*European Journal of Medicinal Plants*
*9(1): 1-11, 2015, Article no.EJMP.17093*
*ISSN: 2231-0894*

**SCIENCEDOMAIN** *international*
www.sciencedomain.org

# Bioactivities of *Pachypodium lamerei* Drake, Family Apocynaceae, Cultivated in Egypt

**Dina Faek El-Kashef[1], Ashraf Nageeb El-Sayed Hamed[1*], Hany Ezzat Khalil[1] and Mohamed Salah Kamel[1]**

*[1]Department of Pharmacognosy, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. All authors designed the study and wrote the protocol. Author DFEK wrote the first draft of the manuscript. Authors DFEK, ANESH and HEK managed the literature searches. Author DFE managed the analyses of the study. Authors MSK, ANESH and DFEK revised the written manuscript. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/EJMP/2015/17093
Editor(s):
(1) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Anonymous, Universidade Federal De Uberlândia, Brazil.
(2) Anonymous, Cumhuriyet University, Turkey.
(3) Simon Bernard Iloki Assanga, Lab. de Investigaciones en Bioactivos y Alimentos Funcionales (LIBAF), Sonora, México.
(4) Abdullahi M. Nuhu, College of Science and Technology, Kaduna Polytechnic, Kaduna, Nigeria.
(5) Anonymous, National Institute of Pathology, Romania.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1190&id=13&aid=9452

***Original Research Article***

***Received 25th February 2015***
***Accepted 16th April 2015***
***Published 27th May 2015***

## ABSTRACT

**Aims:** The current study estimated the anti-inflammatory, anti-pyretic, gastroprotective and anti-hyperglycemic activities in various rat models of total methanol extracts (TMEs) of different plant parts; mixture of leaves & stems (MLS) and subterranean organs (SO) and different fractions of TME of MLS of *Pachypodium lamerei* Drake. Additionally, we evaluated the median lethal dose ($LD_{50}$) of TME of MLS.
**Place and Duration of Study:** The study was carried out for nine months in 2012 in the Department of Pharmacology, Faculty of Medicine and the Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia, Egypt.
**Methodology:** The preliminary phytochemical screening of TMEs of both MLS and SO of *P. lamerei* was carried out to determine secondary metabolites in the extracts. The TMEs of both MLS and SO and different fractions of TME of MLS of *P. lamerei* were used in the current biological study. The pharmacological activities, such as anti-inflammatory, anti-pyretic, gastroprotective, anti-

**Corresponding author: E-mail: ashrafnag@mu.edu.eg, ashrafnag@yahoo.com;*

hyperglycemic effects and $LD_{50}$ were determined in male albino rats using various models.
**Results:** The phytochemical screening of TMEs of both MLS and SO of *P. lamerei* contained carbohydrates and/or glycosides, unsaturated sterols and/or triterpenes, flavonoids, saponins and tannins. While, the biological activities are summarized as follows; in the carrageenan-induced paw edema method, TME of MLS had the highest anti-inflammatory activity (****P<0.001*), whereas in the yeast-induced pyrexia method, the highest anti-pyretic effect was exerted by TME of SO (****P<0.001*). Furthermore, in indomethacin-induced gastric ulcer, TME of SO exerted the most potent gastric protection. Moreover, in the alloxan-induced diabetes method, the most active extract was again TME of SO (****P<0.001*). Finally, no toxicological symptoms of TME of MLS of *P. lamerei* were observed in graded single doses up to 5 gm/kg, during 24 hrs follow-up.
**Conclusion:** From the aforementioned results, *P. lamerei* proved to be a promising candidate to be used in the development of various pharmaceutical herbal drugs viz., anti-inflammatory, anti-pyretic, gastroprotective, anti-hyperglycemic activities, since it exhibited a wide margin of safety.

