Phytochemical Screening and Analgesic Effect of Methanol Root Extract of *Datura Metel* in Experimental Animals

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ABSTRACT

Owing to the fact that plants have been used from ancient times in almost all cultures as a source of medicine; they are considered to be the backbone of traditional medicine and are widely used to treat acute and chronic diseases. Hence, the approval by WHO in the use of natural products for national policies and drug regulatory measures in order to strengthen research and evaluation of the safety and efficacy of herbal products. It is reported that of the 119-plant derived drug listed by WHO study, 74% were discovered as a result of chemical studies to isolate the active compounds responsible for the medicinal properties. *Datura metel* is a bitter narcotic plant that relieves pain and inflammation when taken as concoctions. It has a long history of use as native medicine, commonly used for management and treatment of chest complaints, including asthma, cough, tuberculosis and bronchitis etc. Phytochemical results revealed the presence of alkaloids, tannins etc. that might be responsible to the analgesic activity of the plant parts. Lorke's method was used to establish the dose of the plant extract as well as determine the safety margin (LD_{50}) of the root extract as greater 5,000 mg/kg. Twenty-five healthy albino miceof both sexes were randomly assigned into five groups of five each. The mice in group 1 served as the control, which were given Normal saline, group 2 were administered with standard drug (ibuprofen 400 mg/kg), while groups 3, to group 5 served with 218, 436, 654 mg/kg of the methanol root extracts respectively; which is 5%, 10% and 15% of the extracts LD_{50} .The result of the work shows that analgesic activity of methanol root extract of *D. metel* is dose dependently effective. Hence, the need to isolate and characterize the lead compound in order to produce new drugs.

Keywords: Analgesic, *Daturametel*, Extract, Medicine, Phytochemicals.

BACKGROUNDS

Over the years, there has been a resurgence of interest in the research of natural products as sources of potential drug substances (Manas *et al*., 2012). Most countries are now applying native medicinal strategies that involve the use of herbs as remedies. Hence, many countries have incorporated plants as a source of medicinal agents into their primary modality of health care (Imo and Uhegbu, 2015).

The medicinal value of plants lies in their bioactive phytochemical constituents that produce definite physiological actions in the human and other animals' bodies respectively (Akinmoladun *et al.*, 2007). These chemical constituents include; alkaloids, flavonoids, saponins, terpenes, phenolic compounds etc.(Oseni *et al*., 2010). *D. metel* (Baba Jijji in Hausa) is reported as a bitter narcotic plant that relieves pain and inflammation (Gana *et al.*, 2021). It has a long history of use as native medicine, especially for the treatment of chest complaints including asthma, cough, bronchitis and tuberculosis (Gana *et al.*, 2021).

Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Ridderikhof, 2019). It is always a warning signal and primarily protective in nature but often causes a lot of discomfort and leads to many adverse effects (Yam*et al.*, 2018). It is a disabling

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accompaniment of many medical conditions, that is why pain control is one of the most important therapeutic priorities (Pongan*et al.,* 2017). It is usually initiated by noxious stimuli and transmitted over specialized neuronal networks to the central nervous system (CNS) where it is interpreted as such (Yam*et al.* , 2018). A wide range of different analgesic drugs are available for use to man. This may be classified in two broad groups: (i) the centrally acting agents such as morphine and pethidine and (ii) the peripherally acting agents such as aspirin and diclofenac. The centrally acting agents are generally the most potent ones. Those of clinical importance are narcotic or opiate analgesics, and are subdivided into drugs with morphine-like actions (agonists), those with some antagonist actions (partial agonists) and those which are antagonists to morphine (Raffa *et al*., 2017).This study is aimed at investigating the analgesic effect and phytochemical constituents of methanol root extract of *D. metel* using mice.

METHODS

Sample preparation:

The Methanol root extract was prepared by soaking 100 grams of the powdered sample in 400 mL of 99.8 % methanol for 72 hours, then the soaked sample was filtered using What man filter paper no. 1, the filtrate was evaporated in a rotary evaporator at 40^{o}C.

