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Safety, Analgesic, and Anti-Inflammatory Effects of Aqueous and Methanolic Leaf Extracts of *Hypericum revolutum* subsp. *keniense*

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Abstract:- In light of the enduring obstacles encountered in analgesia and anti-inflammatory therapeutics despite the strides made in contemporary medical sciences, the quest for alternative efficacious strategies is an imperative pursuit. Thus, this study investigated the safety profiles, as well as the analgesic and anti-inflammatory attributes, of aqueous and methanolic leaf extracts derived from *Hypericum revolutum* subsp. *keniense*—a botanical entity entrenched in historical ethnomedicinal practices in the Kenyan context. The assessment of acute oral toxicity of these extracts was conducted in accordance with the Up-and-Down method advocated by the Organisation for Economic Cooperation and Development (OECD), utilizing Swiss albino mice as the experimental subjects. Subsequent investigation entailed the evaluation of antinociceptive and anti-inflammatory properties employing the acetic acid-induced writhing and carrageenan-induced paw oedema methodologies, respectively, in the same murine model. The findings of this study evince the safety of both aqueous and methanolic extracts, with LD50 values surpassing 2000 mg/kg body weight and the absence of discernible signs of toxicity. Furthermore, a notable dose-dependent (P<0.05) manifestation of analgesic effects was observed for both extracts, with the aqueous variant demonstrating heightened potency compared to its methanolic counterpart (P<0.05). Moreover, the anti-inflammatory efficacy escalated significantly with escalating extract doses and prolonged treatment duration (P<0.05), wherein the aqueous extract showcased superior effectiveness vis-à-vis the methanolic extract across all dosage regimens and temporal checkpoints (P<0.05). Therefore, this investigation underscores the latent promise of the examined extracts as reservoirs of safe and efficacious analgesic and anti-inflammatory agents, thereby meriting further meticulous exploration. Subsequent research endeavours ought to pivot towards elucidating the phytochemical constituents underpinning the observed effects, alongside unravelling their mechanistic underpinnings across a spectrum of animal models and clinical milieus.

Keywords:- *Acetic Acid-Induced Writhing; Carrageenan-Induced Paw Oedema; Toxicity; Pain; Inflammation; Phytochemicals.*

I. INTRODUCTION

Pain and inflammation are debilitating conditions that have extensive consequences, affectingvarious aspects of life. They bring about disabilities, impose substantial economic burdens, andlead to a diminished overall quality of life [1,2]. Chronic inflammation is now recognized as a crucial factor in the development and progression of numerous diseases like rheumatoid arthritis, allergies, diabetes, dementia, cancer, cardiovascular diseases, and many other complex health challenges [3]. Moreover, pain is often the primary clinical manifestation in many diseases that involve inflammatory processes, hence the main symptom routinely addressed by healthcare institutions worldwide [4,5].

The traditional approaches to pain and inflammation management rely on Non-steroidal Anti-inflammatory Drugs (NSAIDs), which inhibit the cyclooxygenase enzyme in the prostaglandin synthesis pathway [6,7]. While these medications show effectiveness, they come with significant disadvantages such as high costs, limited accessibility, and undesirable side effects. For instance, the available analgesic and anti-inflammatory drugs, such as diclofenac,and acetylsalicylic acid, among others present serious side effects including high dependence, hepatoxicity, nephrotoxicity, gastric ulcerations, among other life-threatening sequelae [8–11]. Therefore, the need for new analgesic and anti-inflammatory drugs that are safe, potent, easily accessible, and affordable, especially in low-income regions, is warranted.

Throughout history, medicinal plants have played a crucial role in relieving various human ailments, particularly pain and inflammation; however, despite over 80% of the global population relying on medicinal plants for therapeutic purposes, only a few have undergone rigorous scientific investigation and validation [12]. Moreover, concerns about their safety arise due to the lack of standardized dosage

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guidelines, limited information on interactions with conventional drugs, and the absence of regulatory frameworks governing herbal medicine practices [13–15]. Therefore, it is crucial to subject traditional medicinal plants to comprehensiveevaluation to assess their toxicity and safety profiles effectively, to avert potential adverse effects, and to direct subsequent characterization and possible development.

In Kenyan traditional medicine, the application of diverse elements from *Hypericum revolutum*subsp. *keniense*, notably its leaves, has long been widespread as a means of treating various diseases including arthralgia and other inflammatory disorders [16–18]. However, despite such extensive ethnomedicinal usage, there is a dearth of empirical evidence and endorsement from the scientific community regarding the alleged remedial attributes imputed to this plant species. Furthermore, an assessment of its toxicological and safety profile has yet to be undertaken with rigour commensurate to its potential significance. The present study examined the immediate toxic effects when orally consumed, as well as the pain-relieving and anti-inflammatory properties of the aqueous and methanolic leaf extracts of *Hypericum revolutum* subsp. *keniense*. These extracts were investigated as possible starting points for developing more effective and safe analgesic and anti-inflammatory medications. Additionally, the study sought to confirm the traditional medicinal use of these extracts.

II. MATERIALS AND METHODS

A. Plant Material

With the assistance of a well-known traditional herbalist, fresh leaves of *Hypericum revolutum* subsp. *keniense* were procured from Kamae village in Kiambu County, Kenya, where the plant naturally flourished. The plant was recognised and validated by a taxonomist from the University of Nairobi (VMO01/2021) and preserved in the herbarium. The leaves were naturally dried at room temperature before being ground into a powder using a lab plant grinder.