*Keywords: Pachypodium lamerei; Apocynaceae; leaves & stems; subterranean; anti-inflammatory; anti-pyretic; gastroprotective; anti-hyperglycemic; phytochemical screening.*

## 1. INTRODUCTION

Apocynaceae is considered as one of the largest family of Angiosperms, where it contains nearly 402 plant genera and 5031 species [1]. It is a flowering family that includes trees, shrubs, herbs, stem succulents and vines, commonly called the dogbane family [2]. Its plants showed various biological activities viz., *Funtumia elastic* showed significant anti-inflammatory activity [3]. While, *Hunteria umbellate* produced significant anti-edematogenic effect [4]. Also, *H. umbellate* produced anti-pyretic effects up to 60 min [5]. Furthermore, gastroprotective effect was shown by *Alstonia scholaris* [6]. While, *Catharanthus roseus* reduced the levels of glucose, protein, cholesterol, lipid peroxidation and liver enzymes [7]. One of the plants of this family is *Pachypodium lamerei* Drake. Reviewing the available literature on it, the unsaponifiable and saponifiable matters of *P. lamerei* were investigated by GC/MS [8] and two botanical studies showed the botanical features of leaves [9] and stems [10], but nothing could be traced about the biological studies on it. This provoked us to carry out several pharmacological studies viz., anti-inflammatory, anti-pyretic, gastroprotective, anti-hyperglycemic and $LD_{50}$ for this plant.

## 2. MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Plant material and preparation of extracts and fractions

The plant was cultivated in El-Orman Botanical Garden, Giza, Egypt. The MLS and SO were collected in October 2011. It was identified by Agr. Eng. Tereez Labib, consultant of plant taxonomy at the Ministry of Agriculture and ex. director of El-Orman Botanical Garden, Giza, Egypt. It was kept in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Minia University, Minia, Egypt with voucher specimen number (Mn-Ph-Cog-006).

The air-dried, finely powdered MLS (1.4 kg) and SO (280.5 gm) were each separately extracted by maceration with absolute methanol (MeOH) till exhaustion and then concentrated till dryness under reduced pressure by using rotary evaporator to yield 265.3 and 51.9 gm, respectively. The concentrated TME of MLS was suspended in the least amount of distilled water, transferred to a separating funnel and partitioned successively with petroleum ether (pet. ether) and chloroform ($CHCl_3$). The obtained fractions were concentrated under reduced pressure to afford two fractions; pet. ether (40.0 gm) and $CHCl_3$ (78.7 gm). The residual aqueous mother liquor was also concentrated by the same method to yield aqueous fraction (126.0 gm). It was suspended in the least amount of distilled water then subjected to diaion HP-20 open column chromatography then eluted successively with water, 50% MeOH and MeOH to give three corresponding fractions. The aqueous fraction (from diaion HP-20) was excluded and the other two fractions were concentrated by the same method to afford two fractions; 50% MeOH (3.7 gm) and MeOH (3.6 gm).

#### 2.1.2 Animals

The animals used in this study were male albino rats weighing 180-250 gm. The animals were housed under standardized environmental conditions, fed with standard diet and water *ad*

*libidum* and left to acclimatize to the environment for at least one week prior to inclusion in the experiments. The animals were handled only at the time of experiments and during cage cleaning. All conditions were ensured to minimize animal suffering.

## 2.2 Techniques

### 2.2.1 Phytochemical screening

Preliminary qualitative phytochemical screening of TMEs of both MLS and SO of *P. lamerei* was carried out on portions of the residual material, using standard phytochemical procedures [11-14]. The results are shown in (Table 1).

**Table 1. Results of preliminary qualitative phytoche*m*ical screening of TMEs of both MLS and SO of *P. lamerei* [(+) present and (-) absent]**

| Phytoconstituent | Result | |
|---|---|---|
| | MLS | SO |
| Carbohydrates and/or glycosides | + | + |
| Unsaturated sterols and/or triterpenes | + | + |
| Flavonoids | + | + |
| Saponins | + | + |
| Tannins | + | + |
| Crystalline sublimate substances | - | - |
| Alkaloids and/or nitrogenous compounds | - | - |
| Cardenolides | - | - |
| Anthraquinones | - | - |

### 2.2.2 $LD_{50}$ determination

The dried TME of MLS of *P. lamerei* was suspended in 0.5% carboxymethyl-cellulose (CMC) in distilled water and then administered orally to male albino rats in graded single doses up to 5 gm/kg [15]. Male albino rats (180-200 gm) were divided into two groups (four animals each). The control group received the same volumes of the vehicle. The rats were observed for any signs of toxicity and mortality for the first critical 4 hrs and observation was continued till the end of the 24 hrs survey [16]. Signs of toxicity to be observed include paw-licking, stretching, respiratory distress and diarrhea, or death [17].

