Analgesic and Antioxidant Effects of Chenopodium ambrosioides L. Ethanolic Extract in Post-Castration Pain Management in Rabbits

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SUMMARY

Effective post-surgery pain management is vital for animal welfare. *Chenopodium ambrosioides*, known for its analgesic and anti-inflammatory properties, was evaluated for its potential antioxidant and post-castration pain ameliorative effects in rabbit. Twenty-five mature bucks were surgically castrated and randomly assigned to five groups: Group A (oral 0.9% normal saline control), Group B (oral meloxicam, 0.1 mg/kg), Group C (oral *C. ambrosioides* extract, 500 mg/kg), and Group D (topical Ketoprofen 2.5% gel), and Group E (topical *C. ambrosioides* extract). Blood glucose level was measured immediately after the surgery and 15-, 30-, and 60-minutes post treatment while plasma malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPX) were measured in blood samples collected at 1-, 2-, and 3-hours post treatment in order to evaluate the plasma oxidant and antioxidant level Pain was evaluated using the Grimace scale based on still images extracted from 20-minute video recordings taken one hour after treatment. One-hour post-treatment, glucose levels were significantly higher in the control group (267.67 ± 18.77 mg/dL). At two hours, MDA levels were also significantly elevated in the control group (44696 ± 19071 nMol/mg), indicating greater oxidative stress. Grimace scores for oral meloxicam (0.51), oral *C. ambrosioides* (0.71), and topical *C. ambrosioides* (0.55) did not significantly differ from pre-surgical levels, while topical Fastum gel (Ketoprofen) (0.8) and oral *C. ambrosioides* (0.71) did not differ from the control group (0.92). *C. ambrosioides* showed antioxidative and analgesic properties, especially in its topical form, which yielded a faster pain-relief response and thereby have high potential in post-surgical pain management.

Keywords: *Chenopodium ambrosioides*, Castration, pain, Grimace Scale, Analgesic

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INTRODUCTION

Pain is a significant global health challenge and has been identified as one of the leading causes of disability and increased healthcare costs worldwide (Kola-Mustapha *et al.*, 2020). In West Africa, the prevalence of pain-related conditions is rising at an alarming rate, contributing to both economic and social burdens in affected communities (Kola-Mustapha *et al.*, 2020). Effective pain management remains a critical aspect of healthcare, particularly in regions with limited access to advanced medical treatments. The choice of analgesic therapy plays a pivotal role in balancing efficacy and safety, particularly in veterinary medicine and post-surgical care.

Among the various approaches to pain management, topical and oral analgesics are commonly employed. Topical analgesics are applied directly at the site of pain, making them particularly effective for conditions affecting muscles, bones, and nerves (Kraychete *et al.*, 2016). These agents offer targeted pain relief while minimizing systemic absorption, thereby reducing the risk of adverse effects commonly associated with oral medications (Brayfield, 2018). Conversely, oral analgesics, which are administered through the buccal cavity, are widely used for the treatment of both acute and chronic pain, example of such analgesic is the famous Non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs remain one of the most widely used classes of analgesics in both human and veterinary medicine (Flood and Stewart, 2022). These drugs exert their effects by inhibiting the metabolism of arachidonic acid via cyclooxygenase (COX) enzymes, specifically COX-1 and COX-2, leading to a reduced production of prostaglandins responsible for inflammation and pain (Flood and Stewart, 2022).

However, NSAID use and systemic exposure is frequently associated with significant adverse effects, including gastrointestinal ulceration, renal impairment, and cardiovascular risks (Brayfield, 2018; Oguntibeju, 2018).. Given these limitations, there is a growing interest in exploring alternative pain management strategies (acute and chronic pain) that are both effective and safe, especially the medicinal plants. Medicinal plants such as *C. ambrosioides* offer a potential alternative with reduced toxicity, fewer side effects, and a lower risk of drug residues in edible animal products (Drioua *et al.*, 2024). Additionally, phytotherapeutic agents are generally more cost-effective, making them particularly suitable for use in resource-limited settings.

Chenopodium ambrosioides (*C. ambrosioides*) is a tropical plant species indigenous to the Americas but is now widely distributed across the globe (Riou and Althaus, 2020). It has been traditionally used in various cultures for medicinal purposes. In Mexico, it is commonly brewed as an herbal tea to promote milk secretion and enhance blood circulation in women (Bary and Amraoui, 2020). In Morocco, it has been utilized in the management of symptoms associated with coronavirus disease (COVID-19), particularly fever and influenza-like illnesses (Ainane, 2020). Additionally, when consumed orally at low oral doses of 400mg/kg, *C. ambrosioides* has been reported to exhibit antioxidant and anti-inflammatory properties (Ouadja *et al.*, 2021). The plant possesses a broad spectrum of pharmacological activities, including analgesic and anti-rheumatic effects (Okuyama *et al.*, 1993), sedative and antipyretic properties (Gadano, 2006), as well as antimicrobial and antifungal activities (Lohdip *et al.*, 2019; Althobaiti, 2020; Fakehha *et al.*, 2023). Furthermore, studies have demonstrated its anti-helminthic activity (Araújo *et al.*, 2023), antioxidative and immunostimulant properties (Maldonado-Garcia *et al.*, 2019), and it's potential as a natural source of essential oils with sedative, analgesic, and antimicrobial properties (Ez-Zriouli *et al.*, 2023). These diverse pharmacological attributes make *C. ambrosioides* a promising candidate for pain management, particularly in conditions requiring prolonged analgesic intervention with minimal adverse effects.