B. Extraction Procedures

The extraction procedure adhered to established methodologies as delineated by Harborne [19], with refinements introduced by Moriasi et al. [20]. To obtain the methanolic extract, 250 grams of powdered material were immersed in 1 litre of high-purity methanol within a covered 2-liter conical flask. The mixture was subjected to daily agitation over a two-day period followed by concentration utilizing a rotary evaporator set at 50°C after filtration through Whatman filter paper No. 1. The resultant extract underwent further desiccation in a hot-air oven maintained at 35°C for a duration of five days.

For the aqueous extract, 50 grams of powdered material underwent a heating process at 58°C for five minutes in 500 milliliters of distilled water. Following this, the solution underwent filtration through Whatman filter paper No. 1 and was subsequently subjected to lyophilization for a period of 48 hours utilizing a freeze dryer.

C. Experimental Animals

The research used Swiss-albino mice that were five weeks old and weighed an average of 25±2 g. The mice were procured from the animal husbandry section of the Department of Public Health, Pharmacology and Toxicology laboratory at the University of Nairobi's Kabete campus. The mice were housed in typical rectangular cages made of polypropylene, measuring 30 cm by 20 cm by 13 cm, and were kept in regular laboratory conditions with a light-dark cycle of 12 hours. To provide warmth, soft wood chips were inserted inside the cages. Regular laboratory rodent pellets and fresh water were given to the animals on an as-needed basis. This study adhered to the standards for laboratory animal handling, care, and use.

D. Determination of Acute Oral Toxicity

We conducted a rigorous assessment of acute oral toxicity employing the Up-and Down method in accordance with guidelines set forth by the OECD [21]. Our study entailed the random allocation of experimental mice into seven distinct groups, each comprising three individuals, adhering strictly to the designated study protocol. Prior to experimentation, the mice's fasting period of four hours was implemented. The initial group (Group 1) served as the control cohort, receiving solely a dosage of normal saline (10 mg/kg BW) via oral administration. Subsequent groups received oral administrations of aqueous extracts (Groups 2-4) and methanolic extracts (Groups 5-7) of *H. revolutum* subsp. *keniense*, at varying concentrations (175 mg/kg BW, 550 mg/kg BW, and 2000 mg/kg BW), following a stepwise escalation method in line with OECD-approved standards [21]. The dosages of extracts were meticulously prepared in accordance with established procedures [22]. After administration, close monitoring of the animals' physiological parameters ensued, with observations recorded at intervals of 30 minutes, followed by hourly assessments spanning a duration of four hours, and subsequently at 6-hour, 12-hour, 24-hour, 48-hour, 7-day, and 14-day intervals [21].

E. Determination of in Vivo Antinociceptive Activities

The assessment of antinociceptive efficacy of the extracts in Swiss albino mice followed a methodology delineated by Koster et al. [23], albeit with nuanced adaptations. Mice were methodically chosen and divided into discrete cohorts in line with the experimental framework. The control group received an oral administration of normal saline (10 mg/Kg BW; p.o.). Following a 30-minute interlude, the negative control cohort underwent intraperitoneal (i.p.) injections of 100 µl of acetic acid (0.6 % w/v) alongside oral administration of normal saline (10 mg/Kg BW; p.o.). After another 30 minutes, the positive control ensemble received i.p. injections of 100 µl of acetic acid (0.6 % w/v) concomitant with oral administration of acetylsalicylic acid (Aspirin; 4 mg/Kg BW; p.o.). Subsequently, the remaining mice were administered aqueous or methanolic leaf extracts of *H. revolutum* subsp. *keniense* at dosages of 2 mg/Kg BW, 10 mg/Kg BW, 50 mg/Kg BW, and 250 mg/Kg BW, prepared in accordance with established protocols [22]. Following a 30-minute interval, all groups treated with extracts received i.p. injections of 100 µl of acetic acid (0.6 % w/v). Following this administration, the writhing frequency of each animal was

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observed five minutes post-injection of acetic acid and recorded for a duration of 15 minutes, and the percentage writhing inhibition was calculated according to a previously reported method [22] according to equation 1 (Eqn. 1). The percentage inhibitions of writhing were then used to estimate the antinociceptive effectiveness of the study extracts.

$$\%\ \text{writhing inibition} = \frac{C-T}{C} \times 100 \ldots\ldots\ldots (\text{Eqn. } 1) \quad [1]$$

Where *C* stands for the average frequency of writhing in the mice in the control group, while T stands for the frequency of writhing in the animals in the extract-treated, positive control, and normal control groups.

F. Determination of in Vivo Anti-Inflammatory Activity

Employing the Carrageenan-induced paw oedema method [25] with slight modifications [26], the present study delved into assessing the anti-inflammatory properties inherent in aqueous and methanolic leaf extracts of *H. revolutum* subsp. *keniense*. Swiss albino mice, meticulously randomized into eight groups, each comprising five specimens, served as the experimental subjects. Mice within the normal and negative control cohorts were subjected to oral administration of normal saline, dosed at 10 ml/kg body weight (BW). As for the positive control cohort, oral administration of indomethacin, a recognized anti-inflammatory agent, was carried out at a dose of 10 mg/kg BW. Experimental groups, on the other hand, received various doses of *H. revolutum* subsp. *keniense* leaf extracts (2 mg/kg BW, 10 mg/kg BW, 50 mg/kg BW, and 250 mg/kg BW), prepared meticulously following established protocols.

