



Evaluation of local anaesthetic efficacy of the crude extract of *Sterculia tragacantha* using West African Dwarf Goats

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Abstract

The crude extract of *Sterculia tragacantha* was subcutaneously infiltrated at the scrotum and left flank of West African dwarf goat prior to orchidectomy and non invasive flank procedures respectively. To assess the local anaesthetic efficacy of the extract post orchidectomy, the pain threshold, changes in vital parameters and blood glucose were monitored. Post flank infiltration, the durations of local anaesthesia, degree of analgesia and distances of tissue diffusion of the extract were monitored. Data obtained in the extract group were compared with those of lignocaine group and a control group. Mean heart rate (HR) of the extract group were significantly ($p < 0.05$) lower than HR obtained in the control group at 30 and 120 min while mean respiratory rate (RR) of extract group was significantly ($p < 0.05$) lower than RR of control group at 30, 120 and 240 min. Post orchidectomy, glucose values of extract group were significantly ($p < 0.05$) lower than those in control group. The mean post operative pain scores in extract group were significantly ($p < 0.05$) lower than those obtained in control group. Duration of local anaesthesia at the flank due to lignocaine infiltration was significantly ($P < 0.05$) longer compared to that due to the extract. Avoidance responses to pain exhibited post flank infiltration of the extract and lignocaine were not significantly ($p > 0.05$) different. Similarly, the rate and distances of tissue diffusion of the extract and lignocaine were not significantly different ($p > 0.05$). On scrotal infiltration, *S. tragacantha* extract significantly attenuated post operative rise in the vital parameters and blood glucose. The extract was as efficacious as lignocaine when infiltrated on the flank. These results showed that an active component with potent local anaesthetic activity could be isolated from the extract if further purified.

Keywords: Anaesthesia, Flank, Infiltration, Lignocaine, Orchidectomy, *Sterculia tragacantha*.

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Introduction

Goats are generally not stoic animals and have low pain threshold (Gray, 1986). These animals thus tolerate few surgical procedures without the use of general or local anaesthesia (Gray, 1986). These anaesthetic techniques help to minimize pain, movement and vocalization during surgery (Taylor, 1991; Adetunji & Ogunyemi, 1998). General anaesthesia in ruminants is associated with a lot of side effects such as passive regurgitation, ruminal tympany, increased salivation as well as cardiovascular depression (Taylor, 1991). Goats are thus often operated on under local anaesthetic techniques (Taylor, 1991).

Infiltration anaesthesia is the most common form of regional anaesthesia and involves subcutaneous injection of small volumes of local anaesthetic solution into the tissues (Ritchie & Greene, 1990; Haddox & Baumann, 1994; Hall *et al.*, 2001a). By this method, the nerve fibres at the site of operation are desensitized (Hall *et al.*, 2001a). The local anaesthetic also diffuses into surrounding tissue from the site of injection anaesthetizing nerve fibres and endings (Mama & Steffey, 2001). In ruminants

the line block, inverted “L” or “L” block is commonly used for abdominal surgeries such as rumenotomy, celiotomy and cesarean section (Skarda, 1986). Local anaesthetic solutions are also infiltrated subcutaneously in the testicles to enable quick castration, reduce stress and provide post-operative analgesia (White *et al.*, 1995; Nyborg *et al.*, 2000; Haga & Ranheim, 2005).

The local anaesthetic solutions most frequently used in veterinary practice are lignocaine, mepivacaine and bupivacaine (Gray, 1986). Lignocaine is the most commonly used and tolerated local anaesthetic in goats and sheep (Gray, 1986; Taylor, 1991). Various studies have demonstrated the advantage of carrying out orchidectomy post intra testicular infiltration of lignocaine (McGlone & Hellman, 1988; VonWaldmann *et al.*, 1994; Horn *et al.*, 1999). Among the enumerated advantages of this practice is the ability of lignocaine to ameliorate the neuroendocrine and behavioural changes such as decreased activity, inappetence and abnormal vocalization associated with orchidectomy (Molony & Kent, 1997; Mellor *et al.*, 2000; Prunier *et al.*, 2006; Keita *et al.*, 2010). Also lignocaine have been shown to provide adequate analgesia of the flank for rumenotomy (Eze *et al.*, 2004).

