PRELIMINARY PHYTOCHEMICAL SCREENING AND IN-VIVO ANTI-INFLAMMATORY PROPERTY OF ETHANOL EXTRACT OF EGYPTIAN CROWFOOT GRASS

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Abstract

In low- and middle-income nations, many essential medicines remain out of reach and unaffordable, creating room for opportunists to wreak havoc. In sub-Saharan Africa alone, about 267,000 deaths per annum are usually linked to falsified and substandard drugs. The current study therefore evaluated the phytochemical constituents as well as the anti-inflammatory property of ethanol extracts of Egyptian Crowfoot Grass and its effect on the level of Glutathione (GSH) in rats using established protocols. The phytochemical screening revealed that the extract contained phytochemicals such as flavonoid, tannins, phenol, alkaloids, reducing sugar, and cardiac glycosides steroids and terpernoid. Acute toxicity test revealed that the oral administration (up to 2000 mg/kg b.w) of studied plant ethanol extract did not cause any physical toxicological effects in rats. The anti inflammatory result revealed that different concentrations (200 and 400 mg/kg b.w.) of ethanol extract of Egyptian Crowfoot Grass significantly reduced cellular infiltration to the inflammatory region and showed anti-inflammatory action in rats' paw inflammation. Significant alteration was also observed in GSH antioxidant enzyme level of the paw tissue homogenate of rats treated with the test plant. These results revealed that ethanol extract of Egyptian crowfoot grass can be used as a replacement for anti-inflammatory drugs.

KEYWORDS: *Egyptian Crowfoot Grass, Ethanol extract, Phytochemicals, Invivo antiinflammation*

Introduction

Inflammation is a crucial survival mechanism and a process of defense that has been retained throughout evolution. It is made up of intricately coordinated changes in the tissue that remove the underlying factor that led to the cell injury. This factor could have included infectious agents, products of their metabolism (microorganisms and toxins), physical agents (radiation, burns, and trauma), or chemicals (Fialho *et al*., 2018; Liu *et al*., 2017). In most cases, this intricate biological reaction results in the return of homeostasis. However, the inflammatory process endures and a modest but persistent pro inflammatory state may occur when harmful signal-transduction pathways are active and inflammatory mediators are generated over an extended period of time (Nisar*et al*., 2023).Numerous problems and long-term medical concerns, including obesity, cardiovascular diseases, cancer and diabetes are linked to low-grade inflammation (Kim et al., 2018; Singh et al., 2019). It is therefore desirable to find a new generation of therapeutic drugs to use in the reduction of inflammation. Several non-steroidal anti-inflammatory medications are being used to treat inflammation; however they frequently have side effects such as toxicity and the recurrence of symptoms after stopping treatment. In addition because some of these drugs are inaccessible to and unaffordable by low- and middle level income earners in most Sub-Sahara African countries. This has given opportunities to many bad-minded individuals to wreck all manners of havocs including the production of fake and substandard drugs (Barton, Avanceña, Gounden & Anupindi, 2019). Therefore, it is essential to screen and look for plants with anti inflammatory properties since medicinal plants are crucial in the creation of novel and strong medications because of the secondary metabolites they produce that have clinically favorable effects (Riaz *et al*., 2023).

Egyptian crowfoot grass (*Dactyloctenium aegyptium*) is a grass plant indigenous to Africa. It is commonly used during famine as a traditional food plant and not much is known about this grass plant. Recently a compounded pharmacological potential of Egyptian crowfoot grass has been reported and following the Clarkson's criterion for toxicity assessment, this plant has been certified safe for consumption (Al-Snafi, 2017; Aliyu*et al*., 2017; Harun *et al*., 2022).

However there is a dearth of data to support the use or scientifically establish the use of Egyptian crowfoot grass for folklore medicine. Since the most important phytocompounds responsible for anti inflammatory properties have been identified to be phenolic compounds and terpenoids and are best obtained using ethanol extraction (Asidah *et al*., 2021; Cabral *et al*., 2016; Nunes *et al*., 2020). This study therefore, evaluated the phytochemical constituent of ethanol extracts of Egyptian crowfoot grass and also determined their anti inflammation potential using the carrageenan-induced rat paw inflammation model.

