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Original article

Investigation of anaesthetic potentials of various extracts of *Annona muricata* (sour sop) in Wister albino rat and dog

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ABSTRACT

Objectives: Local and general anaesthetics currently used in clinical practice are not absolutely safe and efficacious. Hence two or more drugs are used for induction and maintenance of general anaesthesia.
Methods: In view of this, local and general anaesthetic potentials of *Annona muricata* extracts were investigated prospectively in laboratory using rat and dog. A total of 15 animals comprising 6 rats (3 males; 3 females) and 9 dogs (5 males; 4 females) were used. Secondary metabolites in root bark, leaf, stem bark and seed of *Annona muricata* were qualitatively and quantitatively determined. Methanol and water extracts were tested for induction and maintenance of surgical anaesthesia in rat and dog. The dose of the extracts (1250 mg/kg body weight of rat) that produced deep anaesthesia in rats was translated to 150, 170 and 150 mg/kg of root bark methanol extract (RBME), stem bark water extract (SBWE) and seed water extract (SWE), respectively. Each extract was administered to female and male dog for induction and maintenance of anaesthesia. Laparatomy was carried out via linea alba, and the length of small intestine (220 cm) and large intestine (32 cm) were measured using measuring ruler.
Results: Phenols and tannins were significantly lower ($p < 0.05$) as compared to saponins, flavonoids, alkaloids and glycosides. The median lethal dose (LD50) of methanol extract was < 5000 mg/kg in female and > 5000 mg/kg in male rats, respectively. Sedation, anaesthesia, male mounting and death were observed within 4 days of extract administration in the female rats, However, 12.5 mg/kg showed significant ($p < 0.05$) local anaesthesia on the test surgical sites. The male administered 170 mg/kg of SBWE showed salivation, significantly decreased respiratory rate, and loss of palpebral reflex, excretion, urination that characterized plane III; stage III of anaesthesia (deep anaesthesia) which lasted for 67 min.
Conclusions: Aqueous extract of *Annona muricata* have very potent local and general anaesthetic effects for a period of over 1 h. The observed effects may be due to presence of secondary metabolites.

1. Introduction

The need for efficacious, safe local and general anaesthetics is of paramount importance in surgery. There is no absolutely safe local and general anaesthetic in surgical practice (Neal et al., 2010) leading to continuous quest for anaesthetics with relatively less toxicity and high effeicacy. *Annona muricata* (sour sop) is a member of Annonaceae family. Other examples of the species are *Annona reticulata* (Custard apple), *A. squamosa* (Sugar apple) and *A. cherimosa.* Unripe roots, seeds, leaves and fruits of the plant are used as insecticides, pesticides and insect repellants respectively (Moghadamtousi et al. 2015). *Annona muricata* contains neurotoxic long-chain fatty acids called acetogenins and annonacin which acts via mitochondria by inhibiting nicotinamide adenosine dinucleotide reductase (NADH) and ubiquinone oxidoreductase. Alkaloids such as isoquinoline (reticuline), aporphines and protoberberine (coreximine), phenols (quercetin and gallic acids) and sesquiterpenes (Coria-Téllez et al., 2016), annonaine, nomuciferine and asimilobine have been isolated from *Annona muricata* (Hasrat et al., 1997a,b). Seeds, leaves and pericarp have acetogenin (annonacin), inhibitor of mitochondrial complex L inhibitor that induces nigral and striated neurodegeneration in rats (Escobar-Khondiker et al., 2007). The leaves have anticonvulsant, anti-inflammatory, antinociceptive and analgesic effects via pathway

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of opioid receptors. Antioxidant, adaptogenic (Hamid et al. 2002) antihypertensive via calcium antagonism (Coria-Téllez et al., 2016 and wound healing via up-regulation of Hsp 70 have been reported (Moghadamtousi et al., 2015). The fruit can cause atypical Parkinsonism among French West Indians (Caparros-Lefebvre and Elbaz, 1999).

The flavor components of *Annona muricata* are methylhexanoate and methyl hex-2-enoate (MacLeod and Pieris, 1981). Hydroalcoholic and chloroform extraction could yield phytochemical principles with hypotensive effect. Such compounds have alkane, ketones and alcohol functional groups as demonstrated by *Hibiscus rosasinensis* Linn (Siddiqui et al., 2005). Water and organic solvents could increase the yield of some phytochemical principles (Sunday et al., 2019). Therefore, local and general anesthetic potentials of various extracts of *Annona muricata* were studied in rat and dog respectively with intent to discovering highly efficacious, safe anaesthetic from the plant.

