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INVESTIGATING THE ANALGESIC PROPERTY OF PETROLEUM ETHER EXTRACT OF *PIPTERDENIASTRUM AFRICANUM* (MIMOSACEA) USING FORMALIN-INDUCED PAIN MODEL

BY

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ABSTRACT

Pain is a very common symptom and it indicates that something is wrong in the body and may give a clue to the nature of disease. Thus, this study was done to determine if there is analgesic activity present in the bark extract of Piptadeniastrum africanum and hence future usefulness in the management of pain.

The extract was subjected to preliminary phytochemical screening. The extract showed the presence of flavonoids, sterols, and glycosides.

The analgesic activity of the bark extract was analyzed using Formalin-induced pain model in which the subjects used were adult male mice with an average weight of 24grams. Different doses were administered to the mice with positive control as Aspirin 100mg/kg, negative control as vehicle (emulsion: since petroleum ether is not soluble in water) and other doses of 250mg/kg, 500mg/kg and 1g/kg.

The results from the graph of duration of paw licks against the different dose groups showed that the duration of paw licks reduced drastically when aspirin was administered to the mice when compared to the other doses, but the duration of paw licks was highest with the vehicle. So it may be concluded that the bark extract has little analgesic activity. But further studies on the P-values show that most of the values were not significant (values have to be significant to indicate the presence of analgesia). From this, we can deduce that the petroleum ether extract of Piptadeniastrum africanum has little or no analgesic activity.

KEY WORDS: *Piptadeniastrum africanum*, phytochemical screening, Formalin-induced pain model, analgesic activity.

INTRODUCTION

Pain is not easily or satisfactorily defined and therefore is often interpreted as a suffering that results from the perception of painful stimuli. It's a common symptom and it indicates that something is wrong in the body and may give a clue to the nature of disease. Hence, "pain is a specific sensation with its own peripheral and central mechanisms independent of other five senses." Pain itself is not a disease; it is by far the most common medical complaint. It is usually perceived as an indication of ill health and most diseases have a component of pain. The control of pain is one of the most important uses to which drugs are put. Pain can be defined as the effect produced in consciousness by the arrival of nerve impulses generated by noxious stimuli in the brain. Drugs, which alter the pain sensitivity or remove pain, are called as painkiller or analgesics (Debasis Mishra, 2011). Pain is the most common symptom prompting patients to seek medical attention and is reported by more than 80% of individuals who visit their primary care provider. Despite the frequency of pain symptoms, individuals often do not obtain satisfactory relief of pain. This has led to recent initiatives in health care to make pain the fifth vital sign, thus making pain assessment equally important as obtaining a patient's temperature, pulse, blood pressure, and respiratory rate. (Marie chisholm-burns, Barbara wells, & Schwinghammer, 2008).

According to IASP (International Association for the study of Pain), Pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Merskey, Albe- Ferssard, Bonica, Carmon, Dubner, & Kerr, 1979)." Pain is universal, complex and very subjective (subjective in the sense that; the patient is the only person that can describe the intensity of the pain). Pain threshold is not uniform, people can respond to pain in different ways for the same condition. Pain is used interchangeably with inflammation although they have similar drugs that can be used to treat both pain and inflammation like the conventional Non-steroidal anti- inflammatory drugs [NSAIDS] which can be used for management of both pain and inflammation but they are not the same while pain is a subjective experience that varies from person to person it generally is an unpleasant sensory and emotional experience associated with actual or potential tissue damage such as inflammation, or described in terms of such damage as stated above. Inflammation is a localized physical condition in which part of the body becomes reddened, swollen, hot, and often painful, especially as a reaction to injury or infection.

This research is designed to obtain petroleum ether extract, confirm the presence of certain phytochemical constituents in the extract and investigate the analgesic activity of *Piptadeniastrum africanum* (Mimosaceae) on adult male mice using the formalin-induced pain model.

MATERIALS AND METHODS

Plant collection: - Fresh bark of *Piptadeniastrum africanum* (mimosaceae) was collected from Kumasi, Ghana, and also authenticated in Kumasi by appropriate standard botanist to ensure that the species of the bark was very accurate

Plant extraction: - the bark of *Piptadeniastrum africanum* was sun dried for 4 weeks and milled using a milling machine. A soxhlet extractor was used in the extraction process. 200grams of the powdered dried bark of *Piptadeniastrum africanum* was weighed using a weighing scale five times (making 1kilogram of powdered dried bark was weighed in total). The extraction process was done five consecutive times because the soxhlet extractor could only take 200grams of the powdered mass at a goal. For each 200grams of the powdered dried bark of *Piptadeniastrum africanum*, 2 litres of petroleum ether were added.