Sterculia tragacantha (family: Sterculiaceae) is a medium sized tree seen in rain forests belt of Eastern Nigeria (Keay, 1989). Decoctions made from its leaves, bark and seeds are used in treating diarrhea, arthritis, edema, gout and whitlow (Iwu, 1993). In Nsukka area, its leaves are used by traditional bone setters to prepare ethno medicines which are administered to orthopaedic patients for pain relief after open fracture reduction (Udegbumam *et al.*, 2011a). Earlier, we had reported that *S. tragacantha* crude extract exhibited local anaesthetic action in guinea pig skin when infiltrated intradermally (Udegbumam *et al.*, 2011b). No report exists on the use of a crude extract as a local anaesthetic in goats thus the need for this study.

This study was aimed at determining the local anaesthetic efficacy of *S. tragacantha* crude extract when subcutaneously infiltrated at the scrotum and the left flank prior to orchidectomy and non invasive flank procedures respectively. Post orchidectomy, the threshold of pain, changes in vital parameters as well as blood glucose were monitored while post flank infiltration of the crude extract, the durations of local anaesthesia and analgesia and the mean distances of anaesthetic tissue diffusion were monitored. Data obtained in the crude extract

treated group were compared with those of lignocaine treated group and a control group.

Materials and methods

Animals

A two phase experiment was carried out to evaluate the efficacy of the methanol extract of *Sterculia tragacantha* (MEST) for orchidectomy and flank anaesthesia. Thirty 4- months old male West African dwarf (WADG) goats of mean body weight 5.7 ± 0.4 kg procured from local breeders were used for the two experiments. The goats were transported humanely by vehicle to the departmental pens of Veterinary Surgery, University of Nigeria, Nsukka where they were acclimatized and conditioned for two weeks before the study. Goats were fed fresh grass (*Cynodon aluifensis*) supplemented with a concentrate (Bambara dusa) and water provided *ad libitum* throughout the period of the study.

Phase I - Evaluation of the anaesthetic efficacy of MEST for orchidectomy

Animal grouping

For this experiment, twenty goats were assigned to four groups (n=5). Group 1 was the negative control (normal saline treated), group 2 was treated with lignocaine while group 3 was treated with crude extract of *S. tragacantha* (MEST). Group 4 served as the positive control during the glucose assessment.

Presurgery evaluation of the goats and protocol for orchidectomy

The goats were fasted for 12 h before the experiment. The heart rates (HR), respiratory rates (RR) and fasting blood glucose values were obtained and recorded for all goats in groups 1, 2 and 3 at 0 minute before the commencement of the experiment as described by Udegbumam *et al.* (2012). All goats of groups 1, 2 and 3 had their hair clipped off 1 hour prior to surgery and the sites scrubbed clean with 2% chlorhexidine gluconate antiseptic solution (Hibitane[®], Chlorhex SSS Int. Ltd, UK) 60 seconds prior to surgery and railed into the theatre. All animals in groups to be castrated were tranquilized using 0.5 mg/kg diazepam (Valium[®], Roche, Germany). Animals were placed on dorsal recumbency and anaesthesia was instituted by subcutaneously (s.c) infiltration of the scrotum of goats in groups 2 and 3 goats with lignocaine hydrochloride (8 mg/kg, 1 %, Lignocaine[®], Rotexmedica, Germany) and crude MEST (8 mg/kg, 1%). Normal saline was infiltrated into the scrotum of goats in group 1. The sites were finally scrubbed

with antiseptic soaked gauze sponges and then draped. Goats in groups 1, 2 and 3 were castrated as previously described (Kumar, 2002; Tibary & Van Metre, 2004). Briefly, an incision was made parallel to the median raphe down the anterior surface of the scrotum. The testicle was lifted out of the scrotum. A ligature was tied (chromic catgut) around the spermatic cord and the cord was severed distal to the ligature. The testicle was then removed. The second testicle was lifted out of the scrotum through the previous incision and the procedure of removal was repeated as for the first testicle. Goats in groups 4 did not receive any scrotal injection and were not castrated.

Evaluation of intra operative and post operative pain
Intra-operative pain was evaluated by determining the HR and RR of goats in groups 1, 2 and 3 at 10 min during surgery while post operative pain was evaluated by determining the HR, RR and blood glucose of goats at 30, 120, 240, 480 and 720 min post surgery. These parameters were later compared between groups.