Despite the well-documented pharmacological benefits of *C. ambrosioides*, there is a paucity of information regarding its therapeutic efficacy in animal models following surgical procedures. Given the need for safer and more effective analgesic alternatives, this study seeks to evaluate the analgesic and antioxidative effects of *C. ambrosioides* administered both orally and topically in surgically castrated bucks. The study aims to compare the analgesic efficacy of *C. ambrosioides* with meloxicam and ketoprofen, by assessing oxidative stress and antioxidant biomarkers, and Grimace pain scores. Through this investigation, we aim to explore the potential of *C. ambrosioides* as a viable alternative to conventional NSAIDs for post-procedural pain management in laboratory animals,

thereby contributing to the growing body of research supporting the use of herbal analgesics in veterinary medicine.

MATERIALS AND METHODS

Experimental Animals Management

Twenty-five sexually matured rabbits with mean body weight of 2.5kg and average age of 6 months old were used. The rabbits were sourced from a private rabbit farm located in Samaru, Zaria, Kaduna State. The rabbits were kept in metal cages individually. They were fed commercially available rabbit feed with water ad-libitum. The rabbits were kept for two weeks before the surgery for pre-surgical acclimatization. The rabbits were made to be accustomed to daily routine activities of the animal handlers such as cleaning, feeding, weighing, video-taping, presence of observers and the video monitoring equipment as described by Keating *et al.* (2012). The animals were fed with palletised feed (vital feed®) twice daily and given water *ad libitum*.

Plant Material and Extraction

Chenopodium ambrosioides (Figure 1A and B) was collected from Ago, Asa Local Government area, Kwara State. The plant was identified at the Herbarium of the Department of Biological Sciences of Ahmadu Bello University, and a voucher specimen number (1725) was obtained. The plant was air dried by spreading them on a paper on a concrete floor at room temperature. Once dried, the plant was then crushed into powder using an electric blender. The dried powder sample was extracted with 75% ethanol as described by Prashanthi *et al.*, (2012). Briefly, 500g of dried powder of the plant was soaked in 1liters of 75% ethanol in a glass jar, it was occasionally stirred for even mixture. The mixture was allowed to stay for 48hours. Thereafter it was filtered using a Whatmann No 1 filter paper. The filtrate was then evaporated in a waterbath at 45^{O}C. The pasty extract was then stored in an airtight container until use. Fifty grams (50g) of each extract was dissolved in 100ml of distilled water (500mg/ml) for use.

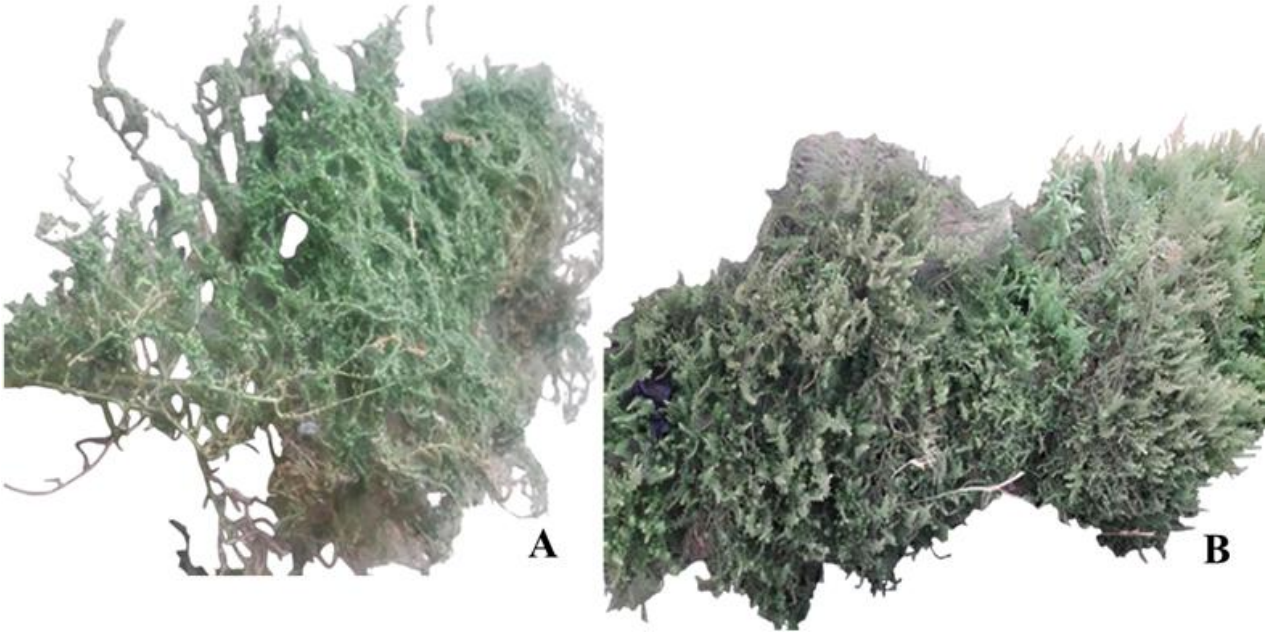

Figure 1A &B. Appearance of whole plant of *C. ambrosioides Linn*, **a small shrublike medicinal herb used in this study**

Determination of Acute Oral Toxicity

The LD_{50} of the ethanol extract of the *C. ambrosioides* was determined as described by Imafidon *et al.* (2016). The procedure was conducted in two phases. In the first phase, nine Wistar rats were divided into three groups of three rats each. Group 1, 2 and 3 received oral doses of 50% (w/v) ethanol extract of *C. ambrosioides* at 10mg/kg, 100mg/kg and 1000 mg/kg respectivelly using cannula. All the rats were kept under the same conditions and monitored for signs of toxicity and mortality for 24 h. In the second phase, a total of three wistar rats were used. The rats were divided into three groups and were administered 1600, 2900 and 5000 mg/kg orally of the ethanol extract of *C. ambrosioides*. They were also observed for signs of toxicity and mortality for 24 h.

