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IN VIVO ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF METHANOL AND N-HEXANE EXTRACTS OF *Phoenix dactylifera* FRUITS

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ABSTRACT

Phoenix dactylifera fruits, commonly known as date fruits are consumed worldwide because of their nutritional and pharmacological values owing to their nutrients and phytoconstituents compositions. This study was undertaken to evaluate and compare anti-inflammatory and analgesic activities of methanol and n-hexane extracts of *P. dactylifera* fruits in Wistar rats. Egg albumin and carrageenan-induced inflammation models were used to evaluate the anti-inflammatory activities, while acetic acid and formalin-induced pain models were used to evaluate the analgesic activities in Wistar rats. Each model comprised of eight groups of three rats each. Groups administered with 300 mg/kg bw and 600 mg/kg bw of the n-hexane and methanol extracts, respectively exhibited maximum percentage inhibition. The maximum percentage inhibitions of methanol extract on egg albumin and carrageenan, acetic acid and formalin-induced pain at 600 mg/kg bw. (67.24% and 64.22%, 63.33% and 65.32%, respectively) were significantly higher ($p < 0.05$) than that of the n-hexane extract at 300 mg/kg bw. (56.90% and 52.94%, 50.00% and 52.56%, respectively). Conclusively, both extracts possess anti-inflammatory and analgesic activities, with methanol extract showing greater activity. These activities may be due to the presence of tannins, saponins, but most especially flavonoids in both extracts.

Keywords: *P. dactylifera* fruits, anti-inflammatory, analgesic, flavonoids, tannins, saponins.

Introduction

Inflammation is a vital part of the immune response to injury or infection elicited by xenobiotic often characterized by symptoms which include redness, pain, swelling, heat and fever (Cadirci *et al.,* 2016). Inflammatory reaction is initiated in order to fight, remove and repair damaged tissue after injury while pain develops in order to protect the affected tissues from further damage (John & Shobana, 2012). As reported by Hossain *et al*. (2011), inflammation and pain are often non-specific manifestations associated with numerous diseases. Different endogenous mediators which include histamine, serotonin, bradykinin, prostaglandins among others are most abundant in inflammatory cells, and among them, prostaglandins are ubiquitous mediators that indicate and modulate cell and tissue responses involved in pain and inflammation (Hossain *et al.,* 2011). Anti-inflammatory drugs elicit their therapeutic

effects by blocking the synthesis of these inflammation mediators. The most common used anti-inflammatory drugs used in modern medicines are cyclooxygenase (COX) inhibitors i.e. Non-steroidal Anti-inflammatory Drugs (NSAIDs) and opioids which are effective for the treatment of inflammation and pain (Wahyuni *et al.,* 2017). These drugs block COX-1 and COX-2 enzymes involved in prostaglandins biosynthesis (Wahyuni *et al.,* 2017). However, chronic use of these drugs is usually accompanied by adverse side effects which include gastrointestinal disorders, ulceration, bleeding and renal toxicity among others, owing significantly to the blockade of COX-1 (Paramita *et al.,* 2017). It has been shown that non-steroidal anti-inflammatory agents may even attenuate the healing process (Mansouri *et al.,* 2014).

Due to the adverse side effects associated with the synthetic drugs, natural remedies have been the focus of many researchers, attempting to find more potent anti-inflammatory and other pharmacological agents with little or no adverse side effects over the years (Chen *et al.,* 2015). Date palm fruit is an ancient plant used in folk medicine for the treatment and/or management of several health complications (El Hadrami & Al-Khayri, 2012). People of the East and world at large consume the fruit as principal foods and ingredients that form basis of their diet (Al-Juraisy *et al.,* 2010, Friedman *et al.,* 2010). It has been reported to have high nutritional value and health benefits (Barron *et al.,* 2007, Sadiq *et al.,* 2013), due to its high essential nutrients; vitamins and mineral contents which are required for the development, growth and overall well-being (Al-Orf *et al.,* 2012, Al-Harrasi *et al.,* 2014). Date palm fruit is a readily available fruit which is cost-friendly and serve as a natural cure to many diseases with little or no adverse side effects (Rahmani *et al.,* 2014).

