DRUG DISCOVERY

Phytochemical constituent, acute toxicity and analgesic activities of *Dichrostachys cinerea* leaves methanol extract

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ABSTRACT

There are several pain relievers available on the market, but current medications are associated with certain side effects, such as gastrointestinal irritation, bronchospasm, fluid retention and prolonged bleeding time. Therefore, it is necessary to find new drugs with fewer side effects. The aim of this research was to investigate the analgesic activity of a methanol extract of *Dichrostachys cinerea* leaves by central and peripheral methods. An acute toxicity study on *Dichrostachys cinerea* leaf methanol extract (DCME) revealed that the extract was not toxic up to 3000 mg/kg, indicating that the test extract was within the safe range. Acetic acid-induced writhing results show a significant dose-dependent decline in the number of writhing times compared to the negative control. In addition, Doclofenac (100 mg/kg) significantly ($p<0.01$) reduced the number of writhing (increased inhibition rate). For the hot plate, the extract had a significant increase ($p<0.01$) the incubation time at the high dose of 400 mg/kg compared to the control at 90 and 120 minutes. In addition, diclofenac (100 mg/kg) significantly ($p<0.01$) increased the incubation period from 30th min to 120th min. These activities may be as a result of the existence of flavonoids, alkaloids, tannins, glycosides, phenols and more. Also, may be due to the presence of plant compounds. The extractable fraction may act as a free radical inhibitor or scavenger or may act as a primary oxidant. This study showed that the plant compound *D. cinerea* is effective against neurological disorders, cancer, inflammation, aging, etc. It gives an idea of how it can aid in the treatment of various diseases.

Keywords: Analgesic, phytochemicals, acute toxicity, *Dichrostachys cinerea*, diclofenec.

1. INTRODUCTION

Pain (analgesea) is a vague, unfriendly sensory and emotional feeling linked with real or potential tissue damage that varies from person to person and sometimes even within the same person. Untreated acute pain can lead to chronic pain and persistent pain can lead to anatomical and even genetic changes in the nervous

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system. Pain is a warning sign and is often protective, but it can also cause discomfort and pain. Excessive pain can cause other effects such as depression, anxiety, sweating, nausea, palpitations, increased or decreased blood pressure and tachypnea (Eric and Visser, 2009).

Pain management requires analgesics, including anti-inflammatories, with maximal doses of analgesic properties (Nowakowska, 2007). Several analgesics are available in the market, but current drugs are associated with certain side effects such as gastrointestinal irritation (Osadebe and Okoye, 2003), bronchospasm, fluid retention and prolonged bleeding time. Therefore, it is necessary to find new drugs with fewer side effects. Thus, people turn to medicinal plants to discover and develop new drugs (Raza et al., 2001). Scientists have also found that herbal extracts can be a vital source of fresh natural and safe drugs for pain management (Kuete and Efferth, 2010). The aim of this research was to analyze the phytochemical elements and evaluate the acute toxicity and analgesic activity of the methanol extract of *Dichrostachys cinerea* leaves.

2. MATERIALS AND METHODS

Sample Collection and Identification

Fresh plant samples were collected from the Kebbi State University of Science and Technology (KUST) site in Aliero. The plant was identified and certified by the Faculty of Plant Sciences and Biotechnology of KhMSTU, where the herbarium was kept for future reference. This plant has been recognized as *Dichrostachys cinerea* with the identification number: KSUSTA/PSB/H/VOUCHER NO: 281A *Dichrostachys cinerea* (L) Wight & Arm.

Sample Preparation and Extraction

The leaves were carefully separated from the stems, air-dried for 7 days and ground with an electric grinder. Ground plant material (150 g) was soaked in 1.5 L of methanol for 72 h at room temperature and then filtered. The filtrate was evaporated and dried in a water bath. The resulting dry extract was placed in a sealed glass container and stored in a refrigerator at 4°C until use. The weight and percentage yield of the extract were calculated.

Acute Oral Toxicity Studies (LD_{50})

Acute toxicity tests were performed using the high-low method (Bruce, 1985). Five albino rats were injected with the extract at a dose of 3000 mg/kg each. Animals were closely monitored for any serious effects or deaths over a 24-hour period. Observation was made for 15 days for signs of delayed toxicity.