Experimental Animals

Thirty-four (34) healthy adult Wister albino mice of both sexes weighing between 20 to 30 g were purchased from Usman Danfodiyo University, Sokoto and used for this study. The mice were housed in ventilated plastic cages. They were allowed to acclimatize for 7 days and were given access to commercial animal feed and Normal saline at *libidum*.

Phytochemical Screening

The phytochemical screening of methanol root extract of *Datura metel* (MREDM) for the presence of secondary metabolites such as saponins, tannins, alkaloids, flavonoid, steroid, terpenoids, anthraquinones and glycoside was carried out using standard laboratory methods.

Acute Toxicity Studies

The method of Lorke was used to establish the dose of the plant extract as well as determine the safety margin of the MREDM, which is divided into two phases:

Phase 1: In this phase nine mice were used. They were divided into three groups of three mice each. Each group of mice were administered different doses (10, 100 and 1000 mg/kg) of the MREDM respectively. The mice were closely observed for behavioral changes and possible mortality or survival for 24 hours.

Phase 2; involves the use of four mice, which were distributed into four groups of one rat each. The mice were administered higher doses (1600, 2900, 3800 and 5000 mg/kg) of the MREDM and then observations were made for 24 hours just as above.

The Median Lethal Dose (LD_{50}) using Lorke's method will be calculated as follows:

$\text{Median Lethal Dose} = \sqrt{\text{Do}} \times \text{D100}$; where Do= Highest dose that showed no mortality and D100 = Lowest dose that produced mortality.

Analgesic Screening

The healthy albino mice were randomly assigned into five groups of five mice each. The mice in group 1

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served as the control, which were given Normal saline, group 2 were administered with standard drug (ibuprofen 400 mg/kg), while groups 3, to group 5 served with 100, 200, 400 mg/kg of the MREDM respectively. The extracts were administered through intraperitoneal route while ibuprofen was administered orally.

Analgesic Activity

The analgesic activity was determined by measuring the drug-induced changes on the pre-screened mice to heat stress; using a hot plate at temperatures of 55°C, applied to their tails. The animals were pre-treated 60 minutes before subjecting them to heat stress as follows:

Table 2.1 Determination of Analgesic Activity of Methanol Root Extract of *D. metel*

Groups	**Dose**
Group 1 Control	Normal saline (5 mL/kg)
Group 2 Standard Drug	Ibuprofen (400 mg/kg)
Group 3	MREDM (218mg/kg) 5% of the LD_{50}
Group 4	MREDM (436mg/kg) 10% of the LD_{50}
Group 5	MREDM (654mg/kg) 15% of the LD_{50}

The distance between the heat source (hot plate) and the tail skin was 1 cm and the cut-off reaction time fixed at 20 seconds to avoid tissue damage. The time taken for mice to react to the heat stimuli introduced was measured for each group that was set up for the experiment.

RESULTS

Phytochemical Screening

The preliminary qualitative phytochemical screening of *D. metel* methanol root extract revealed the presence of some secondary metabolites (Table 4.1); Alkaloids, tannins, saponins, phenols, flavonoids, steroids and terpenoids were detected while anthraquinones and glycosides were not detected

Table 3.1 Qualitative Phytochemical Compositions of Methanol Root Extract of *Datura metel*.

Phytochemicals	Results
Alkaloids	+
Tannins	+
Saponins	+
Phenols	+
Glycosides	–
Flavonoids	+
Steroids	+
Terpenoids	+
Anthraquinones	–

Key; + = Present, – = Absent

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Acute Toxicity Studies

There was no mortality recorded, no toxic signs; including autonomic nor central nervous system effects (perspiration, defecation, urination, salivation dizziness etc.) in the animals. Therefore, the LD_{50} was estimated to be greater than 5000 mg/kg.