Estimation of post operative pain
To estimate the amount of pain felt post orchidectomy, percentage changes in respiratory rates, heart rates and blood glucose were calculated and scored. Also changes in appetite and posture of goats post surgery were scored as described below:

Vital parameters - The HR and RR were determined at 30, 120, 240, 480 and 720 min post surgery. The percentage changes of these values from their baseline readings were then calculated. Percentage increases less than 10 %, 11-20 %, 21-30% and greater than 30% were scored 0, 1, 2 and 3 respectively as described by Grisneaux *et al.* (1999).

Blood glucose - The blood glucose readings of goats in groups 1, 2 and 3 were determined at 30, 120, 240, 480 and 720 min post surgery. The percentage changes of these values from their respective baseline readings were calculated. These percentages were allotted scores as was done in the scoring of the physiologic parameters. All goats were fed after taking the 240 min glucose reading.

Posture - Post orchidectomy at 30, 120, 240, 480 and 720 min, recumbent animals were assigned a score of 0, animals standing were scored 1 while goats standing with a hunched posture were scored 2.

Appetite - The animals were observed at 30, 120, 240, 480 and 720 min post orchidectomy. Animals observed to be eating were scored 0 while those not eating were scored 1. The total post operative pain scores and mean pain scores for each group were later calculated (Udegbunam *et al.*, 2012).

Phase 2 - Evaluation of anaesthetic efficacy of the extract for flank procedures
Phase two of the experiment involved the use of ten goats assigned to two groups (n=5) namely lignocaine and MEST groups. A marker was used to draw a line 8 cm long, 2 cm away from the thirteenth thoracic vertebrae on the left flank of each goat. 3 ml of 1% MEST and lignocaine were infiltrated to groups 1 and 2 goats as described by Hall *et al.* (2001b). The following were determined after the injections.

Onset of anaesthesia
This was calculated as the latency from the time of infiltration to loss of sensation to needle prick on the flank.

Duration of anaesthesia
This was calculated as the latency from the time of loss of sensation to needle prick on the flank to time of its return.

Degree of analgesia
The degree of analgesia obtained by the injection of the MEST and lignocaine were determined by inserting a 21 G needles through the skin, subcutaneous (s.c) tissues (s.c) and muscle layers of the flank as described by Skarda & Muir (1994). Avoidance response to the needle insertion was scored 1, 2, 3 and 4 (Skarda & Muir, 1994).

Distance of diffusion
At 2, 7 and 12 min post infiltration, a meter rule was used to measure the distance (in cm) the drugs had diffused away from the lines of infiltration.

Statistical analysis
All data on HR, RR, blood glucose and mean pain scores were recorded and compared using analysis of variance (ANOVA). The duration of anaesthesia were compared between the two groups using K-independent sample T- test in SPSS 12.0.1 software. The degrees of analgesia obtained in the groups were compared using Mann Whitney U test. DMRT was used to separate variant means at $p < 0.05$.

Results

Evaluation of intra operative and post operative pain

The changes in HR and RR of the groups are presented in Figures 1 and 2. As shown in Figure 1, the mean HR of the groups at 10 min during orchidectomy were not significantly different. Mean HR of MEST treated group was significantly ($p < 0.05$) lower than the mean HR obtained in control group at 30 and 120 min post surgery. The mean HR of the LIG treated group was significantly ($p < 0.05$) lower than that of the other groups throughout the post operative period. The mean RR of MEST group was significantly ($p < 0.05$) lower than RR of control group at 10 min during surgery and at 30, 120 and 240 min post surgery (Figure 1). The LIG treated group had significantly ($p < 0.05$) lower RR compared to control and MEST groups from 10 min of the study. The blood glucose of LIG, MEST and non orchidectomized groups decreased at 30, 120 and 240 min post orchidectomy. The glucose level of control group increased at 30, 120 and 240 min post orchidectomy. The blood glucose values obtained in MEST and LIG groups were significantly ($p < 0.05$) lower than the glucose level in control group at 30, 120 and 240 min post surgery (Figure 3). The blood glucose level of LIG, MEST and non orchidectomized groups were not significantly ($p > 0.05$) different from 120 min of the study.