Phytochemical Screening of *Dichrostachys cinerea* Leaves Methanol Extract

Phytochemical screening of extracts was performed using standard procedures designated by Trease and Evans, (1989). The extracts were selected for the identification of saponins, phenols, glycosides, flavonoids, alkaloids, tannins, terpenoids, anthocyanins and cardiac glycosides.

Determination of analgesic activity

Acetic Acid Induced Writhing Assay

We used the method described by Koster et al., (1959). Wister albino rats were randomly distributed into 5 groups of 5 rats each. Extract (100-400 mg/kg), saline (10 ml/kg) or diclofenac (100 mg/kg) was administered orally to each group 30 minutes before intraperitoneal injection of acetic acid (1% v/v), normal saline (10 ml/kg). The number of coils consisting of the contraction of the abdominal muscles with the stretching of the hind limbs was counted cumulatively for 30 min after the administration of acetic acid. Pain inhibition was expressed as percent protection using the formula:

$$\text{Injection of pain (\%)} = \frac{\text{Mean writhes (control)} - \text{Mean writhes (treated)}}{\text{Mean writhes (control)}} \times \frac{100}{1}$$

Where Mean writhes (control) is the mean writhes of the normal saline treated animals and mean writhes (treated) is the mean writhes of the animal given Diclofenec or each dose of methanol leave extract of *D. cinerea*

Hot plate assay

Male and female albino rats were tested for the corresponding reaction times 24 h before the reaction. The experimental animals were randomly distributed into 5 groups of 5 rats each. Baseline latencies were obtained on a hot-plate for each rat 1 h before treatment. This represents a significant delay before treatment. The extract (100 – 400 mg/kg, oral) was administered to rats with normal saline (10 ml/kg, orally) or Diclofenac (100 mg/kg, oral), depending on the group. After 1 hour 30 minutes of treatment, nociceptive responses were recorded using hot plate analgesia (Ugo Basile, Italy) at 55±1°C (Woolfe and Macdonald, 1944). A time of 45 seconds was used to avoid tissue damage. The time (in seconds) between the placement of the rat and the licking or biting and jumping of the hind paw was recorded as the response expectancy index. Response latencies were measured between 30 and 120 minutes after treatment.

Statistical Analysis

Statistical analysis was done using graph pad prism version 4. The data was analysed using one- and two-way analysis of variance (ANOVA) followed by turkey post hoc test. Data was represented as Mean ± SEM (n = 5). Statistical significance was considered at $p<0.01$.

3. RESULTS

Physical Characteristics of the Extract

The physical characteristics of *D. cinerea* leaves methanol extract are presented (Table 1). It indicates that the extract is dark-green in color, solid at room temperature and is tasteless. The weight measured was 150g and the percentage yield was 39%.

Table 1 Physical characteristics of the Extract

Physical characteristics	Result
Nature at room temperature	Solid
Color	Dark green
Weight	150g
Percentage yield	39%

Phytochemical Constituents present in *D. cinerea* Leaf Extract

The result of phytochemical screening of *D. cinerea* leaves methanol extract is presented (Table 2). It indicates the presence of phenols, saponins, glycosides, flavonoids, alkaloids, tannins, terpenoids and cardiac glycosides. However, anthocyanin was not detected.

Table 2 Phytochemical constituents present in *D. cinerea* Leaves Methanol Extract

Phytochemicals	Results
Saponins	+
Phenols	++
Glycosides	+
Flavonoids	+
Alkaloids	+
Tannins	+
Terpenoids	+
Anthocyanins	-
Cardiac glycosides	+

Plus (+) indicates the presence and minus (-) signifies not detected (ND)

Acute Oral (LD_{50}) Toxicity of *D. cinerea* Leaves Methanol Extract

The acute toxicity investigation of *Dichrostachys cinerea* methanol extract (DCME) carried out revealed that the extract was not toxic up to 3000 mg/kg. Therefore, the LD_{50} of *D. cinerea* leaves methanol extract is estimated to be greater than 3000 mg/kg. Hence, the tested extract happened to be within the safe margin.