Materials and method

The entire plant of Egyptian crowfoot grass was collected from the schoolyard of Federal Polytechnic Ilaro, Ogun State and was subsequently confirmed at the University of Lagos by Dr. Nodza George. A voucher specimen (LUH 9738) has been deposited at the Department of Botany and Microbiology. In this present work, only analytical-grade reagents were employed.

Plant preparation/Preparation of ethanol extract

Following botanical identification, the grass were divided up, thoroughly pre-washed with water, and were left to dry at room temperature in the shade for two weeks. The healthy dried leaves that had shed their skin were mechanically crushed and filtered through a sieve with a 40 m mesh size (Pandiyan & Ilango, 2022). After that, 140 g of the powdered plant material was added to a flask with 2000 mL of 80% ethanol and macerated for three days. Whatman® filter paper was used to filter the macerated extract, which was then dried fully in an oven set to 60ºC (Abubakar and Haque, 2020; Gloria *et al*., 2017).

Figure 1-Egyptian crowfoot grass (*Dactyloctenium aegyptium*)

Phytochemical Analysis

The method of Panchal and Charuben, (2017) was followed to screen for the presence of active constituents contained in Egyptian crowfoot grass ethanol extract.

Assessment of Anti-inflammatory Property

Experimental Animal

The animals (Fifteen Male albino rats) employed for this experiment were sourced from Department of Zoology, University of Lagos, Nigeria. Rats were kept in the animal house, University of Lagos under environmental conditions of 25 ± 1°C and 12 hr dark-light cycle with free access to water and food. They were allowed to acclimatize before the study began. The animals were all sacrificed at the end of the experiment.

Acute Toxicity

Rats were starved the previous night to carry out the oral acute toxicity test. Three rats were administered with 2000mg/kg b.wt, po of ethanol extract of Egyptian crowfoot grass. The rats were watched to monitor their behavioural, neurological, and autonomic profiles every two hours during the day, rats were also monitored for three days to determine its lethality (OECD, 2021).

Carrageenan-induced paw edema

Experimental rats (except those in group I) were given 0.1 ml injection of carrageenan suspension one hour after receiving the test sample to create inflammatory response in them (Boubekri *et al*., 2014; Kosala, Widodo, Santoso, Karyono, 2018). Following the injection of carrageenan, the rat's paw volume was measured every hour for six hours.

Table 1: Experimental Design

GROUPS	**TREATMENTS**	**Number of Rat**
GROUP 1 (normal control)	Distilled	3
GROUP 2 (negative control)	carrageenan + distilled water	3
GROUP 3 (positive control;STD)	carrageenan + diclofenac (20 mg/kg)	3
GROUP 4	carrageenan + 200mg/kg Ethanol extract	3
GROUP 5	carrageenan + 400mg/kg Ethanol extract	3

Determination of Anti inflammatory Activity
Egyptian crowfoot grass ethanolic extract's anti inflammatory activity was determined based on their % inhibition of carrageenan induced paw edema model in rats and it was calculated following the formular;

$$\% \text{ inhibition} = \frac{\text{mean paw volume (Carr group)} - \text{Mean paw volume (treated group)}}{\text{Mean paw volume (Carr group)}} \times 100$$

GSH Enzymatic Antioxidant Assay
Glutathione (GSH) level was assessed in the soft paw tissues of the experimental rats based on changes in absorbance brought on by NADPH's conversion to NADP. The GSH content was given as µlmol/ml of protein (Zammel *et al*., 2021).

Statistical Analysis
Data was presented as mean ± SD. Obtained data was analyzed with two-way analysis of variance (ANOVA).