Experimental Design

The research was carried out in two phases; Phase 1 comprised of 15 rabbits divided into three groups, A, B, C, with 5 rabbits each. All rabbits in groups A, B and C were castrated and treated orally with 5mls 0.9% normal saline, 0.75% meloxicam tablet (0.1mg/Kg) (Melocap, Bahrat perenteral Ltd, India) and oral 50% *C. ambrosioides* extract (500mg/Kg) respectively. In the phase 2 of the experiment, ten rabbits were divided into 2 groups, D and E with 5 rabbits each. Rabbits in the two groups were castrated and treated with topical administration of 2.5% Ketoprofen gel (Fastum gel®, A-Menarini Ltd, Italy) and *C. ambrosioides* ethanolic extract (500mg/Kg) respectively. Blood sample for glucometry was taken immediately after surgery before treatment, then 15, 30 and 60 minutes after treatment. Blood sample for oxidative stress indices was collected 1-hour, 2hours and 3hours following treatment. Grimace scale evaluation was carried out 1 hour post treatment for twenty minutes as described by Langford *et al.*,(2010);

Surgical techniques

The lower abdominal along with the scrotal area was shaved liberally and then scrubbed with a 2%diluted chlorhexidine solution followed by application of povidone iodine on the surgical site. Each rabbit was restrained on a dorsal recumbency on the surgical table and prescrotal approach was used for castration as described by Murray, (2006).

The evaluation of plasma oxidative stress

The evaluation of plasma malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPX) was as described by Rahman et al, (2017).

- *Evaluation of plasma malondialdehyde (MDA)*

To the mixture of 500 µl of plasma, 100 µl of 0.2 mM ferric chloride solution was added to initiate the peroxidation at 37 °C for 30 min. The reaction was stopped by adding 2 ml of ice cold mixture of 0.25 N HCl containing 15% TCA (Trichloroacetic acid), 0.30% TBA (Thiobarbituric acid) and 0.05% BHT (Butylated hydroxytoluene). The reaction mixture was heated at 80 °C for 60 min, the samples were cooled and centrifuged at 3500 rpm for 15 min and the optical density (O.D.) of the supernatant was measured at 532 nm. Lipid peroxidation was expressed as malondialdehyde (MDA) equivalents in nanomoles per milligram of protein.

- *Estimation of plasma superoxide dismutase (SOD)*

The reaction mixture was prepared by mixing 0.5 ml plasma, 1 ml 50 mM sodium carbonate, 400 µl of 25 µM NBT (Nitro Blue Tetrazolium) and 200 µl 0.1 mM EDTA (Ethylene diamine tetraacetic acid), simultaneously control was prepared without plasma. The reaction was initiated by the addition of 400 µl of 1 mM hydroxylamine hydrochloride solution. The change in absorbance was recorded at 560 nm. Units of SOD activity were expressed as the amount of enzyme required to prevent the reduction of NBT by 50%.

- *Estimation of plasma glutathione peroxidase (GPX)*

An aliquot of 1 ml of plasma was precipitated with 1 ml of sulphosalicylic acid (4% w/v). The samples were kept at 4 °C for 1 h and then centrifuged at 3500 rpm for 15 min at 4 °C. The reaction mixture in a total volume of 3 ml consisted of 100 µl supernatant, 2.7 ml 0.1 M phosphate buffer (pH 7.4) and 200 µl of DTNB (40 mg dissolved in 10 ml of 0.1 M phosphate buffer, pH 7.4) The optical density of the yellow colour developed was measured at 412 nm.

Evaluation of acute pain using rabbit Grimace scale

Following surgery, each rabbit was treated and kept in its metal cage. At 1 hour post-treatment, rabbits

were video-taped in a clear transparent 6″x8″x6″ dimension glass chamber for 20 minutes using 2 High-definition video camera. The cameras (HD 1080p, Sony, Japan) were strategically placed outside glass to capture every movement and behaviour of each rabbit. The method described by Keating *et al.* (2012) for rabbit grimace scale was used. From each video sequence of the rabbits' head and face. Still, clear and high quality images were extracted (Figure 2) and pain scoring was done using grimace scale pain scoring technique (NC3R^S, 2025). Treatment-blind participants were involved in the scoring of the images taken before and during each procedure.

Data Analysis.

All measured data were expressed as means ± SEM (standard error of mean). One way analysis of variance (ANOVA) was used to analyse difference between different treatment group and pain control in each group. It also tested the difference in the oxidant and antioxidant level in different treatment group. Two-way analysis of variance (ANOVA) was used to compare data between the groups treated orally and those treated topically. Multiple pairwise comparisons test (Duncan's Multiple Range Test) was performed as post-hoc test after one-way ANOVA. P-Values ≤ 0.05 was considered statistically significant. Statistical analyses were performed using the GraphPad prism 8.4.2 for sciences software.