### 2.2.3 Evaluation of anti-inflammatory activity

The different extracts and fractions of *P. lamerei* were tested for their anti-inflammatory activity using the carrageenan-induced paw edema method. Male albino rats (180-200 gm) were divided into eight groups (four animals each). The tested extracts and fractions were suspended in 0.5% CMC solution and administered orally to the rats at a dose of 100 mg/kg, one hr prior to the subcutaneous injection of carrageenan. The control group received an equal volume of the vehicle of the plant extract (0.5% CMC solution) per oral (p.o.), while the reference drug (indomethacin 8 mg/kg) was given orally to another group [18,19]. Paw thickness (mm) was measured by a vernier caliper [20] immediately (0 hr) and 1, 2, 3 and 4 hrs after administration of the tested TMEs, fractions and reference drug.

The results are presented in (Table 2). The groups were specified as follows:

**Group 1:** Control.
**Group 2:** TME of SO.
**Group 3:** TME of MLS.
**Group 4:** Pet. ether fraction of TME of MLS.
**Group 5:** $CHCl_3$ fraction of TME of MLS.
**Group 6:** MeOH fraction of TME of MLS.
**Group 7:** 50% MeOH fraction of TME of MLS.
**Group 8:** Indomethacin.

### 2.2.4 Evaluation of anti-pyretic activity

The different extracts and fractions of *P. lamerei* were evaluated for their anti-pyretic activity using the yeast-induced pyrexia method [19,21,22]. Male albino rats (220-250 gm) were divided into seven groups (four animals each). They were subcutaneously injected in the back, below the nape of the neck with 20% aqueous suspension of yeast (10 ml/kg). Rectal temperature of each animal was measured before and after 19 hrs of the yeast injection by inserting a digital thermometer (China) into the rectum to a depth of 2.0 cm. After 19 hrs, only the animals that showed an increase 0.5 °C were selected for the experiment [19,21,22]. The tested extracts and fractions were administered orally to the rats at a dose of 100 mg/kg (suspended in 0.5% CMC solution). The control group was given the vehicle only (0.5% CMC solution), while the reference group was given acetylsalicylic acid (100 mg/kg p.o.). The rectal temperature of the animals was registered at a regular interval of 30 min for 3 hrs following the administration of the tested drugs [19,21,22]. The results are summarized in (Table 3).

The groups were specified as follows:

**Group 1:** Control.
**Group 2:** TME of SO.
**Group 3:** TME of MLS.

**Group 4:** Pet. ether fraction of TME of MLS.
**Group 5:** $CHCl_3$ fraction of TME of MLS.
**Group 6:** Aqueous fraction of TME of MLS.
**Group 7:** Acetylsalicylic acid.

### 2.2.5 Evaluation of gastroprotective activity

For the determination of the gastroprotective activity of different extracts and fractions of *P. lamerei*, the indomethacin-induced gastric ulceration model was used [23]. Male albino rats (200-220 g) were divided into eight groups (four animals each). They were fasted for 24 hrs before the experiment in mesh-bottomed cages to minimize coprophagia, but the animals had free access to water except for the last hour before the experiment [24]. The tested extracts and fractions were suspended in CMC (0.5% solution in distilled water) and administered to the animals at a dose of 100 mg/kg. The control group was given the vehicle of the extracts and fractions (0.5% CMC in distilled water). The reference drug (ranitidine) was administered at a dose of 50 mg/kg [25]. All these treatments were given orally one hr before the induction of gastric ulceration [25] by a large oral dose of indomethacin (40 mg/kg) [26].

