* in the tail clip and hot plate tests, a central mechanism of action is confirmed for its observed anti-nociceptive effect.

In the tail immersion test, oral pre-treatment with the crude extract caused a profound and dose dependent analgesia in the treated animals and the analgesic effect was comparable to that produced by morphine (**Table 2**). The animal response in this test is usually integrated at the lower level in the central nervous system, thus, giving information about the pain threshold. It is therefore, used to detect narcotic and non-narcotic analgesics. It is well established that thermal nociceptive tests are more sensitive to opioid μ-agonists and non-thermal tests to opioid κ-agonists [26]. The data generated in the present study suggest the involvement of both κ and μ opioid receptors in the analgesic activity of *S. hispidus*, from which the central involvement is further established.

Anti-nociception can be mediated via cholinergic and dopaminergic mechanism [27]. Dopamine has been demonstrated to play a role in pain processing in multiple levels of the central nervous system including the spinal cord, periaqueductal gray (PAG), thalamus, basal ganglia, and cingulate cortex. Accordingly, decreased levels of dopamine have been associated with painful symptoms that frequently occur in Parkinson's disease [28]. In general, the analgesic capacity of dopamine occurs as a result of dopamine $D_2$ receptor activation [28]. However, anti-nociception elicited by *S. hispidus* was blocked by metoclopramide (dopamine $D_2$ antagonist), indicating the involvement of dopaminergic receptor in the anti-nociceptive effect of *S. hispidus* (**Table 5)**.

In the same vein, naloxone, the universal opioid receptor antagonist, curtailed the anti-nociception induced by *S. hispidus* indicating that the anti-nociception is also mediated via opioid mechanisms.

In a previous study, Agbaje and Fageyinbo (2011) reported the safety of oral administration of the aqueous extract of *S. hispidus* based on the fact that no mortality and visible signs of toxicity were recorded given up to 2 g/kg (2000 mg/kg). Hence, its 1/10th dose i.e 200 mg/kg, 1/20th dose i.e 100 mg/kg and 1/40th dose i.e 50 mg/kg were used as the therapeutic doses for the study.

**CONCLUSION:** The results obtained in this study demonstrated that the aqueous root extract of *Strophanthus hispidus* possesses analgesic property mediated through peripheral and central mechanisms. The anti-nociceptive activity may be mediated via opioid and dopamine receptors modulation.

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