The extraction took two days after which a uniform extract was obtained. The extract was then collected into a round bottom flask and then concentrated using the rotary evaporator. A lemon green concentrate was obtained after the rotary evaporation process. And this was poured into an evaporating dish and was air dried for 2 days.

PRELIMINARY PHYTOCHEMICAL TESTS

Phytochemical analysis was performed on the petroleum ether extract of *Piptadeniastrum africanum* to identify the bioactive chemical constituents present in the crude extract of the plant material. The chemical constituents were determined as stated in (Trease, 2002), (Harborne, 1993) and (Sofowara, 1993). The chemical constituents analyzed were: flavonoid, alkaloids, tannins, coumarins, triterpenoids, steroids, glycoside (saponin, anthracene, cardiac, cyanogenetic glycosides).

PHARMACOLOGICAL ANALYSIS

Experimental Animals: - The ICR mice were obtained from CSRPM (Center for Scientific Research into Plant Medicine) in Mampong in Eastern Region, Ghana and they were male ICR mice and were kept inside cages containing wood shavings in the pharmacology lab, randomly selected and grouped into the positive control study group, negative control study group, the plant extract of different concentration study group (250mg/kg, 500mg/kg and 1000mg/kg). Aspirin was used as a positive control or the reference drug and water was used as a negative control drug, they were fed and given water daily.

Drugs and chemicals: -

- Acetylsalicylic acid (Aspirin) Tablets BP 300mg (GML, ACCRA, GHANA, and BATCH NUMBER- B101/M05),
- Formalin injection,
- Petroleum ether,
- Petroleum ether extract *Piptadeniastrum africanum.*

Reagent preparation

Formalin injection: - 2.5 ml of formalin was measured into a 100ml volumetric flask and topped up to the 100ml volumetric flask using NaCl (2.5% formalin in NaCl).

Emulsion for the petroleum ether extract: - in the preparation of the emulsion, the dry gum method was used. Emulsion was prepared for 4 different doses of the extract. 30grams of acacia gum was weighed using a weighing balance into a clean porcelain mortar and triturated clock wisely into fine particles. 50ml of arachis oil (frytol) was measured using a measuring cylinder and added to the fine particles with an immediate continuing stirring of the mixture in a clockwise direction for 15 minutes, a little amount of water was added to the mixture and triturated. The already made emulsion was transferred into an amber coloured bottle to prevent photo degradation of the emulsion (cracking). And this procedure was repeated four times for the four different doses. Reason behind the preparation of the emulsion is that petroleum ether plant extract is not soluble in water, so an emulsion was prepared to enhance the solubility of the petroleum ether plant extract.

2.6 Method for Pharmacological analysis

The animals were kept in three cages 15 animals per cage at room temperature in which they had access to food and water. Prior to testing, the animals were weighed using a weighing balance and placed in an observation chamber where there was no access to food and water. 3 animals were observed for each dose, for each dose (250mg/kg, 500mg/kg, 1g/kg, positive control, negative control), 0.2ml of each dose were administered to the animals (mice) orally.

After 30 minutes, 0.05ml of formalin injection was injected under the skin of the dorsal surface of the right hind paw using a micro syringe. The amount of time the animal spent on licking the injected paw was recorded.

To determine the course of the responses, experimental naïve animals were observed for 30 minutes after the administration of the formalin injection. Where there was the early phase of observation (15 minutes) and the late phase of observation (15 minutes). The animals for the negative control test were only administered with the emulsion without the extract being present in it and 30 minutes later, they were injected with formalin injection like the other test animals. The animals for the positive control test were administered with aspirin and 30 minutes later, they were injected with formalin injection and the early phase and late phase were also observed. In this experiment only one concentration of formalin injection was used (2.5% formalin in 100ml NaCl).

Pain intensity was rated using one single objective response: licking the injected paw, either the dorsal surface of the paw, the toes or the leg. The duration at which the animal spent licking was recorded.15 experimental animals were used with 3 assigned for each dose group

STATISTICAL ANALYSIS OF RESULTS

3.0 RESULTS

3.1 RESULTS FROM EXTRACTION PROCEDURE

3.1.1 WEIGHT RECORDED

1. Mass of milled plant bark
    - Mass of powered plant + beaker = 1584.1g,
    - Mass of beaker only = 584.15g,
    - Mass of sample only = 100g,
2. Mass of petroleum ether extract
    - Mass of petroleum ether extract + evaporating dish = 171.99g,
    - Mass of empty evaporating dish = 149.96g,
    - Mass of petroleum ether extract
      = (mass of extract + evaporating dish − mass of empty evaporating dish)
      =22.03g.