RESULTS

Effect on Blood Glucose

The toxicity studies conducted in this research revealed no mortality among the Wistar rats in both phases of the study. Additionally, the evaluation of blood glucose concentration in the rabbit models post-surgery (Figure 2A) demonstrated variations across different treatment groups. Notably, rabbits treated with oral meloxicam exhibited a blood glucose concentration of 251.67 ± 18.00 mg/dL, whereas those administered topical ketoprofen gel recorded a concentration of 259.67 ± 18.85 mg/dL. In contrast, oral and topical administration of *C. ambrosioides* extracts resulted in blood glucose levels of 242.67 ± 1.86 mg/dL and 262.33 ± 13.87 mg/dL, respectively. However, the group A treated with oral 0.9% normal saline had higher plasma glucose of 278.34 ± 16.25 mg/dL

Effect on Oxidative stress markers

Plasma malondialdehyde (MDA) concentration (Figure 2B), a key marker of oxidative stress, displayed considerable differences among the treatment groups. One hour after surgery, rabbits treated with oral meloxicam had an MDA concentration of 18,381 ± 11,236 µmol/L, whereas those treated with topical ketoprofen exhibited significantly lower levels at 3,218 ± 340 µmol/L. The administration of *C. ambrosioides* extracts resulted in MDA concentrations of 8,280 ± 551 µmol/L for oral treatment and 3,731 ± 419 µmol/L for topical treatment. In contrast, rabbits treated with normal saline showed an MDA concentration of 5,837 ± 2,615 µmol/L.

Superoxide dismutase (SOD) activity, another critical marker of oxidative stress, was also evaluated (Figure 2C). One-hour post-surgery, rabbits receiving oral meloxicam exhibited SOD activity of 13 ± 6.9 mg/mL, while those treated with topical ketoprofen had significantly lower activity at 2.8 ± 1.1 mg/mL. In contrast, oral administration of *C. ambrosioides* extracts resulted in a higher SOD activity of 19 ± 4.3 mg/mL, while topical administration yielded a value of 5.8 ± 0.39 mg/mL. Rabbits in the normal saline treatment group exhibited an SOD activity of 15 ± 7.3 mg/mL.

The analysis of glutathione peroxidase (GPX) concentration (Figure 2D) further corroborated these observations. One hour after surgery, rabbits in the oral meloxicam treatment group exhibited GPX levels of 80 ± 34 Ug/mL, while those treated with topical ketoprofen recorded 63 ± 11 Ug/mL. The oral administration of *C. ambrosioides* extracts resulted in a GPX concentration of 48 ± 3.5 Ug/mL, whereas topical administration led to a significantly higher value of 93 ± 49 Ug/mL. Interestingly, rabbits in the normal saline group exhibited a GPX concentration of 86 ± 41 Ug/mL.

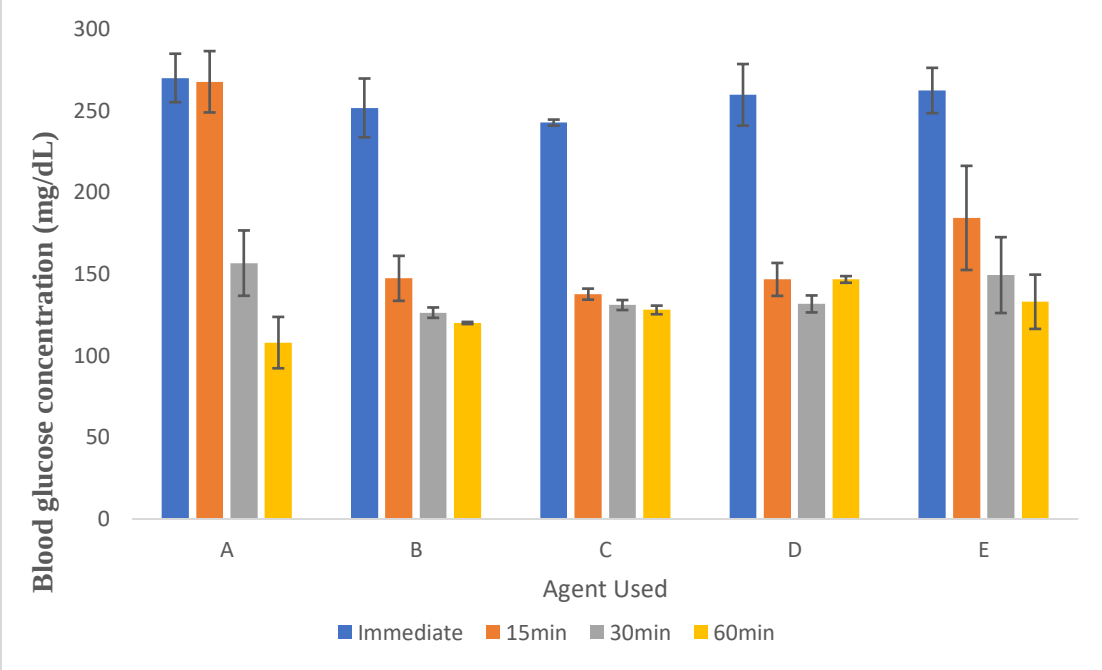

Figure 2A: The blood glucose concentration (mg/dL) of castrated rabbits treated with different agents. [ab]Means in the same column with different superscripts alphabets were significantly different ($p \leq 0.05$). KEY: A: Normal saline (oral); B: Meloxicam (0.1mg/kg; oral); C: *C. ambrosioides* extracts (500mg/Kg; oral); D: Ketoprofen (Liberal; topical); E: *C. ambrosioides* extracts (Liberal; topical); F: Baseline data before surgery