Numerous literatures have reported that date palm possess neuro-protective (Pujari *et al.,* 2011), cerebro-protective (Ismail *et al.,* 2013), hepato-protective (Ragab *et al.,* 2013), anti-oxidant (Vayalil 2002) and anti-inflammatory (Mohamed & Al-Okbi, 2004) properties, and so much more. Although the anti-inflammatory and analgesic activities of aqueous extract date fruit have been evaluated, little or no work has been carried out on methanol and n-hexane extracts of this fruit. Therefore, this study aimed at evaluating the anti-inflammatory and analgesic activities of methanol and n-hexane extracts of date fruit in Wistar albino rats.

Experimental

Plant collection

Matured fresh fruits of *P. dactylifera* were purchased from Bosso market, Bosso LGA, Minna, Niger state, Nigeria and identified by a botanist in the Department of Plant Biology, Federal University of Technology, Minna, Niger State, Nigeria.

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade, products of Sigma Aldrich. The chemicals used include sulphuric acid, formalin, sodium carbonate and acetic acid among others. The reagents used were carrageenan, egg albumin, Folin-Denis reagent, normal saline among others.

Experimental animals

A total of fifty (50) healthy male Wistar rats (130 - 160 g) were used in this study. They were obtained from Federal University of Technology, Minna animal house. The animals were housed in standard cages and given access to standard pelleted feed (Vital feed) and water *ad libitum* prior to the commencement of the experiment.

Sample preparation and extraction

The purchased *P. dactylifera* fruits were rinsed with distilled water and air-dried at room temperature (35 °C) for a period of fourteen (14) days. Electric blender (EUROSONIC, ES-242) was then used to powder the dried fruits after removing the seeds. One hundred grams (100 g) of the powdered fruits was separately extracted with n-hexane while another one hundred grams (100 g) was extracted with methanol using Soxhlet extractor. The extracts were filtered using Whatman No 1 filter paper and extracts were collected in separate clean beakers and concentrated on water bath at 70 °C. The concentrated extracts were kept in refrigerator at 4^0C until the commencement of the experiment.

Phytochemicals screening

Total phenol determination

Singleton et al (1999) method was used to determine total phenol content of extracts. Briefly, 0.01 g of each extract was dissolved in 10 mL of distilled water, and 0.5 mL was oxidized by adding 2.5 mL of 10% Folin-Ciocalteu's reagent which was then neutralized by 2 mL of 7.5% sodium carbonate. The reaction mixture was incubated at 45 °C for 40 minutes. Absorbance was read at 765 nm using double beam Shimadzu UV spectrophotometer, UV-1800. Gallic acid was used to prepare the calibration curve.

Flavonoids determination

Flavonoids content of the extracts was determined using the method of Chang et al (2002). In this method, 0.01 g of each extract was weighed and dissolved in 10 mL of distilled water. Then 0.5 mL of each extract was added to a test tube containing 1.5 mL of absolute methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M sodium acetate and 2.8 mL of distilled water and incubated at ambient temperature for 30 minutes. The absorbance was read at 415 nm with double beam shimadzu UV-spectrophotometer, UV-1800. Quercetin was used to prepare the calibration curve.

Alkaloids determination

Alkaloids content of the extracts was determined using method of Oloyed (2005). Briefly, 0.5 g of each of the extract was weighed and dissolved in 5 mL of mixture of 96% ethanol:20% H_2SO_4 (1:1) and then filtered using Whatman No. 1 filter paper. 1 mL of each filtrate was then added to a test tube containing 5 mL of 60% H_2SO_4 and allowed to stand for 5 minutes. Thereafter, 5 mL of 0.5% formalin was added and allowed to stand at room temperature for 3 hours. The absorbance was read at wavelength of 565 nm. Vincristine extinction coefficient (E_{296}, ethanol {ETOH}= 15136 $M^{-1}cm^{-1}$) was used as reference alkaloid.