The groups were specified as follows:

**Group 1:** Control.
**Group 2:** TME of SO.
**Group 3:** TME of MLS.
**Group 4:** Pet. ether fraction of TME of MLS.
**Group 5:** $CHCl_3$ fraction of TME of MLS.
**Group 6:** MeOH fraction of TME of MLS.
**Group 7:** 50% MeOH fraction of TME of MLS.
**Group 8:** Ranitidine.

After 4 hrs of induction of gastric ulceration, the rats were sacrificed by cervical dislocation. The stomachs were removed and opened along the greater curvature, then rinsed with water to remove the gastric contents and blood clots to allow macroscopical examination of the mucosal lesions. They were quantified in terms of ulcer index (U.I.), calculated using a 0-3 scoring system based on the severity of these lesions [24]. The (U.I.) for each group was calculated as the mean ulcer score of all the rats in that group and the preventive index (P.I.) of a given extract or fraction was calculated using the following equation [27]:

$$P.I. = \frac{(U.I.\ of\ ulcerated\ group) - (U.I.\ of\ treated\ group)}{(U.I.\ of\ ulcerated\ group)} \times 100$$

These results are recorded in (Table 4). After recording the ulcers, the stomachs were fixed in 10% formalin solution and after 24 hrs of fixation, the stomachs were embedded in paraffin. The paraffin blocks were cut into sections and stained with hematoxylin-eosin dye for histopathological assessment of the gastric mucosa. The results are illustrated in (Fig. 1).

### 2.2.6 Evaluation of anti-hyperglycemic activity

The anti-hyperglycemic activity of different extracts and fractions of *P. lamerei* were tested on alloxan-induced diabetic rats. The male albino rats assigned to the diabetic group (200-220 gm) were divided into seven groups (four animals each). They were injected intraperitoneally with a freshly prepared alloxan monohydrate solution in sterile normal saline, at a dose of 150 mg/kg [28]. Hyperglycemia was assessed 3 days after alloxan injection by measuring blood glucose level (BGL) [29]. Rats with a fasting BGL higher than 130 mg/dl were considered diabetic [30,31].

All the tested extracts and fractions were given intraperitoneally to the diabetic rats at a dose of 100 mg/kg (in 0.5% CMC solution) [32]. The control group received the vehicle of the plant extracts and fractions (0.5% CMC solution in distilled water p.o.), while the reference drug (glibenclamide, 0.5 mg/kg p.o.) was given to another group [33,34]. BGLs were measured from the tail tip of the rats at time zero (fasting) and at interval of one hr for 4 hrs [33,35], using the AccuCheck® Active Roche analyzer (Model GC, Mannheim, Germany). The percentage of change in BGL from the fasting levels was calculated [36]. The results of the anti-hyperglycemic activity are listed in (Table 5) and illustrated in (Fig. 2).

The groups were specified as follows:

**Group 1:** Control.
**Group 2:** TME of SO.
**Group 3:** TME of TME of MLS.
**Group 4:** Pet. ether fraction of TME of MLS.
**Group 5:** $CHCl_3$ fraction of TME of MLS.
**Group 6:** Aqueous fraction of TME of MLS.
**Group 7:** Glibenclamide.

### 2.2.7 Statistical analysis

GraphPad Prism5 (Graphpad Software, San Diego California, U.S.A.) was used for the statistical analysis of the obtained results. The results were expressed in terms of means ±

S.E.M. (standard error of the mean). Statistically significant difference was determined using one-way analysis of variance (ANOVA), followed by the Dunnett's test. Probability values (*P*) less than 0.05, 0.01 and 0.001 indicated statistical significance (**P<0.05, **P<0.01, *** P<0.001*).

## 3. RESULTS

### 3.1 Phytochemical Screening

From (Table 1), it can be concluded that, TMEs of both MLS and SO of *P. lamerei* contained carbohydrates and/or glycosides, unsaturated sterols and/or triterpenes, flavonoids, saponins and tannins. On the other hand, they are free from crystalline sublimate substances, alkaloids and/or nitrogenous compounds, cardenolides and anthraquinones.