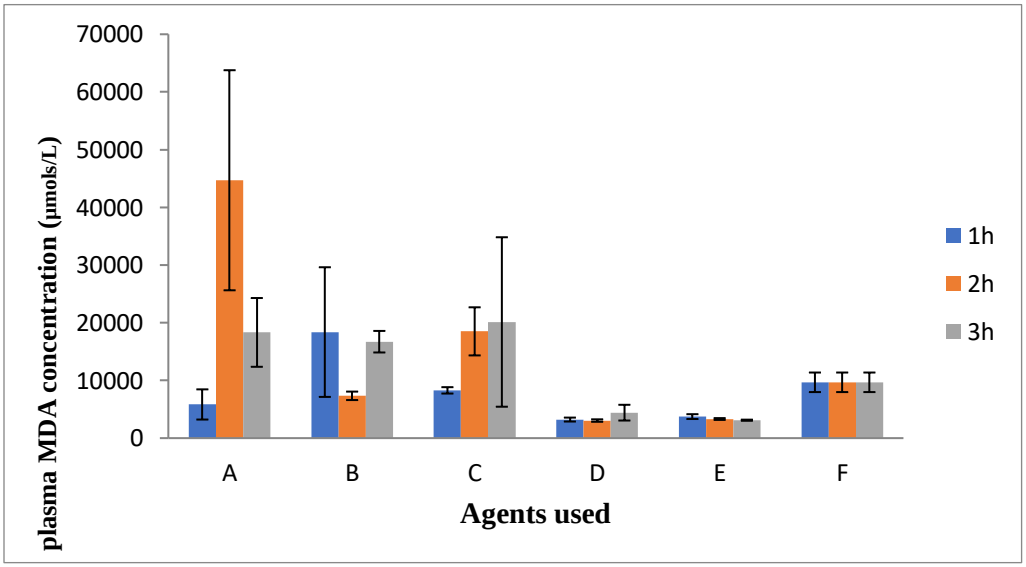

Figure 2B: The plasma MDA concentration (µmol/L) of castrated rabbits treated with different agents. [ab]Means in the same column with different superscripts alphabets were significantly different ($p \leq 0.05$). KEY: A: Normal saline (oral); B: Meloxicam (0.1mg/kg; oral); C: *C. ambrosioides* extracts (500mg/Kg; oral); D: Ketoprofen (Liberal; topical); E: *C. ambrosioides* extracts (Liberal; topical); F: Baseline data before surgery

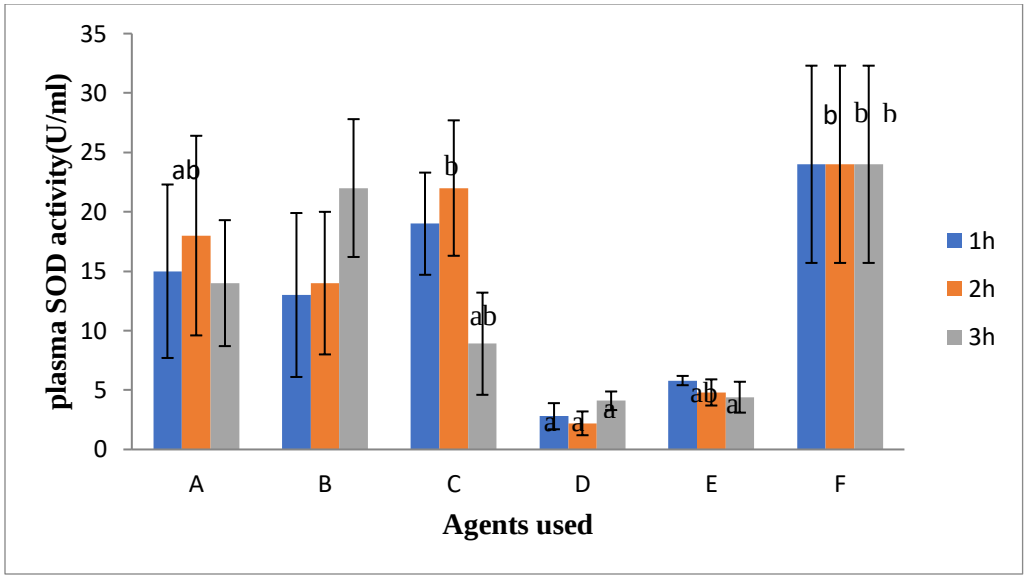

Figure 2C: The plasma SOD activity (mg/mL)of castrated rabbits treated with different agents.
D: plasma GPX concentration (Ug/ml) of the castrated rabbits treated with different agents after the surgery.
[ab]Means in the same column with different superscripts alphabets were significantly different ($p \leq 0.05$).
KEY: A: Normal saline (oral); B: Meloxicam (0.1mg/kg; oral); C: *C. ambrosioides* extracts (500mg/Kg; oral); D: Ketoprofen (Liberal; topical); E: *C. ambrosioides* extracts (Liberal; topical); F: Baseline data before surgery