3.1.2 ANALYSIS OF YIELD OF EXTRACT

- To calculate for the percentage yield
- Mass of total milled sample taken = 1000g
- Mass of concentrated petroleum ether extract = 22.03g
- Percentage yield = $\frac{\text{Mass of concentrated petroleum ether extract}}{\text{Total sample taken}}$,

$$= \frac{22.03g \times 100}{1000g} = 2.203\%$$

3.2 PHYTOCHEMICAL SCREENING

3.2.1 PRELIMINARY PHYTOCHEMICAL TEST

Phytochemical test was carried out on both the petroleum ether extract and the plant bark itself. The tests carried out on the petroleum ether extract showed the presence of phytochemicals including; Steroids, glycosides (cyanogentic glycosides), flavonoids. However, the same test carried out on the bark showed the presence of alkaloids, tannins, flavonids, steroids, glycosides (saponins and cyanogenetic glycosides).

Table 1: Preliminary photochemical tests on plant extract

TEST	OBSERVATION	INFERENCE
Mayer's test for alkaloids a) Wagner's reagent + small portion of extract	A white buff precipitate	Alkaloids may be present
Dragendorff's test for Alkaloids b) 2ml of dragendorff's reagent + 1ml of extract	A purple colour change	Alkaloids may be present
General Test for Glycosides f) 25ml of dil. H_2SO_4 to 5ml extract and boil for 15mins	Formation of a brick-red colour	Glycosides may be present
Froth test for saponins e) 0.5g of extract + 1ml of alcohol diluted with 20ml distilled water. Shake well for 2min.	No froth formed on standing	Saponins may be absent

Test for Anthraquinone glycosides f) 0.5g of extract + heat + HCl. Filtrate + Benzene + NH4OH	No colour change	Anthraquinones may be absent
Test for Cyanogenetic glycosides g) Extract + water moistened on sodium picrate paper + heat	Picrate paper turned brick red	Cyanogenetic glycosides may be present
Test for tannins h) Dilute extract with water, add 3-4 drops of 10% ferric chloride solution	No colour change	Tannins may be absent
Test for coumarins I)0.5g of plant extract +Filter paper treated with 1M NaOH + UV light	No yellow fluorescent	Coumarins may be absent
Test for Triterpenoids J) Extract + 2ml chloroform +3ml Conc.H_2SO_4	No colour Change	Triterpenoids may be absent
Test for Steroids H) Extract + 2ml acetic anhydride+ 3ml H_2S0_4	Green colour Observed	Steroids may be present
Test for Flavonoids I) Extract filtered with petroleum ether + dilute ammonia+ Conc.H_2SO_4	Yellow colour Observed	Flavonoids may be present

Table 2 Preliminary photochemical tests on plant bark

TEST	OBSERVATION	INFERENCE
Mayer's test for alkaloids a) Wagner's reagent + small portion of bark	No colour change	Alkaloids may be absent
Dragendorff's test for **Alkaloids** b) 2ml of dragendorff's reagent + 1ml of bark	No colour change	Alkaloids may be absent
General Test for Glycosides f) 25ml of dil. H_2SO_4 to 5ml bark and boil for 15mins,	Formation of a brick-red colour	Glycosides may be present
Froth test for saponins e) 0.5g of bark + 1ml of alcohol diluted with 20ml distilled water. Shake well for 2min.	froth formed on standing	Saponins may be present
Test forAnthraquinone glycosides f) 0.5g of bark + heat + HCl. Filtrate + Benzene + NH_4OH	No colour change	Anthraquinones may be absent
Test for Cyanogenetic glycosides g) Bark + water moistened on sodium picrate paper + heat	Picrate paper turned brick red	Cyanogenetic glycosides may be present
Test for tannins h) Dilute bark with water, add 3-4 drops of 10% ferric chloride solution	Dark green colour change	Tannins may be present

Test for coumarins I)0.5g of plant Bark +Filter paper treated with 1M NaOH + UV light	No yellow fluorescent	Coumarins may be absent
Test for Triterpenoids J) Bark + 2ml chloroform +3ml Conc.H_2SO_4	No colour Change	Triterpenoids may be absent
Test for Steroids H) Bark + 2ml acetic anhydride+ 3ml H_2S0_4	Green colour Observed	Steroids may be present
Test for flavonoids I)Bark filtered with petroleum ether+ dilute ammonia to filtrate + Conc. H_2S0_4	Yellow colour Observed	Flavonoids may be present