### 3.2 $LD_{50}$

Results of $LD_{50}$ determination revealed that investigated TME of MLS of *P. lamerei* did not induce toxic symptoms such as paw-licking, stretching, respiratory distress, diarrhea or death [17] in graded single doses up to 5 gm/kg during the first 24 hrs after administration.

**Table 2. Results of anti-inflammatory activity of TMEs and fractions of *P. lamerei* using the carrageenan-induced paw edema method**

| Group | Thickness of the paw (mm) after | | | | |
|---|---|---|---|---|---|
| | 0 hr | 1 hr | 2 hrs | 3 hrs | 4 hrs |
| Control | 5.6±0.12 | 5.9±0.17 | 5.0±0.23 | 4.9±0.10 | 4.9±0.12 |
| TME of SO | 4.9±0.13 | 4.6±0.21** | 4.1±0.11** | 4.0±0.10** | 3.9±0.11*** |
| TME of MLS | 5.0±0.35 | 4.2±0.26*** | 4.3±0.06* | 3.8±0.15*** | 3.5±0.09*** |
| Pet. ether fraction | 5.1±0.17 | 4.6±0.11** | 4.3±0.12** | 4.2±0.10 | 3.9±0.08*** |
| $CHCl_3$ fraction | 5.0±0.08 | 4.3±0.15*** | 4.3±0.08* | 4.1±0.02* | 4.0±0.90*** |
| MeOH fraction | 4.7±0.25 | 4.4±0.34*** | 4.4±0.22* | 4.2±0.27* | 4.0±0.19*** |
| 50% MeOH fraction | 5.2±0.08 | 4.4±0.15*** | 4.7±0.03 | 4.2±0.21* | 4.1±0.15** |
| Indomethacin | 5.2±0.17 | 4.3±0.10*** | 4.5±0.16 | 4.0±0.11** | 3.9±0.14*** |

*(*P<0.05, **P<0.01, ***P<0.001) compared to the control group. Data are presented as mean ± S.E.M. in each group. (n=4) rats in each group*

**Table 3. Results of anti-pyretic activity of TMEs and fractions of *P. lamerei* using the yeast-induced pyrexia method**

| Group | Rectal temperature ($^oC$) after | | | | | | |
|---|---|---|---|---|---|---|---|
| | Zero min | 30 min | 60 min | 90 min | 120 min | 150 min | 180 min |
| Control | 38±0.50 | 37±0.80 | 37±0.35 | 38±0.30 | 37±1.00 | 38±0.25 | 38±0.10 |
| TME of SO | 38±0.26 | 37±0.26 | 36±0.52 | 36±0.15** | 35±0.19* | 36±0.26*** | 35±0.39*** |
| TME of MLS | 38±0.38 | 37±0.44 | 37±0.29 | 36±0.32** | 36±0.29 | 36±0.18** | 36±0.15** |
| Pet. ether fraction | 39±0.67 | 38±0.36 | 38±0.77 | 36±0.44 | 36±0.34* | 36±0.31*** | 36±0.32** |
| $CHCl_3$ fraction | 39±0.56 | 38±0.65 | 37±0.68 | 35±0.23** | 35±0.07* | 36±0.47** | 36±0.06*** |
| Aqueous fraction | 39±0.59 | 37±0.90 | 36±0.20 | 36±0.44** | 36±0.09 | 36±0.20*** | 36±0.26** |
| Acetylsalicylic acid | 38±0.49 | 37±0.38 | 38±0.28 | 35±0.23** | 35±0.19* | 35±0.26*** | 35±0.32*** |

*(*P<0.05, **P<0.01, ***P<0.001) compared to the control group. Data are presented as mean ± S.E.M. in each group. (n=4) rats in each group*