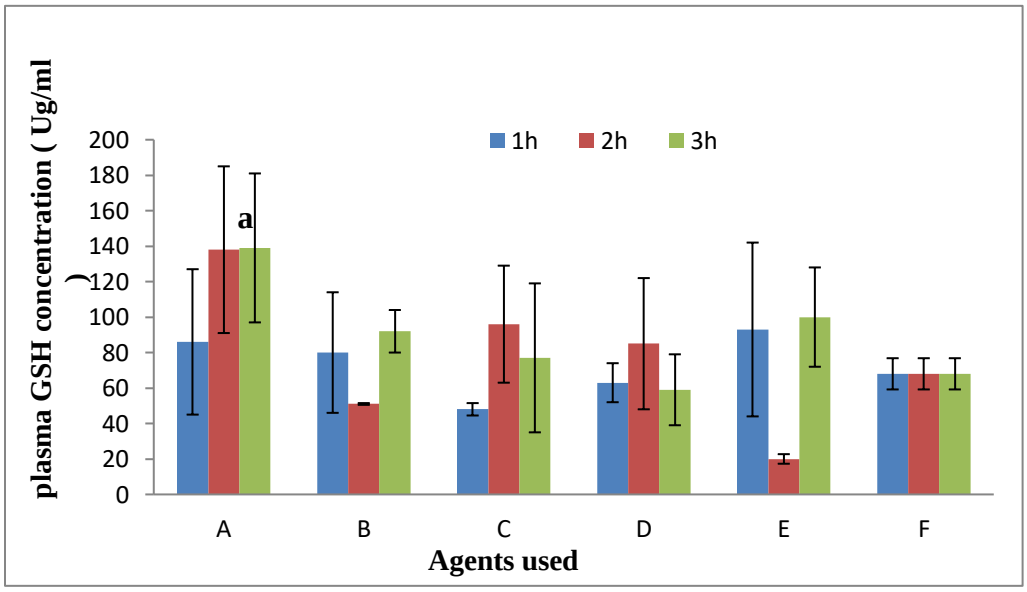

Figure 2D: The plasma GPX concentration (Ug/ml) of castrated rabbits treated with different agents.
[ab]Means in the same column with different superscripts alphabets were significantly different ($p \leq 0.05$).
KEY: A: Normal saline (oral); B: Meloxicam (0.1mg/kg; oral); C: *C. ambrosioides* extracts (500mg/Kg; oral); D: Ketoprofen (Liberal; topical); E: *C. ambrosioides* extracts (Liberal; topical); F: Baseline data before surgery

Pain assessment using facial expression analysis provided further insights into the analgesic efficacy of the tested agents (Figure 3). The mean scores for various facial expressions are presented in Table 1, while the overall pain scores for individual treatment groups are summarized in Table 2. Notably, pre-surgical pain scores were generally lower across all groups, whereas the normal saline-treated group exhibited significantly higher pain scores. The overall mean pain score for the pre-surgical group was recorded at 0.36. In contrast, post-surgical pain scores were as follows: normal saline (0.92), oral meloxicam (0.51), oral *C. ambrosioides* extract (0.71), topical ketoprofen (0.80), and topical *C. ambrosioides* extract (0.55).

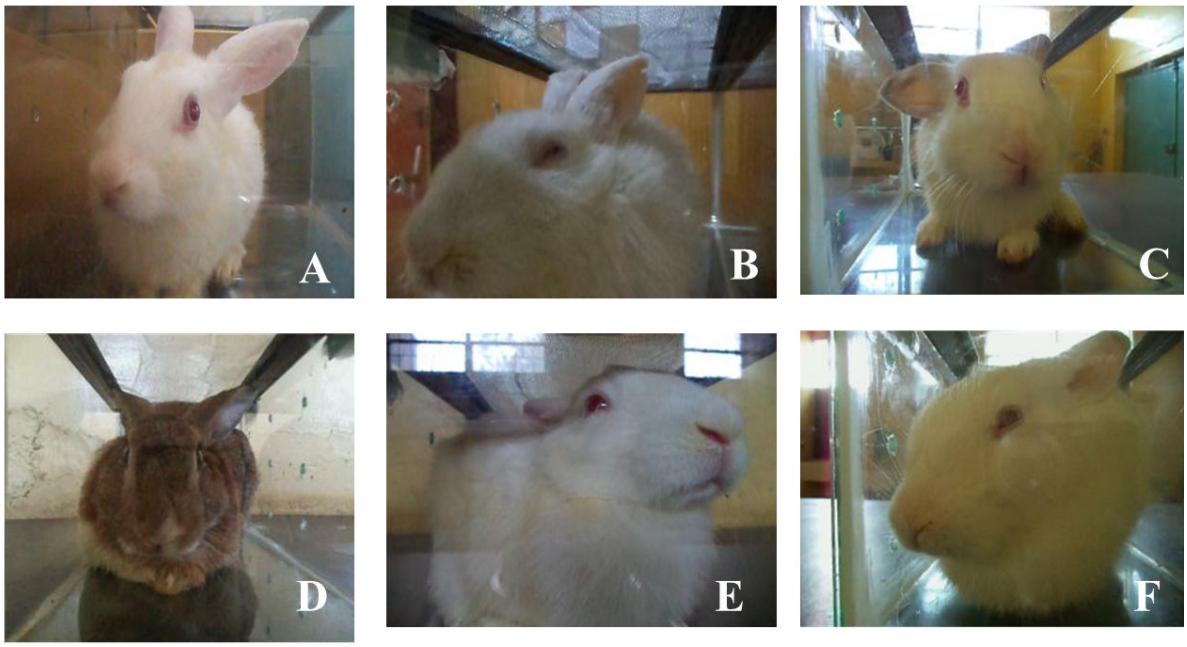

Figure 3 showing facial expression in rabbits before and after castration. A: Pre-surgical facial expression with opened eyelid normal ear shape and carriage; B: Narrowed eyelid and curled ears held closer to the back (dorsum) was seen in group A; C: There was opened eyelid, more rounded face and the nares forming "U" shape in group B; D: There was opened eyelid, rounded face and the "U" shaped nares in group C; E: There was partially closed eyelid, slightly angled face and the curled ears held closer to the back (dorsum) in group D; F: There was partially closed eyelid, slightly angled face and the curled ears held closer to the back (dorsum).