2. Materials and methods

2.1. Qualitative and quantitative analyses

Secondary phytochemicals such as saponins, flavonoids, tannins, alkaloids, glycosides and phenols were detected and quantified from leaves, seeds, stem, stem bark, root bark and root of *Annona muricata* using the method of Harborne (1998).

2.2. Extraction

Whole *Annona muricata* plant was fetched from outskirt of Makurdi metropolis, Benue state, Nigeria and identified by a botanist, Mallam Namadi Sanusi in the Herbarium of Department of Biology, Ahmadu Bello University Zaria where a sample given voucher (ABU0901263) has been deposited. Root barks, stem barks, seeds and leaves were separated from the plant, air dried to constant weight and grounded into fine powder using mortar and pestle. Fifty gramme (50 g) of the root back powder was added to 450 ml methanol, whereas 50 g each of stem bark, seed and leaf powder was added separately to 950 ml of distilled water. The extractions were done many times to obtain large quantities of the yields. The solutions were thoroughly shaken for a period of 24 h, extracted and concentrated in water bath at 45 °C and stored in refrigerator at −4 °C until used. Ten grammes of each extract was reconstituted for administration. The yield of the extracts was 2.5–7.5 %.

3. Methods

3.1. Animals

Six Wister albino rats (3 males; 3 females) of 6 weeks old, and 9 mongrel dogs consisting of 4 females and 5 males of 3 months, weighing 110 ± 5 g and 2.3 ± 0.23 kg respectively were used for the study. The rats were bought from Animal House, College of Health Sciences, Benue State University Makurdi and dogs were bought from private livestock producers in Makurdi. All the animals were kept in the animal house of College of Veterinary Medicine, Federal University of Agriculture Makurdi for a period of 2 weeks for acclimatization, before commencement of the study. Feed and water were provided ad libitum. All the animals were handled according to the institutional and international guiding principle on the use of experimental animals as approved by ethical committee, Department of Veterinary Surgery and Imaging, College of Veterinary Medicine, Federal University of Agriculture Makurdi given the permit number PN 2020–001.

3.2. Acute toxicity study

Acute toxicity study was carried out using the method of OECD (2000). Six albino rats were administered orally, upper limit test dose of 5000 mg/kg body weight. One each of male and female rat was separately dosed 5000 mg per kilogramme of methanol extract and observed for 48 h. Thereafter two each of male and female rats were concurrently dosed 5000 mg/kg and observed for another 48 h. All the rats were further observed for a period of 14 days.

3.3. Induction of local anaesthesia

Six dogs were used for induction and maintenance of local anaesthesia. Prickling method was adopted for assessment of sensation (Boonstra et al. 2016). Aqueous extract of leaf (12.5 mg/kg) was infiltrated into tail base, linea alba, left paralumbar and right paralumbar regions of 3 male and 3 female rats respectively. Five minutes after the administration, each extract infiltrated site was prickled 3 times for scoring of pain sensation using numerical rank score (Flecknel 1984).

3.4. Induction of general anaesthesia

The dose (1250 mg/kg) of the three extracts that produced profound anaesthesia in rat was translated to the doses that could produce similar or more intense anaesthesia in puppies. Human equivalent dose (HED) equals to animal dose (AD) multiplied by animal metabolism constant (AKm) divided by human metabolism constant (HKm) was used to extrapolate rat dose of *Anonna muricata* extracts to dog doses using the method of Saganuwan and Onyeyili (2016). Each of the three puppies was administered root bark methanol (150 mg/kg), stem bark water (170 mg/kg) and seed water extract (150 mg/kg), respectively, for induction and maintenance of general anaesthesia. The extracts were administered via jugular vein by slow transfusion. The puppy administered 170 mg/kg entered deep anaesthesia characterized by salivation, loss of palpebral reflex, excretion, open eye deep sleep and irregular breath, <5 min post administration of the extract and laparatomy was carried out after incision of about 10 cm along linea alba.

3.5. Statistical analyses

Data generated on quantities of phytochemicals present in various parts of *A. muricata* and local anaesthetic effects were presented as average ± standard error of mean (SEM). Repeated measure analysis of variance (ANOVA) was used to analyze the data. Tukey's test was used to detect significance between groups at 5 % level (Daniel 2010).