Except for the subjects in the normal control group, all mice underwent subcutaneous injection of 100 µl of carrageenan solution (1% in normal saline) into the sub-plantar region of their right hind paw, precisely 30 minutes post-treatment administration. Paw diameter measurements were meticulously recorded before carrageenan injection (as baseline) and subsequently every hour for a span of five consecutive hours post-injection, utilizing plethysmography. The percentage inhibitions of inflammation were computed employing a predefined formula [20,22] (Eqn. 2) and duly tabulated. Rigorous statistical analyses were conducted to discern the effects of the extracts on Carrageenan-induced inflammation, considering both dosage and time variables.

$$\%\ Inhibition = \left[\frac{Paw\ size\ of\ control\ mice - Paw\ size\ of\ treated\ mice}{Paw\ size\ of\ control\ mice}\right] \times 100 \ldots\ldots\ldots (Eqn. 2)\ ^{20,22}$$

G. Euthanasia and Disposal of Experimental Animals

At the end of the experimentation, all the animals were placed in a chamber saturated with 40% CO_2 and then disposed of by incineration as per the American Veterinary Medical Association (AVMA) 2020 guidelines [27] adopted by the Faculty of Veterinary Medicine Biosafety, Animal Care and Use and Ethics Committee of the University of Nairobi.

H. Data Analysis

The acute oral toxicity data were assessed in accordance with OECD guideline document No. 425. Antinociceptive and anti-inflammatory activity data were organized into tables and analyzed using Minitab version 21.4 (State College, Pennsylvania). Descriptive statistics were applied, and results were presented as mean ± standard error of the mean (SEM). Statistical differences among means were evaluated via One-Way ANOVA, followed by Tukey's *post hoc* test for pairwise comparison by adopting a significance threshold of $\alpha = 0.05$. Furthermore, independent treatments were compared using the unpaired Student t-test at a confidence level of 95%.

I. Ethical Approval

Approval of ethical considerations was duly secured for the present investigation through rigorous review by the esteemed Faculty of Veterinary Medicine Biosafety, Animal Care, and Ethics Committee (FVM-BAUEC/2021/317) at the venerable University of Nairobi. Furthermore, validation was sought and granted from the eminent National Council for Science, Technology, and Innovation (NACOSTI) under the reference number (NACOSTI/P/22/15796).

III. RESULTS

A. Acute Oral Toxicity Effects of the Aqueous and Methanolic Leaf Extracts of H. Revolutum Subsp. Keniense in Swiss Albino Mice

In this investigation, the aqueous and methanolic leaf extracts of *H. revolutum* subsp. *keniense* were administered at three dosage levels (175 mg/Kg BW, 550 mg/Kg BW, and 2000 mg/Kg BW). The results, depicted in Table 1, reveal no observable acute oral toxicity effects in the experimental subjects. Throughout the 14-day trial period, all subjects exhibited normal behavior without any clinical indications of extract-related toxicity. Moreover, there were no instances of morbidity or mortality among the subjects. Consequently, the LD_{50} values for *H. revolutum* subsp. *keniense*'s aqueous and methanolic leaf extracts exceeded 2000 mg/Kg BW.

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Table 1: Acute Oral Toxicity Effects of the Aqueous and Methanolic Leaf Extracts of *H. revolutum* subsp. *keniense* in Experimental Mice

Examination	**Finding at various time intervals**											
	30 minutes		**4 hours**		**24 hours**		**48 hours**		**7 days**		**14 days**	
	TM	**CM**	**TR**	**CM**	**TM**	**CM**	**TM**	**CM**	**TM**	**CM**	**TM**	**CM**
Skin and Fur appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Faecal matter consistency	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Urination and urine appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Mucous membrane appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Itching	×	×	×	×	×	×	×	×	×	×	×	×
Salivation	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Sleep	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Convulsions and tremors	×	×	×	×	×	×	×	×	×	×	×	×
Breathing	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Coma	×	×	×	×	×	×	×	×	×	×	×	×
Somatomotor activity	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Aggression	×	×	×	×	×	×	×	×	×	×	×	×
Grooming	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Eyes	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Teeth	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Morbidity/Mortality	×	×	×	×	×	×	×	×	×	×	×	×

TM: Test Mice (Administered with either 175 mg/Kg BW,550 mg/Kg BW, or 2000 mg/Kg BW of the aqueous or methanolic leaf extracts of *H. revolutum* subsp. *keniense*); CM: Control Mice (Administered with Normal saline at a dose of 10 ml/Kg BW); LD50 forthe studied plant extracts >2000 mg/Kg BW; ✓: Normal; ×: absent/ not observed.

B. Antinociceptive Effects of Aqueous and Methanolic Leaf Extracts of H. revolutum subsp. keniense in Swiss Albino Mice

The investigation meticulously assessed the analgesic attributes inherent in aqueous and methanolic leaf extracts, employing the acetic acid-induced writhing model in Swiss albino mice as a discerning gauge. Intriguingly, the administration of the aqueous leaf extract of *H. revolutum* subsp. *keniense* engendered a discernible escalation in the percentage inhibition of acetic acid-induced writhing, evincing a notable dose-dependent trend, hence highlighting its substantive efficacy ($P < 0.05$; Figure 1). However, it is noteworthy that the positive control cohort exhibited markedly heightened levels of writhing inhibition vis-à-vis the counterparts subjected to the extract across all dosage regimens ($P < 0.05$; Figure 1).