Pain estimation

The mean post operative pain scores obtained in the treatments groups are shown in Table 1. The results of the experiment showed that at 30, 120 and 240 min, the pain scores of the goats infiltrated with LIG and MEST prior to orchidectomy were significantly ($p < 0.05$) lower than those obtained in control group. The highest pain score was recorded for control group at 120 min post castration. Subsequent decrease in the pain score was recorded thereafter. No significant difference ($p > 0.05$) was seen between the pain scores of the groups given LIG and MEST from 120 min.

Duration of flank anaesthesia

The flank anaesthesia induced by lignocaine lasted for a significantly ($p < 0.05$) longer duration compared to the duration of anaesthesia in the MEST group (Table 2).

Degree of flank analgesia

At 2, 7 and 12 min, the degree of analgesia obtained in LIG and MEST groups were not significantly ($p > 0.05$) different (Table 3).

The distances of tissue diffusion

As shown in Table 4, the distances of diffusion of MEST and LIG away from the lines of infiltration were not significantly different ($p > 0.05$).

Table 1: Mean post operative pain scores of orchidectomized goats

| Treat. | Time (min) | | | | |
|--------|------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| | 30 | 120 | 240 | 480 | 720 |
| Gp. 1 | 2.25±0.29 ^a | 2.55 ± 0.29 ^a | 2.45 ±0.25 ^a | 2.25 ± 0.27 ^a | 1.65 ± 0.25 ^a |
| Gp. 2 | 1.35±0.12 ^b | 1.40 ± 0.80 ^b | 1.50 ± 0.20 ^b | 1.50 ± 0.18 ^b | 1.35 ± 0.21 ^b |
| Gp. 3 | 1.75±0.29 ^c | 1.10 ± 0.07 ^b | 1.20 ± 0.12 ^b | 1.55 ± 0.17 ^b | 1.35 ± 0.20 ^b |

Group 1: Control., Group 2: Lignocaine., Group 3: MEST. Different superscript in a column show significant difference ($p < 0.05$)

Table 2: Duration of flank anaesthesia following lignocaine and MEST infiltration

| Treatments | Duration of anaesthesia (min) |
|------------|-------------------------------|
| MEST | 34.00 ± 11.60 ^a |
| Lignocaine | 70.00 ± 4.71 ^b |

Table 3: Degree of flank analgesia following lignocaine and MEST infiltration

| Treatments | 2 min | 7 min | 12 min |
|------------|-------------|-------------|-------------|
| MEST | 4.00 ± 0.00 | 3.33 ± 0.67 | 2.67 ± 0.67 |
| Lignocaine | 4.00 ± 0.00 | 3.33 ± 0.67 | 3.33 ± 0.67 |

Table 4: Distance of tissue diffusion (cm) of lignocaine and MEST

| Treatments | Distance of diffusion (cm) | | |
|------------|----------------------------|-------------|-------------|
| | 2min | 7 min | 12 min |
| MEST | 1.92 ± 0.14 | 3.37 ± 0.35 | 4.08 ± 0.39 |
| Lignocaine | 2.08 ± 0.26 | 3.85 ± 0.66 | 4.97 ± 0.78 |

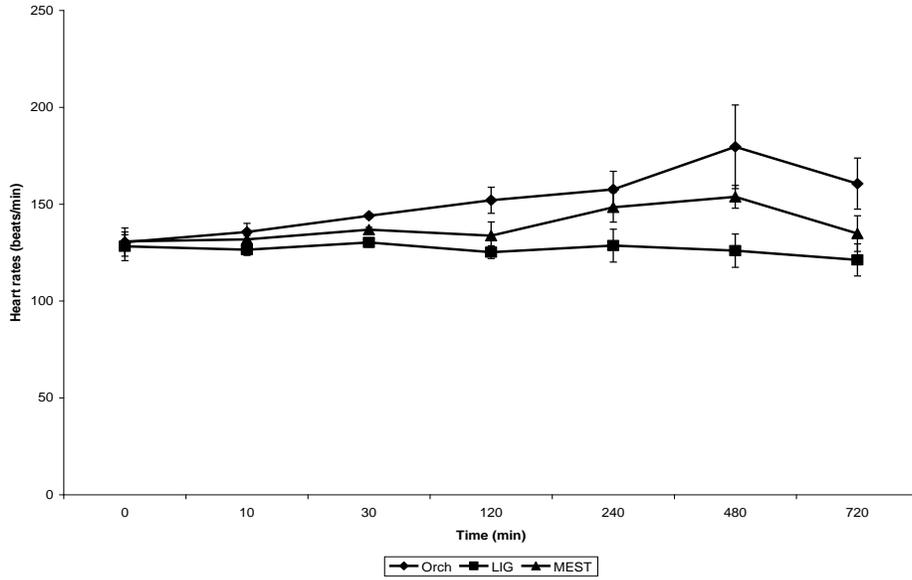


Figure 1: Heart rates (beats/min) of goats in control group (orch), lignocaine group (LIG) and *S. tragacantha* group (MEST)