Determination of tannins

Tannin content of the extracts was determined using the method of Sofowora (1984). Briefly, 0.2 g of each of the extract was weighed into a 50 mL beaker and 20 mL of 50% methanol was added to it and covered with para film and heated in water bath at 80 °C for a period of 1 hour. The reaction mixture was shaken thoroughly to ensure uniformity. Each extract was then filtered into separate 100 mL volumetric flasks, and 20 mL of distilled water, 2.5 mL of Folin-Denis' reagent, and 10 mL of sodium carbonate were added and mixed properly. The reaction mixtures were then allowed to stand for 20 minutes at room temperature for the development of bluish-green coloration. The absorbance was recorded at 760 nm using double beam shimadzu UV-spectrophotometer, UV-1800. Standard tannic acid was used to prepare the calibration curve.

Determination of saponins

Saponins content of the extracts was determined using the method of Oloyed (2005). In this method, 0.5 g of each of the extract was weighed and dissolved in 20 mL of 1 N HCl and boiled in water bath at 80 °C for 4 hours. The reaction mixtures were cooled and filtered. 50 mL of petroleum ether was added and the ether layer was collected and evaporated to dryness. Thereafter, 5 mL of acetone-ethanol (1:1), 6 mL of ferrous sulphate and 2 mL of concentrated sulphuric acid were added and allowed to stand for 10 minutes. The absorbance was taken at 490 nm. Standard saponins was used to prepare the calibration curve.

Determination of Median Lethal Dose (LD_{50})

The LD_{50} of the both methanol and n-hexane extracts was determined according to method of Lorke (1983). This method consists of two distinct phases. In phase I, rats were grouped into three groups of three rats each for each of the extract and administered doses of 10, 100 and 1000 mg/kg bw. of each of the extract, respectively. In the absence of toxicological signs and more importantly mortality, phase II of this method was conducted. In phase II, rats were grouped into three groups of one rat each, for each of the extract and administered higher doses of 1900, 2900 and 5000 mg/kg bw. of each of the extract, respectively.

Evaluation of anti-inflammatory activities of the extracts

Egg albumin-induced paw oedema in rats

The anti-inflammatory activities of the extracts were evaluated according to the method of Sanchez-Maeto et al (2006). Briefly, 0.1 mL of 1% egg albumin prepared in normal saline was administered intraperitoneally into the right hind paw of the rats to bring about inflammation. The thickness of the injected paw was then measured at 0, 1, 2, 3, 4, 5 and 6 hours post egg albumin injection. However, rats in their respective groups were orally administered 150, 300 and 600 mg/kg bw. of the methanol and n-hexane extracts of *P. dactylifera* fruits, 100 mg/kg bw. diclofenac sodium (standard group) and 10 mL normal saline (control group), respectively one hour before the injection of egg albumin solution. Following the procedure described by Okokon and Nwafor (2010), the anti-inflammatory activities was assessed by measuring paw thickness at already-mentioned hours using Vernier calliper. Percentage inhibition of oedema of the treated rats was calculated using the formula:

$$\% \text{ Inhibition} = \frac{Mean\ paw\ thickness\ of\ control\ (Ct - Co) - Mean\ paw\ thickness\ of\ treated\ (Tt - To)}{Mean\ paw\ thickness\ of\ control\ (Ct - Co)} X100$$

Note:

Ct= paw thickness of control at the time of measurement

Co= paw thickness of control rats at 0 hour

Tt = paw thickness of treated rats at the time of measurement

To= paw thickness of treated rats at 0 hour

Carrageenan induced rat paw oedema

According to the procedure described by Winter *et al.* (1962), the anti-inflammatory activities of the extracts were evaluated. In summary, the rats were fasted overnight with free access to water before the commencement of the study. Intraperitoneal administration

50 µL of 1% carrageenan solution was used to induce inflammation in the right hind paw of the rats. However, one hour prior to the administration of carrageenan solution, rats were orally administered 150, 300 and 600 mg/kg bw. of the methanol and n-hexane extracts of *P. dactylifera* fruits, 100 mg/kg bw. diclofenac sodium and 10 mL/kg bw normal saline, respectively (Rahman et al. 2011). At 0, 1, 2, 3, 4, 5 and 6 hours, the paw thickness of the injected paw of each rat was measured using Vernier calliper (Okokon *et al.*, 2012). The percentage inhibition of oedema was calculated using the following formula (Balamurugan et al. 2012: % Inhibition =

$$\frac{Mean\ paw\ thickness\ of\ control(Ct - Co) - Mean\ paw\ thickness\ of\ treated\ (Tt - To)}{Mean\ paw\ thickness\ of\ control\ (Ct - Co)} X100$$