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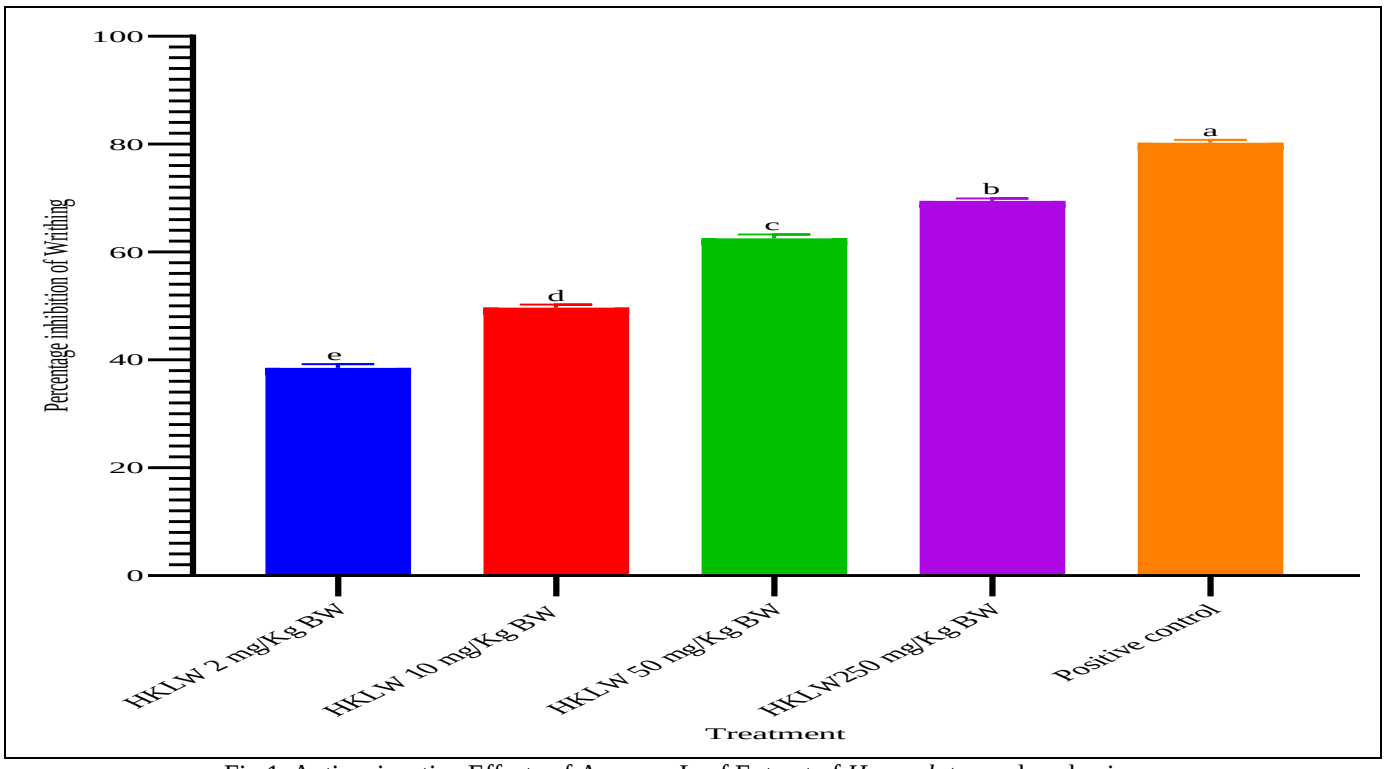

Fig 1: Antinociceptive Effects of Aqueous Leaf Extract of *H. revolutum* subsp. keniense
Values are plotted as $\bar{x} \pm SEM$; Bars bearing dissimilar lower-case alphabets are significantlydifferent (P<0.05; One-Way ANOVA with Tukey's *post hoc* test). HKLW: Aqueous leaf extractof *H. revolutum* subsp. *keniense;* Positive control: Acetylsalicylic acid (4 mg/Kg BW).

The study evaluated the antinociceptive properties of the methanolic leaf extract of *H. revolutum* subsp. *keniense*. Significant dose-dependent reductions in writhing were observed in mice administered with the methanolic leaf extract of *H. revolutum* subsp. *keniense* (P<0.05; Figure 2). It is noteworthy that the positive control, Aspirin, exhibited markedly greater inhibition of acetic acid-induced writhing compared to the methanolic leaf extract of *H. revolutum* subsp. *keniense* (P<0.05), as illustrated in Figure 2.