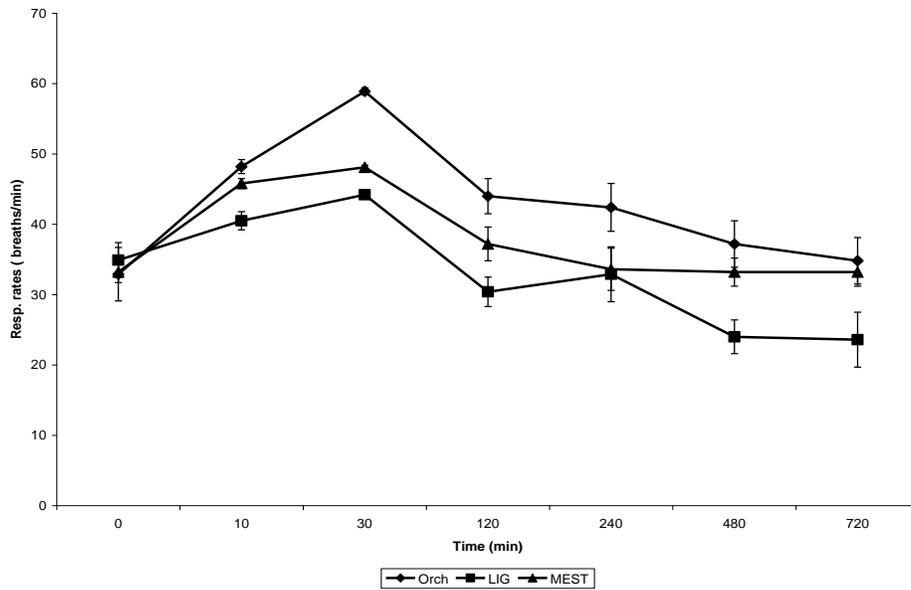


Figure 2: Respiratory rates (breaths/min) of goats in control group (orch), lignocaine group (LIG) and *S. tragacantha* group (MEST)

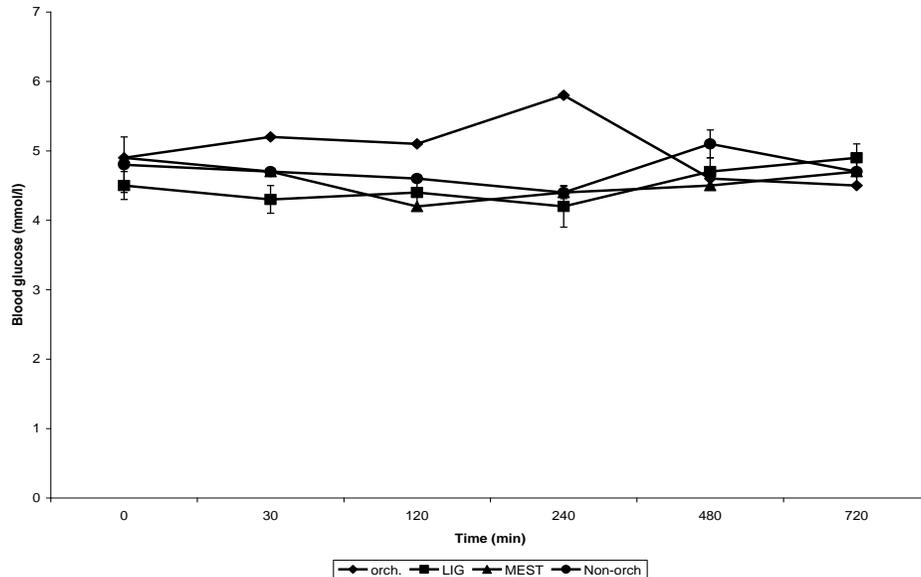


Figure 3: Blood glucose values (mmol/l) of goats in control group (orch), lignocaine group (LIG), *S. tragacantha* group (MEST) and non-orchidectomized group (non-orch)