Note:
C_t = paw thickness of control at the time of measurement
C_o = paw thickness of control rats at 0 hour
T_t = paw thickness of treated rats at the time of measurement
T_o = paw thickness of treated rats at 0 hour

Evaluation of analgesic effect of the extracts
Acetic acid-induced writhing model
The analgesic activities of the methanol and n-hexane extracts of *P. dactylifera* fruits was assessed according to the method of Arul et al (2005) with slight modification. Briefly, Rats were distributed into eight groups of three rats each. Six groups were administered 150, 300 and 600 mg/kg bw. of the methanol and n-hexane extracts, respectively. The remaining two groups were designated positive and negative control groups, and were administered 100 mg/kg bw. diclofenac sodium and 10 mL/kg bw normal saline, respectively. The number of writhes for each rat was counted five minutes after acetic acid injection until 30 minutes. The rats were individually placed in transparent glass container and the abdominal muscles contractions together with stretching of hind limbs were cumulatively counted. The percentage inhibition of writhing was taken as an index of analgesic activities of the extracts (Le Bars *et al.,* 2001; Shreedhara *et al.,* 2009) and was calculated using the following formula:

$$\frac{Cw - Tw}{Cw} X\ 100$$

Where: C_w = mean of number writhing of control group,
T_n = mean of number of writhing of treated group

Formalin-induced hind paw licking model in rats
Analgesic activities of the extracts were also evaluated in Wistar rats using formalin-induced pain model as described by Nwafor *et al.* (2007) with slight modification. Briefly, rats were distributed into eight groups of three rats each. Six groups were orally administered doses of 150, 300 and 600 mg/kg bw. of the methanol and n-hexane extracts of *P. dactylifera* fruits, respectively. The positive control group was given 100 mg/kg bw. diclofenac sodium, while the negative control group was given 10 mL/kg bw normal saline. One hour after the administration of the extracts, diclofenac sodium and normal saline, 20 µL of formalin solution (2.5%) was injected into the right hind paw of the rats. The number of times the rats licked the injected paw was recorded and considered sign of pain. The first nociceptive response usually picks five minutes after the injection of formalin solution and the second phase occurs 15-30 minutes representing the

inflammatory pain response (Padmanabhan & Jangle, 2012). The percentage inhibition was calculated using the following formula:

$$\frac{Cn - Tn}{Cn} X\ 100$$

Where: C_n = mean of number paw licking of control group
T_n = mean of number of paw licking of treated group

Statistical Analysis

The data were analysed by One-way Analysis of Variance (ANOVA) using Statistical Package for Social Science (SPSS). The results were expressed as mean ± SD (Standard deviation). The difference in means of various groups of animals were compared using "Duncan multiple Range Test". *P*-value less than 0.05 was considered significant ($p < 0.05$).

Results

Phytochemical composition of the extracts

Table 1 shows the phytochemical composition of the methanol and n-hexane extracts of date fruit. Phenols, flavonoids, tannins, saponins and alkaloids were found to be present in both extracts. However, concentrations (mg/g) of phenols, tannins and saponins were observed to be higher in n-hexane extract in descending order (297.89.92>83.20>56.36) when compared to the methanol extract (178.92>66.57>42.78), while the concentrations (mg/g) of flavonoids and alkaloids were observed to be higher in methanol extract in descending order (80.46>28.65) when compared to the n-hexane extract (30.71>18.76).

TABLE 1

Phytochemical composition of Methanol and n-Hexane Extracts of P. dactylifera fruit

	Phytochemicals (mg/g)				
		Flavonoids	**Tannins**	**Saponins**	**Alkaloids**
Methanol extract	178.92±0.23	80.46±0.76	66.57±0.87	42.78±0.62	28.65±0.32
Hexane extract	297.89±0.38	30.71±0.85	83.20±0.69	56.36±0.23	18.76±0.22

Values are mean ± standard deviation of three replicates

TABLE 2

Acute Oral Toxicological Test of methanol and n-hexane extracts of P. dactylifera fruits

	n-Hexane extract			**Methanol extract**		
Phase I						
No of rats used	3	3	3	3	3	3
Dosage (mg/kg bw.)	10	100	1000	10	100	1000
Mortality	0/3	0/3	0/3	0/3	0/3	0/3
Sign of toxicity	None	None	None	None	None	None