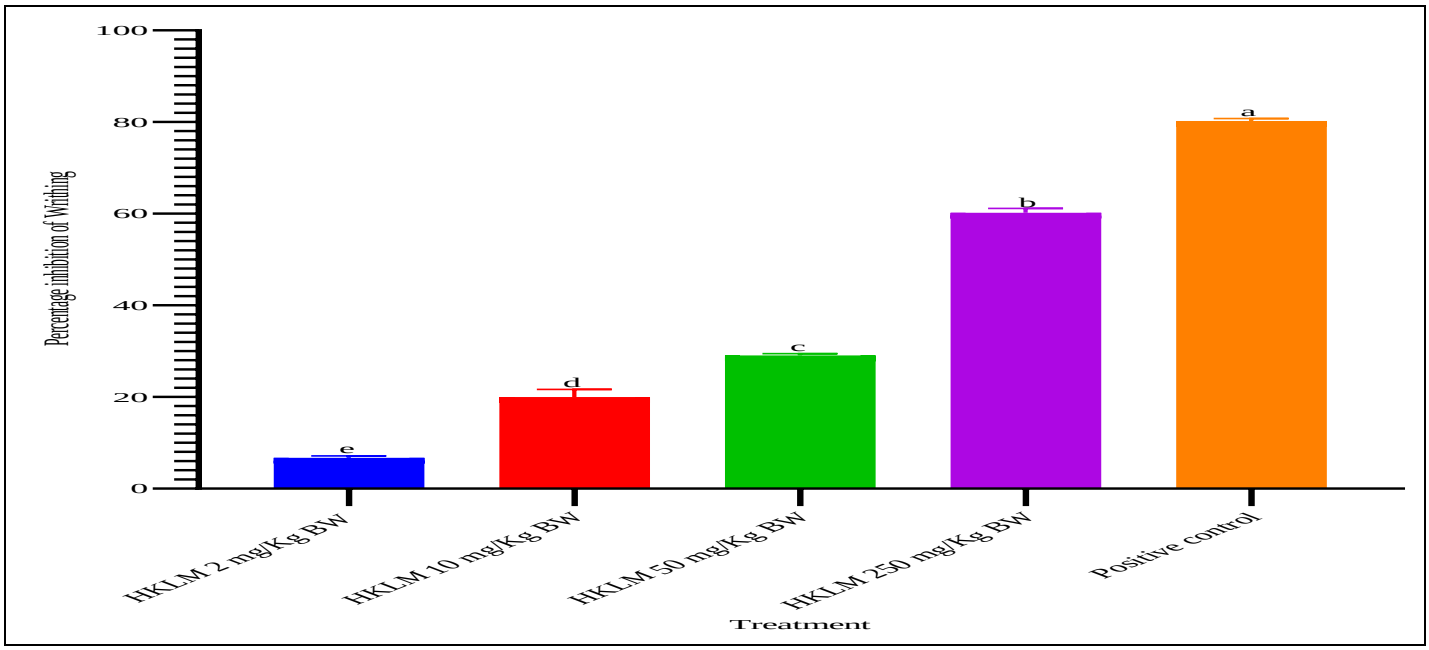

Fig 2: Antinociceptive Effects of Methanolic Leaf Extract of *H. revolutum* subsp. keniense
Values are plotted as $\bar{x} \pm SEM$; Bars bearing dissimilar lower-case alphabets are significantlydifferent (P<0.05; One-Way ANOVA with Tukey's *post hoc* test). HKLM: Methanolic leaf extract of *H. revolutum* subsp. *keniense;* Positive control: Acetylsalicylic acid (4 mg/Kg BW).

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Additionally, a comparison was conducted at each dosage level between the two plant extracts under study in terms of their writhing inhibitions. The aqueous leaf extract of *H. revolutum* subsp. *keniense* significantly reduced acetic acid-induced writhing in mice at all dose levels, compared to the methanolic leaf extract at analogous dose levels (P<0.05; Figure 3).