Table 3.2 Acute Toxicity Studies Determination

GROUP	DOSE	RESULTS
GROUP 1	10 mg/kg	No Death
GROUP 2	100 mg/kg	No Death
GROUP 3	1000 mg/kg	No Death
GROUP 4	1600 mg/kg	No Death
GROUP 5	2900 mg/kg	No Death
GROUP 6	3800 mg/kg	No Death
GROUP 7	5000 mg/kg	No Death

The Lethal Dose (LD50) of MREDM was calculated as follows
Median Lethal Dose =$\sqrt{Do}$×D100
Do= 5000 mg/kg, D100 = 3800 mg/kg
Median Lethal Dose =√5000×3800, Median Lethal Dose =√19000000LD50 = 4358 mg/kg

Analgesic Activity

The result of this work shows that analgesic activity of methanol root extract of *D. metel* is dose dependently effective, except for the Normal saline treated group (negative control) which showed significant difference (P>0.05) when compared to MREDM/drug treated groups. The result also reveals that there is no significant difference (P < 0.05) between the group treated with the standard drug and the group treated with 654 mg/kg of extracts in pain relief. All the same, the 218 and the 436 mg/kg doses showed significant difference (p<0.05) with the normal saline group as well as a dose dependent difference with the standard drug.

Table 3.3 Analgesic Activity of Methanol Root Extract of *D. metel*

Dose Administered	Analgesic Activity (seconds)
Normal saline (5 mL/kg)	5.19 ± 0.23^{a}
Ibuprofen (400 mg/kg)	16.01 ± 0.21^{d}
MREDM (218 mg/kg)	12.20 ± 0.47^{b}
MREDM (436 mg/kg)	13.71 ± 0.38^{c}
MREDM (654 mg/kg)	16.06 ± 0.33^{d}

Values are presented as mean ± standard error of mean (n = 5 per group). Values having the same superscript in the same column are not significantly different (*P*<0.05) analysed by one-way ANOVA followed by Duncan's multiple comparison test. MREDM=methanol root extract of *D. metel*.

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DISCUSSION

Medicinal plants have been the basis of treating various diseases in African traditional medicine as well as other forms of treatment from diverse cultures of the world (Abera, 2014). Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans as medicinal ingredients and nutrients (Chhikara *et al*., 2018). The results of this research revealed the presence of alkaloids, tannins, saponins, phenols, flavonoids, steroids and terpenoids, which is similar to the preliminary phytochemical investigation performed by other scientists on methanolic and hydroalcoholic extract of *D. metel* which revealed the presence of alkaloids, tannins, cardiac glycosides, flavonoids, and phenols. The result is also in agreement with an other work on the phytochemical analysis performed on *D. Stramonium* which also contain alkaloids, saponins, tannins, steroids, flavonoids, phenols and glycosides (Al-snafi, 2017). The median lethal dose (LD_{50}) is defined as the dosage of a substance which kills 50 per cent of the animals in a particular group, it is the toxic effects produced by single exposure of drugs by any route for a short period of time (Chanda *et al.*, 2015). Acute toxicity studies in animals are considered necessary for any pharmaceutical intended for human use (Fattahi *et al*., 2015). The main objective of acute toxicity studies is to identify a single dose causing major adverse effects or life-threatening toxicity, which often involves an estimation of the minimum dose causing lethality (Attah *et al*., 2019).Plants with a medicinal value play a vital part in healing and curing human diseases because of the biochemical constitutes, known as phytochemicals. Phenols exhibit several properties beneficial to humans and its antioxidant properties are important in determining their role as protective agents against free radical-mediated disease processes (Airaodion*et al.*, 2019).

Flavonoids have been shown to have hepato-protective and hepato-curative capacity (Ahmad*et al*., 2017). Flavonoids have been reported to exert multiple biological properties including anti-microbial, cytotoxic, anti-inflammatory and anti-tumor activities; but the best-described property of almost every group of flavonoids is the capacity to act as powerful antioxidants (Melichar*et. al.*, 2015). Alkaloids have pharmacological applications as anaesthetics and central nervous system stimulants (Campbell and Young, 2015). This is suggestive to why methanol root extract of *Datura metel* exhibited analgesic effect almost close to the standard drug (400 mg/kg of ibuprofen), hence the need to isolate and characterize the active component responsible for its analgesic activities.

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