Discussion

There is currently a lot of evidence showing that orchidectomy induces acute pain, discomfort as well as physiological and behavioural changes (Hay *et al.*, 2003; Prunier *et al.*, 2006; Keita *et al.*, 2010). High frequency vocalization of long duration as well as increase in heart rate has been shown by piglets during orchidectomy (White *et al.*, 1995; Weary *et al.*, 1998; Taylor & Weary, 2000; Marx *et al.*, 2003). As shown in figure 2, the RR of goats in all groups increased at 10 min during orchidectomy with significantly higher increase recorded in the control group compared to MEST group. This result suggests that less pain was felt intra operatively in the MEST group compared to the control group. A similar study carried out to evaluate the physiologic responses of pigs to surgical castration reported a consistent increase in the RR of pigs orchidectomized without the use of lignocaine (White *et al.*, 1995).

In all pain assessment techniques used in animals, it is assumed that any change in a variable after a procedure is related to pain in the animal (Flecknell & Liles, 1991; Liles & Flecknell, 1993; Scott *et al.*, 1994). According to these researchers, the administration of analgesics prior to the procedures prevented the occurrence of these changes. Thus the lower HR of goats in MEST and LIG group throughout the post operative period suggests that use of MEST and LIG prior to orchidectomy

significantly reduced the pain response to orchidectomy (White *et al.*, 1995; Haga & Ranheim, 2005).

Post operative pain leads to increased production of epinephrine, nor-epinephrine, cortisol, growth hormones and ACTH (Bailey & Child, 1987; Bailey & Stanley, 1990). Epinephrine and glucagon activates glycogenolysis leading to increase in blood glucose (Breznock, 1980). Glucocorticoids, ACTH and growth hormones also modify carbohydrate metabolism and induce hyperglycemia and carbohydrate intolerance (Allison *et al.*, 1976). Blood glucose level thus increases in the immediate post trauma period but returns to normal by the second day (Breznock, 1980). In this study, the blood glucose level of LIG, MEST and non orchidectomized group decreased at 30, 120 and 240 min of the study whereas the glucose level of control group increased at these time points. The result obtained in this study was similar to that of Lemke *et al.* (2002) who investigated the effect of preoperative injection of ketoprofen on signs of post-operative pain in dogs undergoing ovariohysterectomy. These authors reported a decrease in the serum glucose of dogs between 4-20 h post surgeries. They concluded that the preoperative use of ketoprofen reduced signs of post operative pain in dogs. We therefore conclude that the use of MEST reduced post operative pain thus preventing post operative rise in blood glucose.

To estimate the amount of pain felt by the goats post orchidectomy, changes in blood glucose level, respiratory rates, heart rates, posture and appetite of goats in all groups were scored. Comparison of the pain scores obtained showed that the pain scores of MEST group were lower than those of control group throughout the post operative period. This finding further supports our claim that administration of MEST before orchidectomy ameliorated the acute post operative pain induced by castration.

The degree of analgesia and distance of tissue diffusion of LIG and MEST on the flank were similar throughout the period of assessment. However the duration of anaesthesia obtained in MEST group was shorter compared to that obtained in LIG group. The shorter duration of flank anaesthesia obtained in the MEST group may be given two interpretations. One is that it can be said that LIG may be more lipophilic in nature compared to MEST. Local anaesthetics which are more lipophilic are more potent and have

a more prolonged duration of action compared to less lipophilic drugs. This is because association of the drug at the lipophilic sites enhances the partitioning of the drug to its site of action and decreases the rate of metabolism by plasma esterase's and hepatic enzymes (Courtney & Strichartz, 1987). The shorter duration of anaesthesia in the MEST group may also mean that LIG produced a longer duration of action since it was a pure compound and contained more active anaesthetic compounds as against the MEST which was still in a crude form.

In conclusion, the extract of *S. tragacantha* on scrotal infiltration significantly attenuated post operative rise in the vital parameters and blood glucose. The extract was also as efficacious as lignocaine when infiltrated on the flank. The results of this study showed that if this extract is further purified, an active component with potent local anaesthetic activity could be isolated.

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