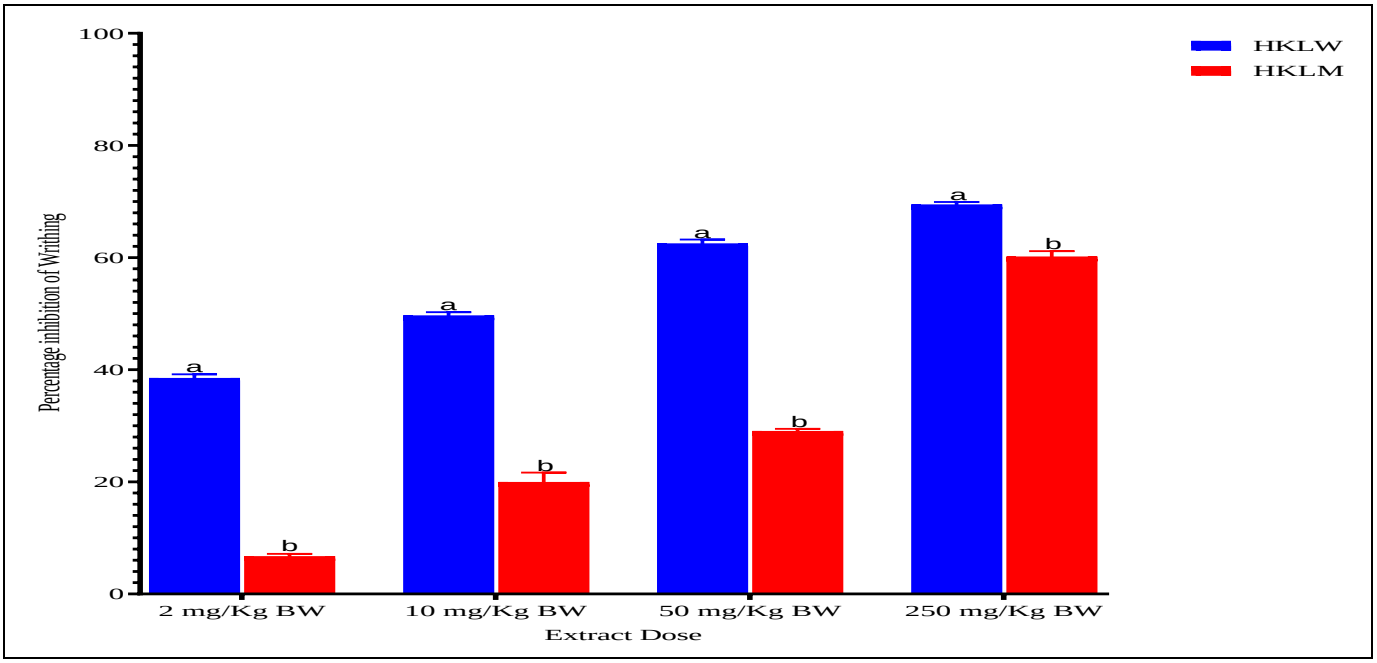

Fig 3: Comparison between antinociceptive effects of the aqueous and methanolicleaf extracts of *H. revolutum* subsp. *Keniense* Values are plotted as $\bar{x} \pm SEM$; Bars bearing dissimilar lower-case alphabets within the same dose level are significantly different (P<0.05, Unpaired student t-test. HKLW: Aqueous leaf extract of *H. revolutum* sub spp. *keniense;* HKLM: Methanolic leaf extract of *H. revolutum* subsp. *keniense.*

C. Anti-inflammatory effects of aqueous and methanolic leaf extracts of H. revolutum subsp. keniense in Swiss albino mice

We investigated the anti-inflammatory properties of *H. revolutum* subsp. *keniense* leaf extracts within a murine model of carrageenan-induced paw edema spanning a five-hour duration. Administered at a dosage of 2 mg/Kg BW, no significant alterations in paw edema inhibition percentages were discerned during the initial and subsequent hours when juxtaposed against the control group (P>0.05; see Table 2). Likewise, mice treated with either 10 mg/Kg BW of aqueous leaf extract or 50 mg/Kg BW of methanolic leaf extract exhibited analogous outcomes throughout the experimental window (P>0.05; Table 2).

Over the initial two hours, the administration of 250 mg/Kg BW of aqueous leaf extract yielded inhibitory percentages akin to those observed with the reference drug (positive control) (P>0.05; Table 2). Generally, a dose-dependent escalation in paw edema inhibition percentages was evident within the first hour following oral administration of both aqueous and methanolic leaf extracts (P<0.05; Table 2).

Consequently, notable reductions in paw edema percentages manifested during the third hour with 2 mg/Kg BW of both aqueous and methanolic leaf extracts (P<0.05; Table 2), with no significant distinctions observed amongst the experimental groups (P>0.05; Table 2). Similarly, no significant fluctuations were observed in inhibition percentages during the third hour with 10 mg/Kg BW of aqueous extract and 50 mg/Kg BW of methanolic extract (P>0.05; Table 2).

By the fourth hour, significant disparities emerged in inhibition percentages (P<0.05; Table 2). Treatment with 250 mg/Kg BW of aqueous leaf extract conferred greater protection against paw edema compared to other groups (P<0.05; Table 2), whereas 2 mg/Kg BW of methanolic leaf extract demonstrated significantly diminished suppression percentages (P<0.05; Table 2).

During the fifth hour, no significant differences were discerned in inhibition percentages between doses of aqueous and methanolic leaf extracts (P>0.05; Table 2). Nevertheless, treatment with 250 mg/Kg BW of aqueous leaf extract exhibited comparable suppression to that of the reference drug (P>0.05; Table 2), with both extracts exhibiting dose-dependent enhancements in inhibition percentages during this interval (P<0.05; Table 2).

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Table 2: Anti-Inflammatory Efficacy of the Aqueous and Methanolic Leaf Extracts of *H. Revolutum* Subsp. *Keniense*