**Table 4. Results of gastroprotective activity of TMEs and fractions of *P. lamerei* using indomethacin-induced gastric ulcer method**

| Group | Ulcer index (U.I.) | Preventive index % (P.I.) |
|---|---|---|
| Control group | 40.0±0.65 | -------- |
| TME of SO | 3.3±0.25*** | 91.75 |
| TME of MLS | 16.0±3.20*** | 60.00 |
| Pet. ether fraction | 27.0±3.10** | 32.50 |
| $CHCl_3$ fraction | 19.0±0.33*** | 52.50 |
| MeOH fraction | 22.0±1.20*** | 45.00 |
| 50% MeOH fraction | 31.0±5.00 | 22.50 |
| Ranitidine | 3.0±1.10*** | 92.50 |

*(*P<0.05, **P<0.01, ***P<0.001) compared to the control group. Data are presented as mean ± S.E.M. in each group. (n=4) rats in each group*

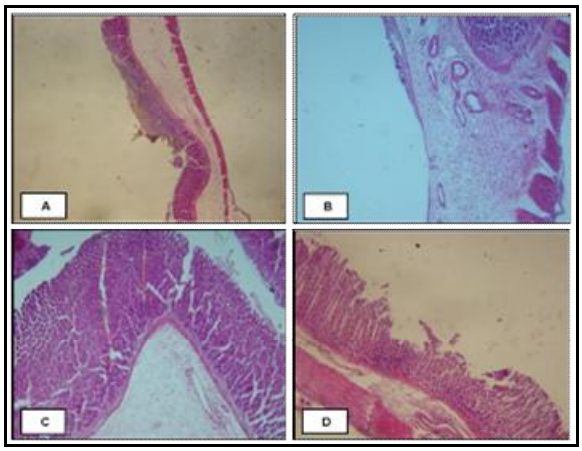


**Fig. 1. Histopathological results; A-Group 1 (Control), B- and C-Group 2 (TME of SO) and D-Group 8 (Ranitidine)**

**Table 5. Results of anti-hyperglycemic activity and percentage of change in (BGL) compared to the fasting levels of the different extracts and fractions of *P. lamerei* using the alloxan-induced diabetes method**

| Group | Blood glucose level (BGL) (mg/dl) | | | | |
|---|---|---|---|---|---|
| | 0 hr (Fasting) | 1 hr | 2 hrs | 3 hrs | 4 hrs |
| Control | 142.8±3.77 | 142.3±3.92 | 143.0±4.38 | 143.0±4.38 | 142.0±4.42 |
| % | 100.00 | 99.64 | 100.10 | 100.10 | 99.40 |
| TME of SO | 138.0±1.20 | 154.0±2.30 | 139.0±0.33 | 139.0±0.33 | 91.0±0.58*** |
| % | 100.00 | 111.59 | 100.72 | 100.72 | 65.94 |
| TME of MLS | 143.0±1.20 | 149.0±1.20 | 136.0±1.70* | 136.0±1.70* | 122.0±1.20*** |
| % | 100.00 | 104.19 | 95.18 | 95.10 | 85.31 |
| Pet. ether fraction | 157.0±0.00 | 169.0±6.35 | 225.3±29.16 | 191.3±21.07 | 175.0±13.28 |
| % | 100.00 | 107.64 | 143.50 | 121.85 | 111.46 |
| $CHCl_3$ fraction | 157.0±0.00 | 201.0±13.28 | 204.3±33.78 | 150.0±23.67 | 141.0±0.58 |
| % | 100.00 | 128.03 | 130.13 | 95.54 | 89.81 |
| Aqueous fraction | 158.0±3.00 | 178.0±15.00 | 212.0±8.10 | 154.0±16.00 | 157.0±10.00 |
| % | 100.00 | 112.65 | 134.18 | 97.47 | 99.37 |
| Glibenclamide | 172.7±1.45 | 166.0±15.01 | 118.7±15.30 | 117.7±16.46* | 101.7±12.41* |
| % | 100.00 | 96.12 | 68.73 | 68.15 | 58.89 |

*(*P<0.05, **P<0.01, ***P<0.001) compared to 0 hr (fasting). Data are presented as mean ± S.E.M. in each group. (n=4) rats in each group*