Phase II

No of rats used	3	3	3	3	3	3
Dosage (mg/kg BW)	1900	2900	5000	1900	2900	5000
Mortality	0/3	0/3	0/3	0/3	0/3	0/3
Sign of toxicity	None	None	None	None	None	None

Acute oral toxicity test of the extracts

No mortality or any toxicological sign was observed during the acute oral toxicity testing. As a result, the LD_{50} of both methanol and n-hexane extract was found to be greater than 5000mg/kg bw (LD_{50}> 5000 mg/kg bw.) as depicted in Table 2.

Evaluation of anti-inflammatory activities of the extracts

Effect of the methanol and n-hexane extracts of P. dactylifera fruits on egg albumin-induced paw oedema in Wistar rats

Fig. 1. shows the effect of the methanol and n-hexane extracts of *P. dactylifera* fruits on egg albumin-induced paw oedema, using percentage inhibition as a measure. Significant reduction ($p < 0.05$) in paw oedema size of experimental rats which corresponds to high percentage inhibition of inflammation, was observed at all tested doses for both extracts. The maximum percentage inhibition observed at dose of 600 mg/kg bw. of the methanol extract (67.24% at 3rd hour), though, this is not significantly different ($p > 0.05$) from the one obtained for 300 mg/kg bw. (65.52% at 3rd hour) of the same extract, is significantly higher than that of the n-hexane extract (56.90% at 3rd hour) when compared to diclofenac sodium (74.14% at 3rd hour).

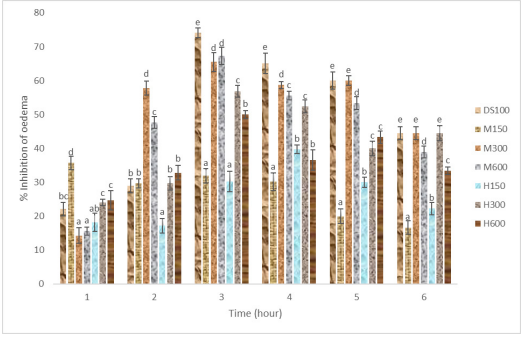

Fig. 1. Percentage inhibition of methanol and n-hexane extracts of *P. dactylifera* fruits on egg albumin-induced paw oedema in Wister rats.

Values are mean ± standard deviation of three replicates Values with different alphabets at the top at the same hours are significantly different at $p < 0.05$

Key:
DS100= Diclofenac sodium 100 mg/kg bw.,
M150, M300 and M600= Methanol extract at doses of 150, 300 and 600 mg/kg bw.,
H150, H300 and H600= n-Hexane extract as doses of 150, 300 and 600 mg/kg bw.

Effect of methanol and n-hexane extracts of P. dactylifera fruits on carrageenan-induced paw oedema in Wistar rats

Significant reduction in size of paw oedema induced by injection of carrageenan in Wistar rats was observed at all the tested doses of both extracts. The maximum percentage inhibition

was observed at 3rd hour for the extracts and diclofenac sodium. Similar to the effect of the extracts on the egg albumin-induced paw oedema, the maximum percentage inhibition (64.22% at 3rd hour), implying maximum effectiveness of methanol extract observed at dose of 600 mg/kg bw. was significantly higher than that of the n-hexane extract (52.94% at 3rd hour) at dose of 300 mg/kg bw. when compared to diclofenac sodium (78.43% at 3rd hour) at dose of 100 mg/kg bw. The maximum percentage inhibition observed at dose of 600 mg/kg bw of methanol extract was not significantly different ($p < 0.05$) from the one obtained at dose of 300 mg/kg bw. (63.18% at 3rd hour) of the same extract.