4. Results

Qualitative and quantitative analyses revealed presence of saponins, flavonoids, tannins, alkaloids, glycosides and phenols in the leaf, stem bark, root, root bark and stem but anthraquinone was absent (Tables 1 and 2). The results of limit dose test of 5000 mg/kg are presented in Table 3. No male rat died throughout the period of experimentation, but all the three female rats died at the same dose level. The observed toxicity signs are pruning of hair; roaming, standing still, calmness, sedation for 3–5 min, male mounting another male, lateral and ventral recumbency and anaesthesia were observed. The scoring of pain sensation to assess local anaesthetic potential of *Annona muricata* aqueous leaf extract is presented in Table 4. Loss of pain sensation that lasted for > 1 h was significantly higher ($p < 0.05$) at all the four anatomical sites:

Table 1
Qualitative analysis of *Annona muricata extracts.*

	Phytochemical principles										
Extracts	Flavonoids	Alkaloids	Saponins	Tannins	Steroids	Glycosides	Saponin glycosides	Cardiac glycosides	Volatile oils	Balsams	Anthraquinone
LWE	++	++	+++	+++	–	–	+++	–	++	+++	–
LME	++	+++	+++	+++	+	+	++	+	+++	+++	–
LHE	+	++	–	+	++	+	++	++	++	+	–
LEE	+++	+++	–	++	–	++	++	–	+++	++	–
SWE	–	+++	++	–	+	++	++	+	+++	–	–
SME	+	+	+++	–	–	+++	+++	–	+	–	–
SHE	+	+	–	+	+	+	+	+	+	+	–
SEE	+	++	++	+	+++	+	+	+++	+	+	–
FWE	+	+	+	–	++	–	+	+++	+	–	–
FME	–	++	+	–	++	–	+	++	++	–	–
FHE	–	+	–	–	++	–	+	++	++	–	–
FEE	+	++	–	++	+++	+++	+	+++	++	++	–
SWE	+	+++	–	+	+++	+++	+	+++	+++	+	–

Keys: – = absent; + = low; ++ = moderate; +++ = high.
LWE = leaf water extract; LME = leaf methanol extract; LHE = leaf *n*-hexane extract; LEE = leaf ethanol extract; SWE = stembark water extract; SME = stembark methanol extract; SEE = stembark ethanol extract; SHE = stembark *n*-hexane extract; FWE = fruit water extract; FME = fruit methanol extract; FHE = fruit *n*-hexane extract; FEE = fruit ethanol extract; SWE = stembark water extract, each analysis was carried out thrice.

Table 2
Quantity of phytochemical principles (ppm) present in leaf of *Annona muricata.*

Plant Part	Saponin	Flavonoids	Tannins	Alkaloids	Glycosides	Phenols
Fruit	0.79 ± 0.03	11.11 ± 0.61	0.12 ± 0.00^{b}	0.94 ± 0.04	289.2 ± 0.0	0.21 ± 0.02^{b}
Leaf	0.72 ± 0.04	9.88 ± 0,62	0.13 ± 0.01^{b}	0.86 ± 0.4^{a}	193.6 ± 0.4	0.16 ± 0.02^{b}
Seed	0.84 ± 0.02	11.68 ± 0.49	0.14 ± 0.01^{b}	1.20 ± 0.13	194.4 ± 0.4	0.18 ± 0.03^{b}
Stem	0.53 ± 0.02^{a}	9.15 ± 0.50	0.11 ± 0.00^{ab}	0.89 ± 0.00^{a}	97.2 ± 11.0^{a}	0.10 ± 0.01^{ab}
Stem bark	0.67 ± 0.11^{a}	10.34 ± 0.15	0.11 ± 0.02^{ab}	0.89 ± 0.01^{a}	118.8 ± 23.3^{a}	0.11 ± 0.01^{ab}

Key: Data are presented in triplicates; a = Significantly lower ($p < 0.05$) along the column; b = Significantly lower ($p < 0.05$) along the row.

Table 3
Limit dose test of *Annona muricata* root bark methanol extract in rats.