Table 1. The mean ± SEM (minimum and maximum) values for the pain scores recorded for various facial expression under different treatment groups using Rabbit Grimace scales (n=25)

	pre	A	B	C	D	E
Orbital	0.1±0.06 (0-0.2)	0.93±0.19[b] (0.7-1.3)	0.23±0.12 (0-0.4)	0.37±0.22 (0.1-0.8)	0.57±0.09 (0.4-0.7)	0.4±0.06 (0.3-0.5)
Cheek	0.3±0.12 (0.1-0.5)	0.83± 0.12 (0.6-1)	0.23±0.09 (0.1-0.4)	0.73±0.24 (0.4-1.2)	0.6±0.06 (0.5-0.7)	0.37±0.07 (0.3-0.5)
Nostril	0.5±0.26 (0.1-1)	0.7±0.1 (0.5-0.8)	0.63±0.07 (0.5-0.7)	0.57±0.23 (0.2-1)	0.5±0.1 (0.4-0.7)	0.57±0.09 (0.4-0.7)
Whisker	0.47±0.03 (0.4-0.5)	0.83±0.03 (0.8-0.9)	0.63±0.09 (0.5-0.8)	0.63±0.15 (0.4-0.9)	0.73±0.18 (0.4-1)	0.6±0.1 (0.4-0.7)
Ear	0.43±0.13[a] (0.3-0.7)	1.3±0.25 (0.8-1.6)	0.8±0.06 (0.7-0.9)	1.2±0.12 (1-1.4)	1.6±0.06[b] (1.5-1.7)	0.8±0.2 (0.6-1.2)

[ab]Means in the same row with different superscripts alphabets were significantly different ($p \leq 0.05$).
KEY: Pre: pre surgical values; A: Normal saline (oral); B: Meloxicam (0.1mg/kg; oral); C: *C. ambrosioides* extracts (500mg/Kg; oral); D: Ketoprofen (Liberal; topical); E: *C. ambrosioides* extracts (Liberal; topical).

Table 2. The total mean ± SEM (minimum and maximum) values for the pain scores recorded for various treatment group using Grimace scales (n=25)

	pre	A	B	C	D	E
Overall mean pain scores (range)	0.36 ± 0.07^{a} (0-1)	0.92 ± 0.08^{b} (0.5-1.6)	0.51 ± 0.07^{ac} (0-0.9)	0.71 ± 0.11^{ab} (0.1-1.4)	0.8 ± 0.12^{bc} (0.4-1.7)	0.55 ± 0.06^{ac} (0.3-1.2)

^{abc}Means in the same row with different superscripts alphabets were significantly different ($p\leq0.05$).

KEY: Pre-surgical values; A: Normal saline (oral); B: Meloxicam (0.1mg/kg; oral); C: *C. ambrosioides* extracts (500mg/Kg; oral); D: Ketoprofen (Liberal; topical); E: *C. ambrosioides* extracts (Liberal; topical).

DISCUSSION

The toxicity studies conducted in this research confirm that *C. ambrosioides* is safe for use at doses up to 5000 mg/kg, when administered orally. The findings show that none of the animals exhibited any overt signs of toxicity, further supporting the safety profile of the tested compounds. These findings are crucial in establishing the non-toxic nature of the studied agents, which holds significance for their potential therapeutic applications. This finding aligns with the report by da Silva *et al.* (2014), who also observed no signs of toxicity in animals subjected to both acute and sub-chronic trials using aqueous extracts of *C. ambrosioides* leaves. However, caution must be exercised when utilizing this plant for therapeutic applications, as Doughmi *et al.* (2021) reported a case of *C. ambrosioides* poisoning characterized by symptoms such as vomiting, epigastric pain, tachycardia, and neurological, renal, hepatic, hemorrhagic, and skin pathologies. These findings proof the importance of dosage regulation and proper clinical evaluation before incorporating *C. ambrosioides* into routine veterinary practice.

The observed increase in blood glucose levels within the normal saline treatment group suggests a heightened distress and pain perception, and this is also documented by Uresin *et al.* (2004) and Sim *et al.* (2012). It is well established that pain and stressors elevate blood glucose levels and negatively impact glycemic control, particularly in diabetic patients. However, two- and three-hours post-treatment, no significant differences in blood glucose concentrations were observed across all treatment groups (A, B, C, D, and E), suggesting that the interventions effectively mitigated the stress-induced hyperglycemia associated with surgical castration.

The analysis of oxidative stress markers revealed that the plasma malondialdehyde (MDA) concentration was significantly ($p \leq 0.05$) elevated in the normal saline-treated group compared to baseline values obtained before surgery and to other treatment groups (B, C, D, and E). Notably, oral administration of meloxicam and *C. ambrosioides* significantly reduced MDA levels. At one-hour post-administration, *C. ambrosioides* effectively restored MDA levels to baseline, whereas meloxicam required two hours to achieve a comparable effect, indicating a more immediate antioxidant action of *C. ambrosioides*. The topical application of ketoprofen and *C. ambrosioides* similarly resulted in a significant ($p \leq 0.05$) reduction in plasma MDA levels relative to the normal saline-treated group, though these levels remained comparable to the pre-surgical baseline values. These findings suggest that both oral and topical formulations of *C. ambrosioides* possess potent antioxidative properties, with topical application potentially providing a more rapid onset of pain relief. Furthermore, the reduction in MDA concentration observed with *C. ambrosioides* further suggests a potential antioxidant role of *C. ambrosioides*, comparable to that of conventional analgesics such as meloxicam and ketoprofen and these findings indicate that oral *C. ambrosioides* administration may enhance endogenous antioxidant defense mechanisms more effectively

than topical administration or conventional analgesics.