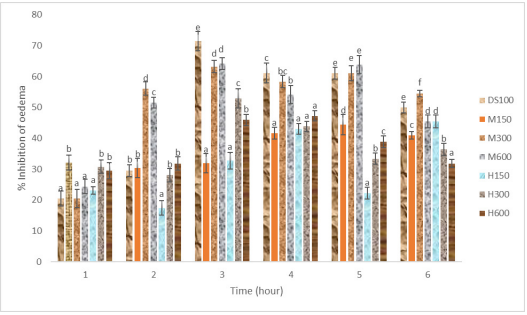

Fig. 2. Percentage inhibition of methanol and n-hexane extracts of *P. dactylifera* fruits on carrageenan-induced paw oedema.

Values are mean ± standard deviation of three replicates
Values with different alphabets at the top at the same hours are significantly different at $p < 0.05$

Key:
DS100= Diclofenac sodium 100 mg/kg bw.,
M150, M300 and M600= Methanol extract at doses of 150, 300 and 600 mg/kg bw.,
H150, H300 and H600= n-Hexane extract as doses of 150, 300 and 600 mg/kg bw.

Evaluation analgesic activities of the extracts
Effect of methanol and n-hexane extracts of P. dactylifera fruits on acetic acid-induced writhing in Wistar rats

The effects of both the methanol and n-hexane extracts on acetic acid-induced writhing in Wistar rats are shown in figure 3. Lower percentage inhibition on acetic acid-induced writhing in Wistar rats was observed for both the extracts treated groups as well as the diclofenac sodium treated group at all tested doses for the first five (5) minutes (first phase). However, higher percentage inhibition was observed for all the treated groups in the second phase. The maximum percentage inhibition was observed at dose of 300 mg/kg bw. at 20th minute for both extracts. No significant difference ($p > 0.05$) was observed in the maximum percentage inhibition of methanol extract at doses of 600 mg/kg bw. (63.33% at 20th minute) and 300 mg/kg bw. (61.28% at 20th minute). On the other hand, the maximum percentage inhibition of methanol extract at dose of 600 mg/kg bw was significantly higher ($p < 0.05$) than the maximum percentage inhibition (50% at 20th minute) obtained for n-hexane extract at dose of 300 mg/kg bw. when compared to diclofenac sodium (78.78% at 25th minute) at dose of 100 mg/kg bw.

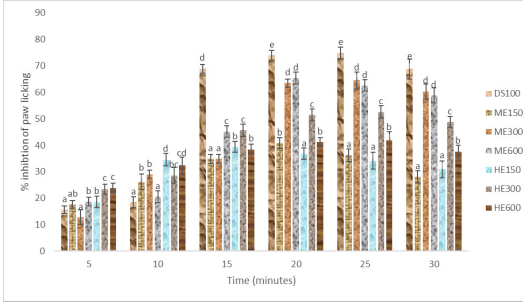

Fig. 3. Percentage inhibition of methanol and n-hexane extracts of *P. dactylifera* fruits on acetic acid-induced writhing in Wistar rats.

Values are mean ± standard deviation of three replicates
Values with different alphabets at the top at the same hours are significantly different at $p < 0.05$

Key:
DS100= Diclofenac sodium 100 mg/kg bw.,
M150, M300 and M600= Methanol extract at doses of 150, 300 and 600 mg/kg bw.,
H150, H300 and H600= n-Hexane extract as doses of 150, 300 and 600 mg/kg bw.

Effects of methanol and n-hexane extracts of P. dactylifera fruits on formalin-induced pain in Wistar rats

Fig. 4 depicts the effect of methanol and n-hexane extracts of *P. dactylifera* fruits measured by percentage inhibition on formalin-induced pain. Low percentage inhibition of both methanol and n-hexane extracts at all tested doses, as well as diclofenac sodium on formalin-induced pain was observed. However, in the second phase, higher percentage inhibition was observed for both methanol and n-hexane extracts with maximum percentage inhibition of methanol and n-hexane extracts being obtained at doses 600 mg/kg bw. and 300 mg/kg bw., respectively. The maximum percentage inhibition of methanol extract (65.32% at 20th minute) was significantly higher ($p < 0.05$) than that of n-hexane extract (52.56% at 25th minute) when compared to diclofenac sodium (74.89% at 25th minute).