Sex	Dose (mg/kg)	Signs	Comment(s)	Survival status
Male	5000	Roaming, standing still, sedation for 3 min, dullness, lying ventrally, anaesthesia, survived	LD50 above 5000 mg/kg	O
Male	5000	Pruning, roaming, sedation, anaesthesia in lateral recumbency, survived	LD50 above 5000 mg/kg	O
Male	5000	Roaming, pruning, male mounting another male, calmness, sedation for 5 mins, lying ventrally, anaesthesia, survived	LD50 above 5000 mg/kg	O
Female	5000	Roaming, pruning, calmness, sedation, anaesthesia, in lateral recumbency, died after 3 days	LD50 < 5000 mg/kg	X
Female	5000	Roaming, pruning, calmness, sedation, anaesthesia, died after 3 days	LD50 < 5000 mg/kg	X
Female	5000	Roaming, pruning, calmness, sedation, anaestheesia, died after 4 days	LD50 < 5000 mg/kg	X

Key: X = Dead; O = Survived.

Table 4
Ranking of analgesia at different anatomical sites of puppies anaesthetized with intravenous aqueous leaf extract of *Annona muricata* (12.5 mg/kg).

	Site of pain sensation and pain score				
Sex	Pretreatment pain score (Control)	Tail	Left paralumbar	Right paralumbar	Linea alba
Male	25 ± 0.00	50 ± 0.00^{a}	75 ± 0.00^{a}	75 ± 0.00^{ab}	75 ± 0.00^{ab}
Male	25 ± 0.00	75 ± 0.00^{ab}	75 ± 0.00^{ab}	25 ± 0.00	75 ± 0.00^{a}
Male	25 ± 0.00	50 ± 0.00^{a}	50 ± 0.00^{a}	75 ± 0.00^{ab}	750.00^{ab}
Female	25 ± 0.00	25 ± 0.00	75 ± 0.00^{ab}	75 ± 0.00^{ab}	40 ± 0.00^{a}
Female	25 ± 0.00	75 ± 0.00^{ab}	75 ± 0.00^{ab}	75 ± 0.00^{ab}	50 ± 0.00^{a}
Female	25 ± 0.00	75 ± 0.00^{ab}	75 ± 0.00^{ab}	75 ± 0.00^{ab}	50 ± 0.00^{a}

Key: a = significantly higher along the row ($p < 0.05$); b = significantly higher along the column ($p < 0.05$); c = significantly lower along the column ($p < 0.05$); Data are of 3 replicates ± SEM; 0–25 = No analgesia; 25–50 = Low analgesia;50–75 = Moderate analgesia;75–100 = High analgesia.

tail, linea alba, left paralumbar and right paralumbar regions in comparison with the pre-treatment sensation test. The vital parameters and clinical signs observed from the puppies administered various doses of *Annona muricata* extracts are presented in Table 5. It was only puppy administered 170 mg/kg of stem bark water extracted that produced a complete state of general anaesthesia that lasted for 1 h 07 min. The signs observed are respiratory rate (32 cycles per min), heart rate (60 beats per min), open eye sedation for about 3 min, full anaestesia similar to that of plane 3, stage 3, deep respiration, salivation which disappeared after

Table 5
Comparative parameters of puppies anaesthetized with intravenous extracts of *Annona muricata.*

Sex	Dose (mg/kg)	Respiratory rate	Heart rate	Anaesthetic signs	Comment(s)
Female	150 (Root bark methanol extract)	–	–	Calmness, sedation (1 min), 6 min after admin, open eye hypnosis (12 mins)	No Surgery
Male	170 (Stem bark water extract)	Before anaesthesia = 32; cycles/min after anaesthesia = 70 cycles/min	Before anaesthesia = 60 beats/min; after anaesthesia = 60 beats/mins	Salivation, excretion, immediate full anaesthesia (1 hr 7 min)	Laparotomy. Small intestine = 220 cm; large intestine; 32 cm
Male	150 (Seed water extract)	66 cycle/min	168 beats/min	Salivation, urination, excretion, lying laterally for 20 min, no sedation	No surgery

Key- = the parameter not measured.

5 min, defecation and urination. The lengths of small and large intestines measured are 220 cm and 32 cm, respectively. The values of vital parameters measured after surgery were heart rate (60 beats per min) and respiratory rate (70 cycles per min), respectively (Table 5). The surgery was successfully done (Figs. 1–4). However, the animal died of bleeding the following day, suggesting haemolytic potential of the extract.Table 5.