Analgesic Activity of *Dichrostachys cinerea* Leaves Methanol Extract

Acetic Acid-Induced Writhing

Effect of *Dichrostachys cinerea* leaves methanol extract in writhing induced by acetic acid is shown (Table 3). Generally, the result in the table shows that, the dose injected in mg orally per Kg of the body weight showed a significant reduction in the number of twists compare to the negative control group. Also, Diclofenec (100mg/kg) produced a significant ($p<0.01$) decrease in the number of writhes (increase percentage inhibition).

Table 3 Effect of *D. cinerea* Leaves Methanol Extract on Writhing Induced by Acetic Acid

Groups	Number of writhing	Percentage of Inhibition
Normal Saline (10ml/kg)	27.2±1.74	………
Diclofenec (100mg/kg)	12.6±1.43*	53.7
D. cinerea extract		
100 mg/kg	22.0±2.35**	19.1
200 mg/kg	17.4±2.09**	36.0
400 mg/kg	13.6/±1.08*	50.0

Values are Mean ± SEM (n=5), *P<0.01 is significantly different compared with normal control.

Hot Plate Assay

The extract produced a significant ($p<0.01$) increase in latency time at a higher dose of 400 mg/kg at the 90th and 120th minute compare to the control group. Also, Diclofenec (100mg/kg) produced a significant ($p<0.01$) increase in latency time from the 30th to the 120th minute (Table 4).

Table 4 Effect of *D. cinerea* Methanol Extract on hot plate test in rats

Group	Dose (mg/kg)	30min	60min	90min	120min
Normal Saline		5.06±0.67	5.76±1.44	6.26±0.91	5.75±0.37
Diclofenec	100	30.07±2.21 (83.17)	30.30±1.25 (80.99)	32.02±2.79* (80.45)	32.82±4.44* (82.48)
D. cinerea	100	6.54±0.17 (22.63)	6.85±0.54 (15.91)	8.08±0.35 (22.52)	8.69±0.44 (33.83)
D. cinerea	200	16.72±1.50** (69.74)	16.76±3.05** (65.63)	20.80±0.59** (69.90)	20.94±0.68** (72.54)
D. cinerea	400	28.36±2.49 (82.16)	30.93±6.22* (81.38)	31.00±5.58* (79.81)	31.49±3.72* (81.74)

Values are Mean ± SEM (n=5), (*P<0.01) is significantly different compared with normal control.

4. DISCUSSION

The physical characteristics of *Dichrostachys cinerea* leaves methanol extract indicates that it is dark in color, solid at room temperature and is tasteless. Qualitative phytochemical analysis of the extract revealed the presence of phenols, saponins, glycosides, flavonoids, alkaloids, tannins, terpenoids and cardiac glycosides, but anthocyanins were not detected. Plants have always been regarded as source of food and medical compounds (Phillipson, 2001). Actually, around 200 species are regarded as medicinal plants and roughly 25% of the medicines have plants ancestry (Gurnani et al., 2014). Most of phytochemicals, constituents of food, beverages and herbal plants are often stated in literature as “nutraceutical”, underlining their health promoting characteristics, including the prevention and treatment of syndromes like cancer, cardiovascular disease, neural disorders and Alzheimer (Winter et al., 2017).

In plants, phenolics take part in H_2O_2 detoxification, provide UV protection and act as enzyme regulators and nutrients for herbivores (Bennett and Wallsgrove, 1994). The wide range of biological functions of phenolics, including antioxidant (i.e., antioxidants, free radical scavengers and singlet oxygen scavengers) and antitumor properties, have been widely recognized in several studies (Kasote et al., 2015; Pandey and Rizvi, 2009). Flavonoids include flavonols, flavones, flavones, flavan-3-ols, anthocyanidins and isoflavones. They portrayed the attention of researchers due to their positive effects on several diseases. For example, anthocyanins and quercetin have been reported to have effect in reducing the development of malignant cells, affecting oncogenic metabolism, reducing tissue inflammatory parameters and inhibiting angiogenesis (Bunea et al., 2013).