TABLE 3: DURATION OF LICK OF HIND PAW IN THE FORMALIN PAIN MODEL TEST

	Dose Groups					
Experimental Parameter	**Vehicle**	**Aspirin(100mg/kg)**	**250mg/kg**	**500mg/kg**	**1g/kg**	
Duration of paw licks in seconds(M1)	273	75	97	0	26	
Duration of paw licks in seconds(M2)	18	36	28	86	91	
Duration of paw licks in seconds(M3)	96	37	64	59	62	
Mean of M1-M3	129	74	63	48.3	59.7	
S.D. of M1-M3	130.66	22.23	34.51	43.98	32.56	

Note: M stands for mouse representing the experimental animals used; **S.D** stands for standard deviation is a quantity expressing by how much the members of a group differs from the mean value for the group.15 experimental animals were used with each dose group having three different mice.

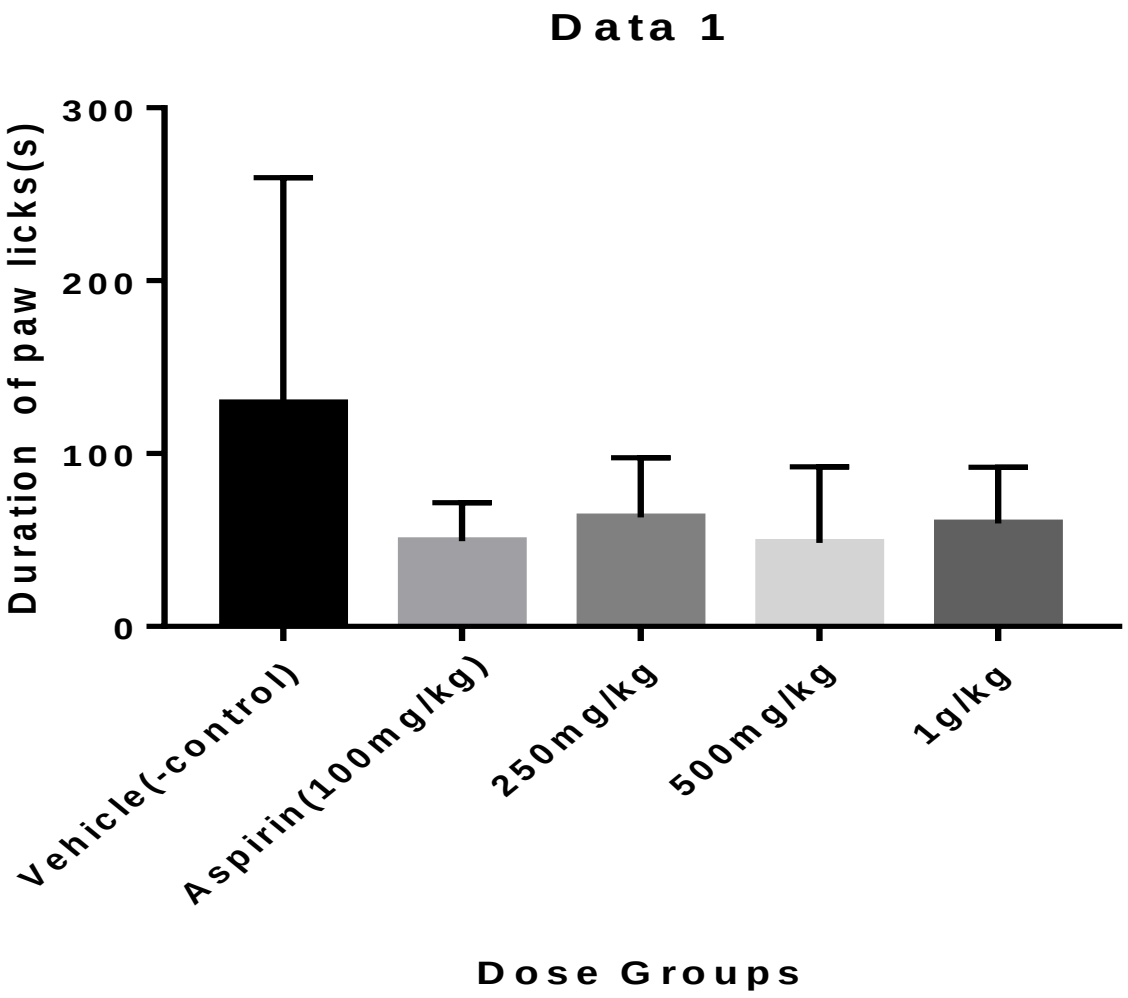

Figure 1: Graph Plot of Duration of Paw Lick Against Dose Groups Mean/Standard deviation

DISCUSSION.