5. Discussion

High quantities of secondary metabolites present in the plant suggest likely vast number of biological activities of the plant. The survival of three male rats administered 5000 mg/kg body weight of *Annona muricata* bark root methanol extract shows that the LD_{50} of the extract is above 5000 mg/kg. The death of three female rats administered 5000 mg/kg of the extract shows that the female rats are more sensitive to the extract than the male rats are. Our finding agrees with the report indicating that plant materials could be toxic and their toxicity depends on the dose, phytochemical principle, sex, species and age of animals (Saganuwan 2016). However, our finding disagrees with the report indicating that the LD_{50} of *A. muricata* leaf extract was 1091.7 mg/kg body weight (Coria-Téllez et al., 2018). The difference in the LD_{50}s may be due to differences in solvents used, part of plant tested and soil chemistry on which the plants are cultivated. The muscle relaxation, local anaesthetic and sedative effects of aqueous leaf and root bark methanol extract respectively observed on male and female dogs show that the plant is a source of potent anaesthetic. Gonzalez-Trujano et al. (2001) had reported that the plant has anticonvulsant activity. Palmitone (16- hentriacontanine) isolated from the leaves of *Annona muricata diversifolia* showed anticonvulsant activity on pentylenetetrazole- induced clonic-tonic seizures. It delayed the onset of seizure, reduced death caused by 4- amino pyridine (4-AP) and bicuculine. However, palmitone didn't produce motor incoordination and loss of righting reflex used as signs of neurological impairment. A dose of palmitone (1.85 mg/kg) was more potent than etosuximide (59.6 mg/kg), sodium valproate (63 mg/kg), carbamazepine (>300 mg/kg) and four folds less potent than diazepam (0.48 mg/kg), respectively (N'gouemo et al., 1997).

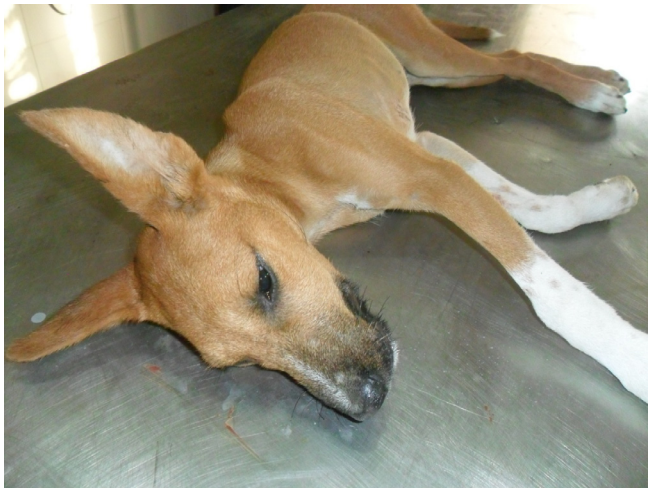

Fig. 1. Mongrel dog treated with 170 mg/kg of *Annona muricata* stem bark water extract in the state of open eye anaesthesia.

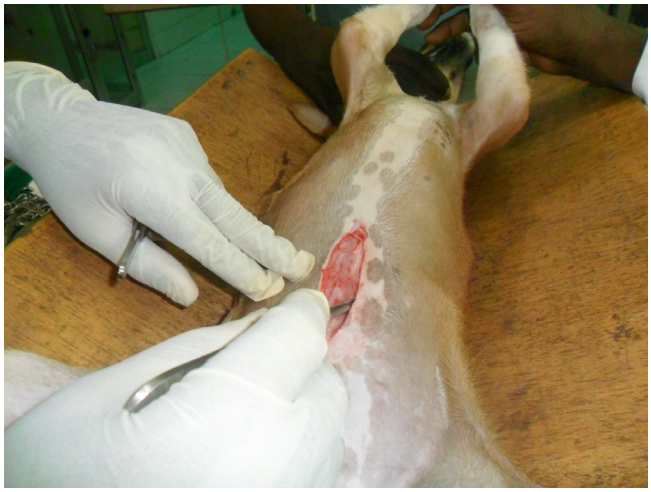

Fig. 2. Mongrel dog anaesthetized with 170 mg/kg of *Annona muricata* stem bark water extract undergoing laparotomy for measurement of large and small intestines.