Results

Phytochemical Screening

The findings of the qualitative screening of the ethanol extract (EE) of Egyptian crowfoot grass are documented in Table 2 below;

Table 2: Qualitative phytochemical screening of ethanol of Egyptian crowfoot grass

Phyto-compounds	**EE**
Tanin	+
Phlobatanin	+
Phenol	+
Alkaloid	-
Flavonoid	+
Steroid	+
Saponin	+
Cardiac glycoside	+
Terpenoid	+
Reducing sugar	+

Acute Toxicity

After receiving a 2000 mg/kg dosage of the tested plant extracts, no fatalities or symptoms associated with the medication were noted. Furthermore, the animals displayed no treatment-related changes in their behavior, skin, eyes, temperature, fur examination, food and drink intake, or respiration. Ethanol extract of Egyptian crowfoot grass is therefore deemed safe to use at a level of 2000 mg/kg, even though the lethal dose (LD50) was expected to be higher than 2000 mg/kg BW. This confirms the findings of Aliyu *et al*., (2017) and Harun *et al*., (2022) following their toxicity assessment that ethanol extract of Egyptian crowfoot grass is safe for consumption. This led to the therapeutic low dose of Egyptian crowfoot grass being chosen to be one tenth of the maximum tolerated dose (200 mg/kg), and the highest dose for this study to be twice this low dose (400 mg/kg).

Anti inflammatory Activity

The anti-inflammatory activity of ethanol extract of Egyptian crowfoot grass and diclofenac is shown in Table 2 below. A significant ($P \leq 0.05$) reduction in paw inflammation was seen in rats pre-treated with the different doses (200 and 400 mg/kg) of the ethanol extract of the studied plant as well as diclofenac. However, the highest paw edema inhibition was exhibited by 400 mg/kg b. w of Egyptian crowfoot grass ethanol extract which exhibited its anti inflammatory power starting from 2hrs of its administration.

Table 3- Effect of different doses of ethanol extract (E.E) of Egyptian crowfoot grass (on carrageenaan-induced paw oedema in rat

	Carr Grp + Dist Water	Carr Grp + E.E(200 mg/kg)	Carr Grp + E.E(400 mg/kg)	Carr Grp +Diclofenac (20 mg/kg)
0 hr	3.58± 0.15	3.55± 0.13	3.58± 0.15	3.57± 0.16
1 hr	5.40± 0.13	5.88± 0.47	5.81± 0.36	5.45± 0.32
2 hrs	7.19± 0.13	7.16± 0.04 (0.42%)	5.50± 0.72(23.50%)**	7.17± 0.10(0.28%)
3hrs	7.98± 0.64	5.24± 0.21(34.34%)****	4.28± 0.17(46.37%)****	6.69± 0.51(16.17%)*

4 hrs	7.83± 0.58	4.72± 0.03 (39.72%)****	4.04± 0.14(48.40%)****	5.48± 0.17(30.01%)****
5 hrs	7.68± 0.53	4.18± 0.16(45.57%)****	3.79± 0.16(50.65%)****	4.26± 0.30(44.53%)****
6 hrs	7.74± 0.30	3.74± 0.12(51.68%)****	3.65± 0.15(52.84%)****	3.96± 0.10(48.84%)****

Values are mean ± SEM (n = 3).*P < 0.05, **P< 0.01, ***P <0.001, ****P<0.0001 significant decrease in the development of oedema in comparism to the carrageenan group.Values in brackets shows (%) inhibition.

GSH Antioxidant Enzyme Assay

Enzymatic antioxidant levels of GSH were measured in rat paw tissue homogenate 24 hours following carrageenan induction. The extent to which this antioxidant enzyme level was altered is shown in Figure 2.