Treatment	Percentage inhibition of Carrageenan-induced paw oedema in mice				
	1st Hour	2nd Hour	3rd Hour	4th Hour	5th Hour
2 mg/Kg BW of HKLW	$1.23\pm0.19^{f}_{e}$	$3.03\pm0.14^{f}_{d}$	$6.29\pm0.18^{f}_{c}$	$12.81\pm0.18^{h}_{b}$	$16.69\pm0.12^{f}_{a}$
2 mg/Kg BW of HKLM	$0.79\pm0.19^{f}_{e}$	$1.67\pm0.14^{f}_{d}$	$3.39\pm0.18^{f}_{c}$	$8.82\pm0.18^{i}_{b}$	$12.56\pm0.12^{g}_{a}$
10 mg/Kg BW of HKLW	$5.14\pm0.24^{d}_{e}$	$7.39\pm0.22^{d}_{d}$	$13.56\pm0.18^{d}_{c}$	$19.39\pm0.19^{f}_{b}$	$24.90\pm0.18^{d}_{a}$
10 mg/Kg BW of HKLM	$2.77\pm0.24^{e}_{e}$	$4.84\pm0.22^{e}_{d}$	$9.57\pm0.18^{e}_{c}$	$14.39\pm0.19^{g}_{b}$	$20.02\pm0.15^{e}_{a}$
50 mg/Kg BW of HKLW	7.93 ± 0.11^{c}	11.42 ± 0.10^{c}	22.13 ± 0.67^{bc}	28.86 ± 0.13^{d}	37.20 ± 0.18^{b}
50 mg/Kg BW of HKLM	$4.81\pm0.11^{d}_{e}$	$7.85\pm0.10^{d}_{d}$	$14.84\pm0.67^{d}_{c}$	$22.96\pm0.13^{e}_{b}$	$28.41\pm0.18^{c}_{a}$
250 mg/Kg BW of HKLW	$12.12\pm0.10^{a}_{e}$	$19.62\pm0.10^{a}_{d}$	$28.02\pm0.17^{a}_{c}$	$39.52\pm0.13^{a}_{b}$	$44.92\pm0.39^{a}_{a}$
250 mg/Kg BW of HKLM	$9.33\pm0.10^{b}_{e}$	$14.69\pm0.10^{b}_{d}$	$20.79\pm0.17^{c}_{c}$	$31.53\pm0.13^{c}_{b}$	$37.02\pm0.39^{b}_{a}$
Reference drug (Indomethacin)	$10.91\pm0.20^{a}_{e}$	$18.67\pm0.24^{a}_{e}$	$22.60\pm0.14^{b}_{c}$	$38.06\pm0.23^{b}_{b}$	$44.84\pm0.41^{a}_{a}$

Values are expressed as $\bar{x} \pm SEM$ of replicate measurements (n=5 mice). Means with dissimilar superscript alphabets within the same column, and those with dissimilar subscript alphabets within the same row are significantly different (P<0.05; One-Way ANOVA with Tukey's *Post hoc* test). HKLW: Aqueous leaf extract of *H. revolutum* subsp. *keniense*; HKLM: Methanolic leaf extract of *H. revolutum* subsp. *keniense*; Reference drug: Indomethacin

IV. DISCUSSION

Over 80 % of the world's population relies on these plants as their primary medicine reservoir; this popularity continues to rise significantly even in highly developed nations [28–30]. The rich content of pharmacologically active secondary metabolites in medicinal plants offers tremendous promise as a source of effective lead molecules for developing analgesic and anti-inflammatory drugs [31,32]. Despite medicinal plants and their products playing such a crucial role in healthcare provision, most scholars have limited their exploration and empiricalvalidation of medicinal plant use, primarily due to the scarcity of essential scientific data and the lack of a comprehensive regulatory framework for herbal medicine practices. Notably, doubts regarding the safety and health implications of plant-derived extracts and products arise from concerns about their preparation, storage, and dosage administration procedures and a lack of critical pharmacological information, such as herb-herb interactions and reactions withsynthetic drugs [15,33–36].

In our research, we employed the Up-and-Down procedure outlined by the Organization for Economic Co-operation and Development (OECD) to assess the acute oral toxicity of the aqueous and methanolic leaf extracts of *H. revolutum* subsp. *keniense* on Swiss albino mice [21]. Our findings demonstrated that these two extracts did not adversely affect any indicators ofwell-being we assessed; this underscores their safety when administered orally. Moreover, both extracts' LD50 values—exceeding 2000 mg/kg body weight—reinforce their safety profile [21]. Scholars have established that medicinal plants with high concentrations of toxic alkaloids, cyanogenic glycosides, and anthraquinones generally exhibit toxicity [37,38]. Therefore, we can attribute the safety of studied plant extracts to either an absence or low concentration of these harmful phytochemicals, which does not induce observable signs of toxicity in experimental mice. A prior investigation indicated this finding concerning aqueous and methanolic extracts from *H. revolutum* subsp. *keniense* genus contains numerous bioactive compounds and typicallyposes no substantial safety concerns [39]. However, certain species in this same category— particularly *Hypericum perforatum*—have links to teratogenic, phototoxicity-, and psychotropic effects; these can be attributed to their hypericin content [40,41]. Despite that acknowledgement, an inadequate amount of toxicological research exists on the *Hypericum* genus. As far as we know, this study provides the first report regarding acute oral toxicity of *H. revolutum* subsp. *keniense* in experimental mice.

We employed the acetic acid-induced writhing technique, initially described by Koster et al. (23) to evaluate the analgesic effects of aqueous and methanolic leaf extracts from *H. revolutum* subsp. *keniense* on Swiss albino mice; this approach allowed us to assess their potential as pain-relieving agents in laboratory settings. In this study, we selected acetic acid as the chemical stimulus: it incites a swift surge in prostaglandin production- a process that triggers the activation of primary pain nociceptors [23,42]. As a result of such induction, animals display involuntary contortions or 'writhes'; these serve reliably to indicate overt pain [24,43–45]. Consequently, extracts or drug agents that can avert or reduce writhing in the induced animals ultimately possess analgesic efficacy and therapeutic potential.

The aqueous and methanolic leaf extracts of the studied plant exhibited significant analgesic potential by inhibiting writhing frequency dose-dependently, thereby demonstrating theirremarkable analgesic efficacy. The analgesic efficacy of the aqueous extract surpassed that of its methanol counterpart consistently across all administered doses. Prior research attributes this observed dose-dependent analgesic effect to variations in the composition of bioactive compounds [24,45,46]. These findings imply that higher concentrations of analgesic phytochemicals are likely present in the aqueous extract, leading to a more pronounced reduction in writhing frequency, denoting higher antinociceptive efficacy in experimental animals.