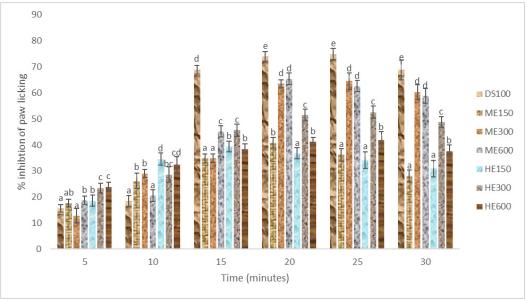

Fig. 4. Percentage inhibition of methanol and n-hexane extracts of *P. dactylifera* fruits on formalin-induced paw licking in Wistar rats.

Values are mean ± standard deviation of three replicates
Values with different alphabets at the top at the same hours are significantly different at $p < 0.05$

Key:
DS100= Diclofenac sodium 100 mg/kg bw.,
M150, M300 and M600= Methanol extract at doses of 150, 300 and 600 mg/kg bw.,
H150, H300 and H600= n-Hexane extract as doses of 150, 300 and 600 mg/kg bw.

Discussion

The use of herbal medicines has been in existence since time immemorial to maintain good health. Identification of bioactive compounds in medicinal plants (advances in Phytochemistry) is the renowned interest in herbal medicines. The bioactive compounds from plants play substantial roles in the discovery and development of drugs (Mercy & David, 2018). It has been estimated that about 25% of drugs are composed of compounds from plant origin (Ebong, 2015). On a global level, about 65-80% of developing countries inhabitants solely depend on herbal medicines to meet their primary health care needs due to enormous physiologic effects exhibited by medicinal plants which may include sedatives, analgesic, antipyretics, cardio-protective, anti-inflammatory, antioxidants, antispasmodics, and immunomodulatory effects among others, according to the World Health Organization (Johnson & Ayoola, 2015).

Owing to the involvement of inflammation in nearly all human and animal diseases, research on inflammation has set to be one of the interests of the world scientific findings. Drugs from plant origin have become focus of current scientific researches because they are used in the treatment of numerous ailments including inflammation, and exhibit greater therapeutic potential with little or no toxic side effects and relatively cheap than the synthetic drugs (Johnson & Ayoola, 2015).

The acute toxicological evaluation of drugs is essential for determining the safety level of such drugs. Therefore, the absence of mortality or toxic signs upon oral administration of dose of 5000 mg/kg bw of both extracts, implies that the LD_{50} of the two extracts is greater than 5000 mg/kg bw ($LD_{50} > 5000$ mg/kg bw) which rendered the extracts safe (OECD, 2000). This is justified by the use of the plant extract in the folklore medicine with no report of acute toxicological signs.

Evaluation of anti-inflammatory activities of substances is carried out via injection of substances capable of causing the release of inflammatory mediators which in turn causes inflammation. These substances include egg albumin and carrageenan among others (Zhao *et al.,* 2013). Egg albumin-induced inflammation is described as a biphasic event. Inflammatory mediators including histamine and serotonin are released and responsible for induction of inflammation immediately after the injection of egg albumin, and this lasts up to 2 hours, this phase is described as first phase (early phase). In the second phase (later phase), the release of bradykinin, protease, prostaglandins and lysosome is responsible for inflammation, and this can keep up to 5 hours post egg albumin injection (Jantzen *et al.,* 2002). Egg albumin-induced inflammation (oedema) occurs as a result of plasma eructation, upsurge tissue water and plasma protein exudation alongside eructation of neutrophils caused by these inflammatory mediators (Yankanchi & Koli, 2010). Hence, the higher percentage inhibition observed in the second phase suggested that the extracts though inhibit the release or action of histamine and serotonin (first phase), however, they showed greater inhibition on prostaglandins, bradykinins, protease and lysosome (second phase).