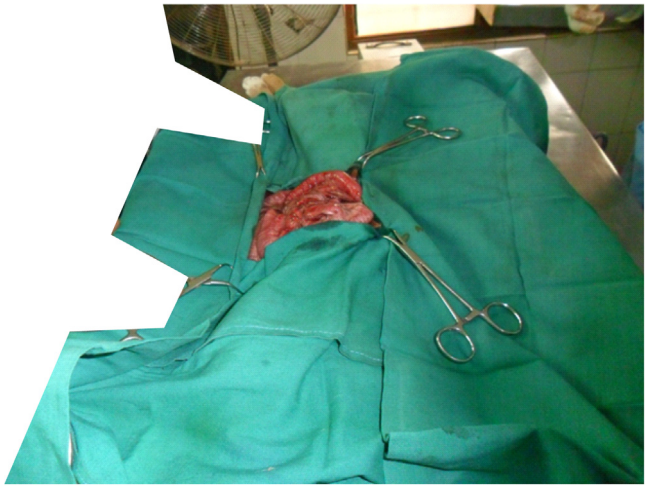

Fig. 3. Mongrel dog anaesthetized with 170 mg/kg of *Annona muricata* stem bark water extract in draping.

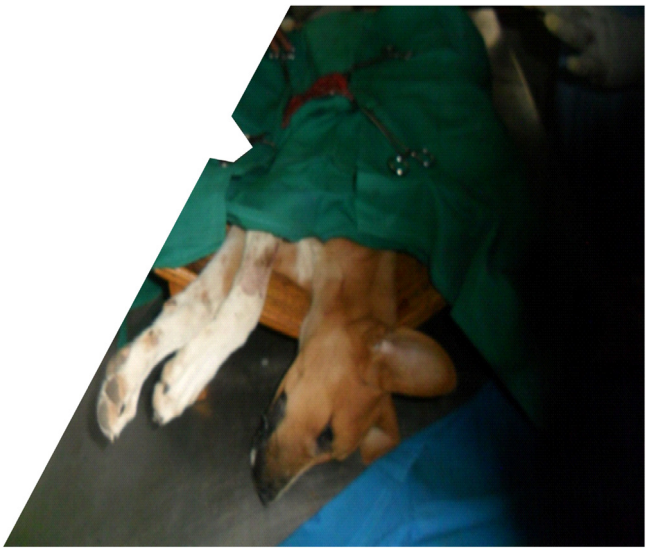

Fig. 4. Mongrel administered 170 mg/kg of *Annona muricata* stem bark aqueous extract recovering from general anaesthesia after laparatomy.

The mechanisms of action may be via gamma aminobutyric acid (GABA) receptors (Gonzalez-Trujano et al. 2001). Single repeated administration of clozapine on phencyclidine-induced hyper locomotion, down regulated 5-HT (2A) receptors and blocked the enhanced phencyclidine-induced neurochemical and behavioural changes (Abekawa et al. 2007). The general anaesthesia shown by the male dog administered 170 mg iv (Figs. 1–4) indicates that the extract has general anaesthetic principle. General anaesthesia (hypnosis, muscle relaxation and analgesia) is a state of unconsciousness in which the animal no longer responds to innate instinct so as to avoid vulnerability of dorsal recumbency known as loss of righting reflex (LORR). The end-state is marked by return of the righting reflex (RORR). Therefore, hypnosis in animals is less straight forward than it is in human (McCarren et al. 2013). Because of neurotoxic tendency of sour sop, it can cause neurodegenerative, atypical Parkinsonism (Caparros-Lefebvre and Elbaz, 1999). Reticuline from the plant inhibits dopamine uptake, at high concentration, it is toxic to GABAergic and dopaminergic receptors. It is a weak neuromuscular (nicotinic) calcium channel blocker, causes uterine relaxation and vasorelaxation activity via L-type Ca^{2+} channel. The analgesic effect of *Annona muricata* is via opioidergic pathway and anti-inflammatory effect is via inhibition of chemical inflammation mediators (Ishola et al. 2014). The low respiratory rate observed during the period of anaesthesia (Table 5) agrees with the report indicating that respiratory sinus arrhythmia could be used for prediction of slow wave brain activity during sleep (Niizeki & Saitoh 2018). Agents that cause deep sleep (hypnosis) are GABA agonists and many other CNS depressants by altering physical properties of lipid in neuronal membranes (Mendelson 2002). Injectable anaesthetic can be used for the induction and maintenance of short-term anaesthesia that prevents detrimental effects of pain by establishing preemptive and multimodal analgesia (Sullivan et al. 2016). The salivation and open eye sleep observed in the present study agrees with the report indicating that some general anaesthetics have salivation as one of the side effects. Dissociative anaesthetics including ketamine have salivation and open eye sleep as side effects (Jud et al.2010). General anaesthesia is induced by intravenous drug and maintained by inhalational anaesthetics. If anaesthesia is maintained by intravenous anaesthetic, autonomic function remains more stable intraoperatively. Hence hypnosis, analgesia and muscle relaxation may improve the welfare of animal undergoing general anaesthesia (Tajeda-Chavez et al. 2018) as observed in the present study. The ability of the extract to cause full general anaesthesia using a single large bolus to fill the volume of distribution of the central compartment, disagrees with the report indicating that the technique of intravenous anaesthesia requires lower dosages of anaesthetic after large volume, to maintain effective drug plasma concentration for the duration of anaesthesia (Waelbers et al. 2009).