Superoxide dismutase (SOD) plasma activity was markedly reduced in the normal saline-treated group compared to baseline values, masking the oxidative stress induced by surgical castration. Moreso, the oral administration of meloxicam and *C. ambrosioides* significantly increased SOD activity, demonstrating their ameliorative effects against oxidative stress. The increase in SOD activity occurred more rapidly in the *C. ambrosioides* treatment group, while the meloxicam-treated group exhibited a delayed but comparable rise after two hours. Surprisngly, topical applications of ketoprofen and *C. ambrosioides* significantly ($p \leq 0.05$) decreased plasma SOD activity relative to baseline, suggesting that topical formulations may be less effective in combating oxidative stress compared to oral administration.

Similarly, the glutathione peroxidase (GPX) concentration was significantly ($p \leq 0.05$) elevated in the oral meloxicam treatment group, the topical ketoprofen treatment group, and both oral and topical *C. ambrosioides* treatment groups compared to pre-surgical baseline values, albeit with minor variations. This increase emphasized the antioxidant potential of these treatments. However, at two- and three-hours post-treatment, the GPX levels in the normal saline-treated group were higher than in all other treatment groups, although statistical significance was only observed between the normal saline and *C. ambrosioides* topical treatment groups at two hours post-treatment. Notably, the oral administration of *C. ambrosioides* demonstrated greater efficacy than the topical application, likely due to differences in systemic absorption. The observed reductions in MDA levels and increases in SOD and GPX activity support the antioxidant properties of *C. ambrosioides*, which may contribute to its analgesic efficacy.

This study establishes that *C. ambrosioides* significantly reduces plasma MDA levels while enhancing SOD and GPX activity, reinforcing its potent antioxidant properties. The antioxidative effects of *C. ambrosioides* are likely attributable to its high flavonoid, phenol, alkaloid, and terpenoid content, as previously reported (Degenhardt *et al.*, 2016; Althobaiti, 2020; Kola-Mustapha *et al.*, 2020; De Garde *et al.*, 2022). Flavonoids are known to scavenge oxygen-derived free radicals, inhibit lipid peroxidation, and reduce inflammation (Christian *et al.*, 2016). Additionally, some researchers have linked the anti-inflammatory activity of *C. ambrosioides* to its ascaridole content (Matos, 2011; Degenhardt *et al.*, 2016). The analgesic, sedative, and anti-inflammatory effects of ethanolic extracts of *C. ambrosioides* leaves have been attributed to the presence of this secondary metabolite (Olajide *et al.*, 1997; Ibironke and Ajiboye, 2007). Kitchen *et al.* (2004) further substantiated these findings using molecular docking studies, which demonstrated the interaction between ascaridole and specific pain receptors, including N-methyl-D-aspartate (NMDA) receptors. These receptors are thought to play a crucial role in central sensitization and chronic pain mechanisms (Ishizuka *et al.*, 2007). Moreover, NMDA receptor antagonists have been shown to attenuate chronic pain (Bary and Amraoui, 2020). Kola-Mustapha *et al.* (2020) also reported that *C. ambrosioides* extract exhibits dose-dependent inhibition of cyclooxygenase-2 (COX-2) enzyme expression, prostaglandin E2 (PGE-2) production, and modulation of inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10), in vitro.

The overall Grimace pain score analysis revealed that the oral meloxicam treatment group, as well as the oral and topical *C. ambrosioides* extract treatment groups, exhibited pain scores comparable to pre-surgical baseline values. In contrast, the topical ketoprofen and oral *C. ambrosioides* treatment groups showed pain scores similar to the normal saline treatment group, suggesting that topical application of *C. ambrosioides* produces a more rapid analgesic effect than its oral counterpart. The Grimace scale is a well-validated tool for assessing pain in various animal models, including rats, mice, and rabbits (Asgar, 2015). Notably, Keating *et al.* (2012) utilized the Rabbit Grimace Scale to evaluate the efficacy of EMLA cream in preventing pain during rabbit tattooing, while Dreancă *et al.* (2017) applied the Rat Grimace Scale to determine the most effective post-ovariectomy analgesic protocol.

CONCLUSION

In conclusion, this study demonstrates that *C. ambrosioides* exhibits significant analgesic and antioxidant properties comparable to those of standard analgesics such as meloxicam and ketoprofen. The immediate reduction in oxidative stress markers and the modulation of pain perception following *C. ambrosioides* administration highlights its potential as a natural alternative for post-operative pain management. While oral administration provides superior systemic absorption and efficacy, the topical application remains an effective, though less potent, alternative. Further research is warranted to optimize dosage formulations and assess long-term safety profiles to facilitate their integration into mainstream veterinary therapeutics.

Availability of data and materials

All datasets related to this manuscript have been provided, however raw data can be provided upon reasonable request from the authors.

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Conflicts of interest

Authors declare no conflict of interest

Ethical statement

This study was conducted at the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine of Ahmadu Bello University, Zaria. Approval for the project was granted by the Ahmadu Bello University Ethical Committee on Animal Use and Care, with the assigned approval number ABUCAUC/2021/036. All ethical protocols concerning the handling, care, and welfare of the animals were meticulously observed throughout the study, from its inception to its conclusion.

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