We adopted the Carrageenan-induced paw oedema technique [25] with slight modifications to evaluate the anti-inflammatory properties of aqueous and methanolic extracts of *H. revolutum* subsp. *keiense*, considering its utilisation as an anti-inflammatory remedy in traditional medicine [39]. Carrageenan is a well-recognised thermal and chemical stimulus that induces inflammation due to its capacity to

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trigger the synthesis of proinflammatory mediators [47,48]. Two distinct phases characterized by our experiment appeared in carrageenan-induced inflammation: an initial phase occurring within one hour - propelled by bradykinin synthesis along with histamine, serotonin, and hydroxytryptamine – then followed by a subsequent phase between one and four hours primarily marked by the secretion of prostaglandin-like mediators alongside activation of cyclooxygenase-2 pathway (Cox-2) by the free radicals [49,50]. Therefore, any substance capable of inhibiting or reducing Carrageenan-induced oedema is considered topossess anti-inflammatory activity.

The study revealed that the plant extracts we investigated had significant anti-inflammatory effects in experimental mice, displaying a pattern dependent on dosage and time in both early and late phases of inflammation. Notably, the aqueous extract had consistently superior anti-inflammatory efficacy to its counterpart, the methanolic extract. These results align with prior investigations conducted by other researchers [51,52]. These results partially validate the ethnomedicinal claims about the plant's anti-inflammatory properties; moreover: they confirma potential for therapeutic applications--specifically in conditions associated with inflammation.

In our investigation, we employed Acetylsalicylic acid (Aspirin) as a reference standard for both analgesic and anti-inflammatory assays; Aspirin: a prevalent non-steroidal anti-inflammatory drug (NSAID), finds extensive utilization in conventional medicine [53,54]. Non-selective inhibition of the Cox-1 and Cox-2 enzymes characterizes its action mechanism—this leads to reduced prostaglandin synthesis, thereby exerting its anti-inflammatory effects [54,55]. Itsclinical utility, however, remains limited: severe side effects—such as gastric ulcers; intestinal bleeding; hepatoxicity and nephrotoxicity pose significant risks [9,56].

Beyond simply inhibiting prostaglandin synthesis, many conventional analgesic and anti-inflammatory agents are also known for their adverse effects, such as toxicity [57]. Recent studies have demonstrated that analgesic and anti-inflammatory drugs [58], such as aspirin, and ibuprofen, potentially induce oxidative stress acetaminophen [9,59]. Oxidative stress is an aetiologic factor for an array of diseases including inflammation; metabolic syndromes; cancer; and neurodegenerative disorders [60]. This recognition implies that paradoxically these conventional agents—while they do provide some efficacy—may have effects that overshadow their benefitsby worsening the conditions under treatment. Therefore, we reckon that antioxidant therapy using natural products, such as the studied plant extracts, may be the most feasible strategy forthwarting pain and inflammation.

Recent reports have shown analgesic and anti-inflammatory properties exhibited by medicinal plants, are attributable to flavonoids, coumarins, phenols, and tannins [61–63]. The aqueous and methanolic extracts we report here exhibit analgesic and anti-inflammatory efficacy, which weattribute to a diverse array of antioxidant-associated phytochemicals; these compounds have been previously documented as exerting their pharmacological effects through various mechanisms [64–66].

Our study thus emphasizes the potentiality of *H. revolutum* subsp: *keniense* aqueous and methanolic leaf extracts as invaluable sources for safe, effective analgesic-anti-inflammatory agents, upon further investigations. Furthermore, through empirical evidence provided by the present study, we partially substantiate the ethnomedicinal assertions about the plant's properties against pain and inflammation: paving a path towards prospective research. Such future studies could encompass isolating-characterizing specific bioactive compounds to develop therapies that are both safer and more efficacious in combating pain-and inflammation-related conditions.

V. CONCLUSIONS AND RECOMMENDATIONS

Our results indicate that the aqueous and methanolic leaf extracts of *H. revolutum* subsp. *keniense* exhibits strong analgesic and anti-inflammatory properties in Swiss albino mice and is safe to be given orally at dosages of up to 2000 mg/Kg BW. Therefore, we urge future empirical studies targeted towards establishing full toxicity profiles of these extracts, especially when coupled with others, to appraise their safety. To further elucidate, identify, and develop safe and effective medications to treat pain, inflammation, and the sequelae associated with these conditions, investigations into the isolation and characterization of analgesic and anti-inflammatory phytochemicals as well as the mechanisms of bioactivity from the examined plant extracts should be conducted.

AVAILABILITY OF DATA AND MATERIALS

All data are included in the publication; however, the authors may offer extra material upon reasonable request.

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AUTHORS' CONTRIBUTIONS

The research idea was conceived, and experimental designs were formulated collectively by all authors. Omambia Vincent conducted the experiments, analyzed the data, and authored the manuscript under the guidance of Moriasi Gervason. Reagents and data analysis tools were contributed by Onyancha Jared. The study was supervised by Nguta Joseph and Mitema Simon. All authors critically reviewed and endorsed the final manuscript for submission and publication.

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