Annona is used for treatment of insomnia, convulsion, anxiety, and causes sedation, parturition, milk let down and spasms. Annonaine and asimilobine (aporphines) are antidepressants whereas coreximine (protoberberine) and reticuline (isoquinoline) are neurotoxic. The mechanism of action of annonaine is via dopamine inhibition. Also, nornuciferine (isoquinoline) has antidepressants activity. The neuroactive principles are present in fruit, leaf, root and stem, but annonacin has neurodegenerative effect (Moghadamtousi et al. 2015). Behavioural and neurological effects of *A. muricata* were via gamma aminobutyric and monoaminergic receptors. The purified plant extracts could be source of a new compound with local and general anaesthetic potential (Souza et al. 2018). The first anaesthetic discovered from plant, *Erythroxylon coca* is cocaine (Biondich and Joslin, 2016). Alkaloids and flavonoids have anaesthetic effects (Udegbunam et al. 2012). Cocaine isolated from *E. coca* is the prototype of local anaesthetics. Thymol and eugenol from *Thymus vulgaris* and *Syzygium aromaticum* are mechanistically and structurally similar to intravenous anaesthetic of phenol origin. The anaesthetic phytochemicals act via gamma aminobutyric acid type A receptor, sodium channel, lipid membranes and *N*-methyl-D-aspartate receptors (Tsuchiya, 2017). The nociceptive impulses are blocked, providing analgesia with minimal risk of adverse effects (Grubb and Lobprise, 2020). The depressant activity of chemical compounds depends on their functional groups and structure–activity relationship (Saganuwan, 2017a,b). The bleeding observed in the present study agrees with the report indicating that many herbal anaesthetic agents cause coagulation, endocrine disruption, cardiovascular problems, hepatotoxicity and prolongation of anaesthetic effects (Cheng et al. 2002). Kaempferol, apigenin, benzoylaconine, aconitine, chalcone, catechin,valtrate, pyrethrin and beta-carboline have anaesthetic and pain relief potential (Sayhan et al., 2017). Hence the anaesthetic potential of medicinal plants depends on the plant parts, solvents and the innervations of injected anatomical sites (Lopez et al. 2016).

Central nervous system disorders could be treated or managed by chemical agents that modify the tight junction for easy transport to the brain (Liu 2008). The reaction may be redox at the level of the lipophylic membrane and it is dependent on molecular weight, pH, physicochemical and pathological condition of blood–brain barrier. Some CNS acting agents may undergo carboxylation, carbonylation, methylation, desulphation and dehydrogenation with low molecular weight (Saganuwan, 2017a,b). But carotid bodies comprise glomus cells and sustentacular cells, sense pH, partial pressure of oxygen, carbondioxide and glucose. Sensory information is sent to brainstem neurons for regulation of respiratory airway ventilation, circulatory and endocrine responses (Saganuwan 2019). Death of the operated dog could be attributed to blood loss observed after surgery. Hence concurrent use of antihaemolytic with the extract may be evident.

6. Conclusion

Annona muricata root bark methanol extract is more toxic in female rats as compared to male rats. A dose of 12.5 mg/kg of the leaf extract caused significant local anaesthesia. However 1250 mg/kg caused deep anaesthesia in rat and was translated to 170 mg/kg that caused deep anaesthesia that lasted for 67 min

and permitted laparotomy in dog. Hence, *A. muricata* may serve as source of less toxic and high efficacious local and general anaesthetic that may provide balanced anaesthesia comprising analgesia, deep hypnosis and muscle relaxation (triad of anaesthesia).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

SAS designed the study and did statistical analysis, AIK did the surgery, whereas both SAS and AIK did phytochemical analyses, tested for local anaesthesia, wrote, proof read and approved the manuscript.

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