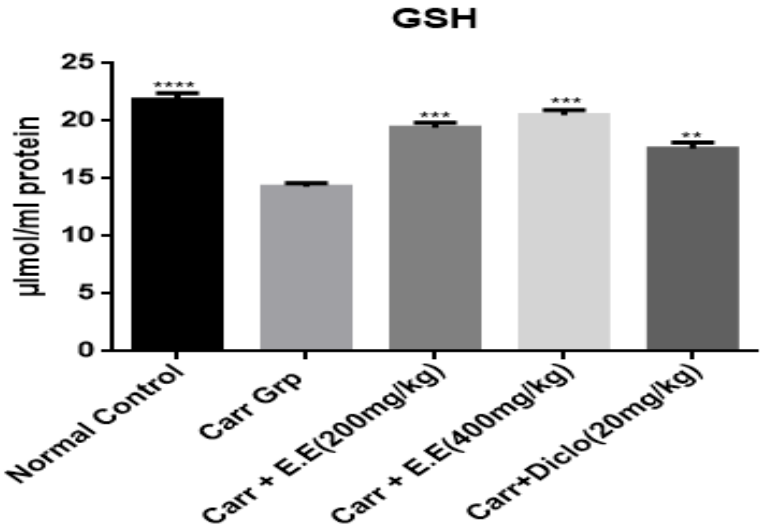

Figure 2-Effect of different doses of ethanol extract (E.E) of Egyptian crowfoot grass on GSH level in rat paw tissue homogenate after 24 h of carrageenan injection.Values are expressed as Mean ± SEM (**P< 0.01, ***P <0.001, ****P<0.0001 significant increase in GSH level compared with carrageenan group.

Discussion

The therapeutic management of inflammation is a big public health issue since it is triggered by diverse mechanisms. However because of the side effects associated with the seeking and use of conventional non-steroidal drugs especially in the developing nations, there is a need to seek new approaches that can reduce inflammation in a way that is homeostatic, modulatory, effective, easily accessible and well tolerated by the body. Medicinal plants have proven their value as a source of chemicals with therapeutic potential throughout history; today, they remain an important source for the identification of new medication leads (Atanasov, Zotchev & Dirsch, 2021; Twaij &Hassan, 2022).

The Phytochemical analysis of ethanol extract of Egyptian crowfoot grass revealed that the plant extract contained phytochemicals such as terpenoids, tannins, flavonoids, saponins, phenols etc that have been reported to have anti inflammatory properties. On the other hand, the result of the acute toxicity test revealed that the plant extract was less

toxic up to 2000 mg/kg and safe for consumption. A similar result for phytochemical screening and toxicity test was reported by Devi, Sravanthi and Reddy (2018).

A well-known animal model for assessing the anti-inflammatory effects of herbal remedies and traditional drugs is carrageenan-induced paw edema (Fatima *et al*., 2021; Pantil *et al*., 2019). The effects of carrageenan on the paw led to the development of edema, which is a biphasic process. In the first phase, which lasts for 1 to 1.5 hours, non-phagocytic edema predominates. This is followed by a second phase, which lasts for 2 to 5 hours and has heightened edema production. Multiple mediators, including histamine, serotonin, and bradykinin, work on vascular permeability to cause the initial phase to be initiated while the overproduction of another mediator, prostaglandins, causes the late phase or second phase edema. The result of the anti inflammatory activity (Table 3) revealed that ethanol extract of Egyptian crowfoot grass significantly reduced the carrageenan-induced rat paw edema when compared to the standard treatment, diclofenac. The pre-treatment results demonstrated that 400 mg/kg of the ethanol extract was effective in the early phases of inflammation. However the anti-inflammatory effects of both doses (200 mg/kg and 400 mg/kg) were noticeable up until the 6th hour exhibiting 51.68% and 52.84% inhibition respectively. Different medicinal plants have shown anti-inflammatory activity in a similar way (Sumaiyah, Masfria, & Dalimunthe, 2020).

According to Labarrere and Kassab (2022), GSH-increasing medications may become crucial for lowering the severity and disastrous effects of inflammatory diseases since GSH depletion plays a central role in inflammatory disorders. GSH antioxidant enzyme level of the paw tissue homogenate of rats treated with the test plant increased significantly when compared with the inflamed untreated group (Carr group). Plants with ability to increase GSH levels as well as having anti inflammatory property has been reported (Bidian *et al*., 2020; Zammel *et al*., 2021). The overall result of this study has revealed fact that ethanol extract of Egyptian crowfoot grass could be considered for use in folklore medicine especially for the treatment of inflammation.

Conclusion

Accordingly, it can be inferred from the current findings that the ethanolic extract of Egyptian crowfoot grass has strong anti-inflammatory action and could be used alone or added to existing anti-inflammatory herbal compositions to increase their effectiveness.

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