The bark of *Piptadeniastrum africanum* was first chopped into smaller sizes and dried by the sun for six weeks to facilitate drying after which it was milled into fine particles and properly stored. Petroleum ether was used as the solvent for extraction from the plant bark. The crude petroleum ether extract was gotten after extraction using the soxlet extractor and concentrating with the rotary evaporator and drying for three days.

Phytochemicals analysis were carried out on the bark of the plant and it showed that glycosides, steroids, tannins, alkaloids, saponins, flavonoids may be present. However; phytochemicals analysis carried out on the crude petroleum ether extract indicated that glycosides, steroids may be present. The absence of the components listed above in the crude petroleum ether extract as compared to the ethanol extract where tannins, alkaloids, flavonoids were present is as a result of the highly polar nature of these components which contain highly polar constituents which are only soluble in polar solvents like ethanol.

Ethanol possesses hydrogen and carbon atom bonds which are non- polar covalent bonds also there is hydrogen-oxygen and carbon-oxygen bonds which are polar covalent bonds.

These combinations of polar and non- polar covalent bonds present in ethanol allow for the extraction of both polar and non-polar phytochemicals constituents unlike petroleum ether. The highly aliphatic C5 and C6 hydrocarbons present in petroleum ether makes it very non polar and therefore, polar photochemical constituents like alkaloids, tannins, flavonoids will not be able to be extracted by petroleum ether because they will not be able to be extracted by petroleum ether.

After the phytochemicals analysis, the crude petroleum ether extract was used for the formalin induced pain model test in mice. The extract was prepared in an emulsion as vehicle to be given to the animals orally due to the insolubility of the crude extract in water. Five different dose groups were used in this test the vehicle (emulsion only) which was the negative control, Aspirin 100mg/kg as the positive control, crude extract + emulsion 250mg/kg, crude extract + emulsion 500mg/kg, crude extract + emulsion 1000mg/kg. The mean duration of licks of paw in seconds for each of the groups was 129s, 74s, 63s, 48.3s, and 59.7s, respectively. From the results of the experiments there was a significant reduction in the duration of lick when the vehicle which contained no extract is compared to the extract dose groups or aspirin which is a known analgesic drug with aspirin. However, the dose group of 500mg/kg showed the lowest mean duration of lick when compared to a higher dose of 100mg/kg this is most likely as a result of this dose group being the most potent and effective analgesic form of this dose group of the petroleum ether extract of the plant bark. Another interesting observation that was deduced from the experiment was that in mouse 1 the duration of licks was 0 seconds this could be as result of the pain threshold for that particular animal being very high which we have seen from this experiment varies between animal to animal.

Furthermore, the graph plot was done with the graph prism Software 7.0.From the Figure 3.1 the graph shows a large error bar in the vehicle dose groups this is due to the high variability of the different values gotten of 273s, 18s, 96s meaning the results gotten could have been by chance and due to this there was a significant overlap in the error bar of the other dose groups since the result of the vehicle could have been as high as 273s or as low as 18s which was even lower than the duration of paw licks observed for some of the crude extract dose groups and aspirin, This high variability was a result of the this been the first dose groups that we performed so there could have been issues in dosing the animal or even the handling of the animal for them to properly open their mouths to insert the vehicle because of the limited number of animals of only 15 and so three per dose group the experiment could not be repeated to narrow the variability. Therefore, upon further analysis of the graph although there was a significant reduction in the duration of licks of paw of the various extract dose groups showing some form of analgesia as compared to the vehicle the results were statistically insignificant to be able to ascertain for certain if the petroleum ether extract has analgesic activity or not.

4.2 CONCLUSION

The conclusion drawn from the phytochemical and analgesia study on the crude petroleum ether extract of bark *of Piptadeniastrum africanum* shows that;

- Steroids, Flavonoids glycosides, may be present in the crude extract
- The absence of Alkaloids, tannins, saponins, flavonoids in the crude extract may be due to the use of the non- polar solvent petroleum ether for the extraction process

- ❖ There may be analgesic property present in the crude extract but the results of the experiment were statistically insignificant to determine for certain if there is analgesic property or not.
- ❖ Further test is needed with a higher number of experimental animals to be able to adequately ascertain the presence of analgesia in the crude extract.

4.3 RECOMMENDATIONS

- ❖ Further research should be carried out with a larger number of experimental animals to be able to ascertain to a higher level of certainty whether the crude petroleum ether extract has analgesic activity or not.

CONFLICT OF INTEREST

We have no conflict of interest to disclose.

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