Terpenoids are another very huge group of plant secondary metabolites (Aharoni et al., 2005). *In vitro* tests have shown that diterpenes, sesquiterpenes as well as monoterpenes in aromatic plants exhibit significant antioxidant activity (Baratta et al., 2008). Different flavonoids with different chemical structures are associated with different mechanistic anti-inflammatory effects (Gomes et al., 2000). *Citrus spp*'s flavonoids and fruit flavones inhibit multiple pro-inflammatory mediators, including mediators derived through the arachidonic acid pathway (Vafeiadou et al., 2009). Indeed, citrus flavanones (eg, naringenin) can modulate neuroinflammation through interactions with the p38 and STAT-1 signaling cascade (Hamalainen et al., 2007) or suppress inflammatory responses in animal models of rheumatoid arthritis, thereby providing anti-inflammatory properties. It exerts an inflammatory effect orally (Rogerio et al., 2010). Licorice root (Glycyrrhiza glabra) triterpenes glycyrrhizins and glycyrrhizic acid: These have various uses, such as gastric protection, regulation of blood pressure through mineralocorticoid activity (Kao et al., 2010) and anti-inflammatory effects through P13K/Akt/ exist. The GSK3-β pathway reduces cytokine production and 18β-glycyrrhetinic acid also stops inflammation by inducing glucocorticoid receptor dissociation (Salminen et al., 2008). There are hundreds of different phytochemicals in the literature with prospective function on the skin, such as anti-aging activity, photo protection, wound healing and anti-infection (Houston et al., 2017; Prasad et al., 2017).

An acute toxicity study on *Dichrostachys cinerea* leaf methanol extract (DCME) revealed that the extract was not toxic up to 3000 mg/kg, indicating that the test extract was within the safe range. Peripheral analgesic activity was detected by the acetic acid-induced writhing test and the hot-plate test was used to assess central analgesic activity. In this study, the number of ripples induced by acetic acid was significantly suppressed in rats treated with *D. cinerea* (Table 4, 3) indicating that *D. cinerea* had an excellent peripheral analgesic effect. Also, *D. cinerea* and Diclofenac can significantly increase the pain threshold in rats (Table 4), indicating that *D. cinerea* has strong central analgesic activities. In addition, the analgesic activity of *D. cinerea* showed dose-dependent values in hot plate test and the acetic acid-induced writhing experiment

Acetic acid-induced writhing experiment is straight forward, sensitive and analytical method to measure peripheral analgesic effect. Acetic acid triggers nociception by releasing endogenous substances such as histamine, bradykinin, prostaglandin and serotonin, which may excite sensory nerve ending. Thus, *Dichrostachys cinerea* could be beneficial in the management of pain. The new findings in the present investigation offer a scientific support to the ethno medicinal property of the plant by the traditional people.

Acetic acid induced rats writhing test is useful for the evaluation of mild analgesic non-steroidal anti-inflammatory compounds for peripheral anti nociceptive or analgesic activity (Berkenkopf and Weichman, 1988). Thus, the significant inhibitory effect exerted by the extract on the acetic acid induced rats writhing suggests that the extract has a peripheral analgesic effect.

In Hot Plate Assay, centrally mediated nociception is often modeled using the thermal stimulus produced by the hot plate. Subsequently, the hot plate experiment is often used for the screening of substances for central analgesic property and centrally active analgesics rise reaction latency period of laboratory animals to induced pain by the hot plate. Hence, the significant rise of the reaction latency period of the rats, 90^{th} and 120^{th} minutes following injection of the extract (400 mg/kg) indicates that the extract, at higher doses must have a strong and long-lived central analgesic activity.

5. CONCLUSION

The safety profile of the methanol extract of *D. cinerea* leaves was investigated and abnormalities were observed at an initial dose of 3000 mg/kg. The results of another experiment show that the methanolic extract of *D. cinerea* has analgesic properties. These activities may be as a result of the occurrence of alkaloids, flavonoids, tannins, glycosides, phenols and more. Also, presence of plant compounds can be another reason. The extractable fraction may act as a free radical inhibitor or scavenger or act as a primary oxidant. This study showed that the plant compound *D. cinerea* is effective against neurological disorders, cancer, inflammation, aging and more. It gives an idea of how it can be used in the management of several diseases.

Informed consent

Not applicable.

Ethical approval

The ethical guidelines for plants & plant materials are followed in the study for sample collection & identification. The Animal ethical guidelines are followed in the study for experimentation.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability
All data associated with this study are present in the paper.

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