Another suitable test for evaluating acute anti-inflammatory activities of drugs is the carrageenan-induced paw oedema model (Zhao *et al.,* 2013). Carrageenan is used in the induction of inflammation due to its ability to cause the release of inflammatory mediators (Solanki *et al.,* 2015). Carrageenan-induced inflammation is manifested in three phases (Rock *et al.,* 2018). In the first phase (the first 90 minutes), histamine and serotonin are released, in the second phase (the first 90-150 minutes), kinins are released, while in the third phase (after 180 minutes), prostaglandins are released after the intraperitoneal administration of carrageenan (Rock *et al.,* 2018). Both steroidal and non-steroidal anti-inflammatory drugs show greater effect on the second phase than first phase since prostaglandins are the most suspected chemicals for inflammation (Patil *et al.,* 2012 and Kumari *et al.,* 2013). The observed greater percentage inhibition in the third phase (at 3rd hour) than in other phases for both extracts also suggests that the extracts showed greater effect on prostaglandins than other mediators as observed in egg albumin-induced inflammation. This implies that the extracts either exerted inhibitory effect on the cyclooxygenase enzyme which synthesizes prostaglandins or disrupt the prostaglandins' receptors thus inhibiting prostaglandins actions. The inhibitory effects exhibited by the extracts may be traceable to the presence of flavonoids, saponins and alkaloids in both extracts as these phytochemicals have been reported to possess anti-inflammatory activity (Afsar *et al.,* 2015 & Kumar *et al.,* 2015). Therefore, it will be rational to infer that the higher anti-inflammatory activity of methanol extract than n-hexane extract may be perhaps, as result of higher concentration of flavonoids and alkaloids found in the methanol extract. The anti-inflammatory results obtained for methanol extract correlates with the report of Mohamed & Al-Okbi (2004), Umar *et al.* (2015), who reported aqueous extract of *P. dactylifera* fruits to possess anti-inflammatory.

Acetic acid-induced writhing test has long been used as model for testing peripherally acting analgesics (Oh *et al.,* 2015). Administration of acetic acid induces pain by instigating increase in the levels of prostaglandins (PGE2 and PGEF2a) in peritoneal fluid, involving in part peritoneal receptors and inflammatory pain by causing capillary permeability (Patil *et al.,* 2012 and Alemu *et al.,* 2012). Therefore, the abilities of the extracts to significantly suppress or inhibit acetic acid-induced pain may be as a result of their action on prostaglandins synthesis/action. This further buttress the fact that the extracts strongly affect prostaglandins synthesis/action as inferred by the results obtained for the anti-inflammatory activities of the extracts.

Similar to acetic acid-induced writhing model, formalin-induced paw licking model is also used for evaluating the analgesic activities of drugs (Gong *et al.,* 2014). However, unlike the acetic acid-induced writhing model, formalin-induced paw licking model is used for testing both peripherally and centrally acting analgesics (Gong *et al.,* 2014). Injection of formalin causes the release of different pain mediators in two distinct phases. In the first phase, formalin directly stimulates nociceptors, resulting in pain induction (this occurs within 5 minutes after formalin injection), while in the second phase (which surfaces between 15 and 30 minutes), formalin causes the release of inflammatory mediators including prostaglandins (Hunskaar & Hole, 1997). Hence, lower and higher percentage inhibitions observed for both extracts in the first and second phases, respectively, suggests that though, the extracts inhibit the activation of nociceptors by formalin, however, both extracts exerted greater inhibitory effect on either prostaglandins synthesis or action. Therefore, it may be suggested that the analgesic effect of the extracts is predominantly via inhibition of inflammatory pain, which in turn implies cyclooxygenase inhibition. The inhibitory effects exhibited by both extracts may be as a result of presence of flavonoids, alkaloids and saponins in them (Afsar *et al.,* 2015 and Kumar *et al.,* 2015), while the higher analgesic activity of the methanol extract may be said to be due to higher concentrations of flavonoids and alkaloids in it. From the results obtained from both acetic acid and formalin-induced pain, it may be suggested that the extracts peripherally act on the nervous system, similar to the Non-Steroidal Anti-inflammatory Drugs (NSAIDs). The analgesic activity of methanol extract concurs with the study of Umar *et al.* (2015), who reported aqueous extract of date fruit to possess analgesic activity. The higher activity observed at 300 mg/kg bw. than 600 mg/kg bw. of the n-hexane extract may be as a result of antagonistic reaction between the bioactive substance(s) responsible for the activity and other constituent(s) of the extract at higher concentration of 600 mg/kg bw.

Conclusion

Both methanol and n-hexane extracts of *P. dactylifera* fruit possess anti-inflammatory and analgesic activities in vivo. However, the activity of methanol extract was significantly greater than that of the n-hexane extract, which may be as a result of higher flavonoids concentration in methanol extract.

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