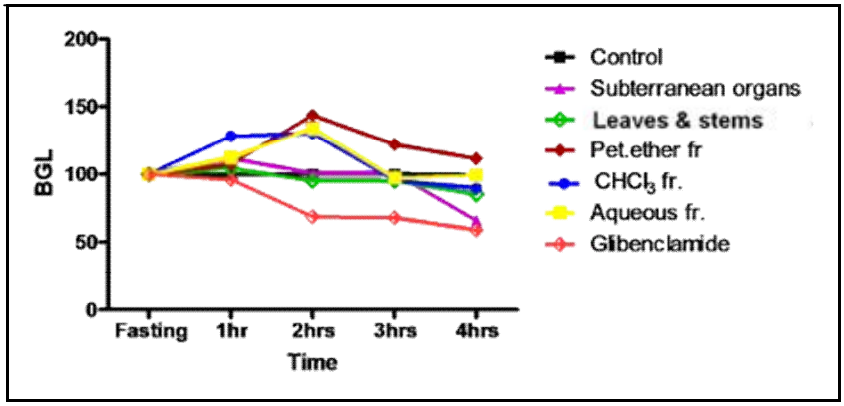


**Fig. 2. Effect of TMEs and fractions of *P. lamerei* on the percentage of the difference in BGL compared to the fasting level**

### 3.3 Anti-inflammatory Activity

The different extracts and fractions of *P. lamerei* were tested for their anti-inflammatory activity using the carrageenan-induced paw edema method [18,19]. The tested extracts and fractions of *P. lamerei* showed significant anti-inflammatory activity as shown in (Table 2), from the 1st hr after administration till the end of the experiment (4 hrs). The TME of MLS, as well as $CHCl_3$, 50% MeOH and MeOH fractions exhibited significant reduction in the paw swelling after one hr (****P<0.001*). After 2 hrs, TME of SO and pet. ether fraction were the most active and showed higher reduction in the paw edema than the reference drug (indomethacin). After 3 and 4 hrs, TME of MLS showed the highest activity, followed by TME of SO, which was comparable to indomethacin. The TMEs and fractions of *P. lamerei*, except 50% MeOH fraction, were found to reduce significantly the paw edema after 4 hrs (****P<0.001*).

### 3.4 Anti-pyretic Activity

The different extracts and fractions of *P. lamerei* were evaluated for their anti-pyretic activity using the yeast-induced pyrexia method [19,21,22]. The tested extracts and fractions exhibited anti-pyretic activity. The activity was observed from the 90 min after administration of the tested extracts and fractions till the end of the experiment. After 120 min, only TME of SO, pet. ether and $CHCl_3$ fractions reduced the rectal temperature, as compared to the reference drug (acetylsalicylic acid). All the extracts and fractions showed high activities after 150 and 180 min, which were comparable to the activity of acetylsalicylic acid. After 180 min, TME of SO showed the highest effect, which was comparable to the reference drug. The results are given in (Table 3).

### 3.5 Gastroprotective Activity

For the determination of the gastroprotective activity of different extracts and fractions of *P. lamerei*, the indomethacin-induced gastric ulceration model was applied [23]. The TMEs of both MLS and SO together with pet. ether, $CHCl_3$ and MeOH fractions displayed significant gastroprotective activity. The highest preventive index (91.75%) was obtained by TME of SO, which was comparable to the protection displayed by the reference drug ranitidine (92.5%). The results are presented in (Table 4).

The paraffin blocks were cut into sections and stained with hematoxylin-eosin dye for histopathological assessment of the gastric mucosa. The results are illustrated in (Fig. 1) as follows:

**Group 1:** Control group.

The picture (Fig. 1A) showed complete ulceration of the mucosa with haemorrhage and congestion of blood vessels. Inflammatory cells infiltrated the mucosa and sub mucosa.

**Group 2:** TME of SO group.

The pictures (Figs. 1B and 1C) illustrated minimal submucosal lymphocytic infiltration that indicated a certain degree of gastroprotection exerted by the extract (Figs. 1B and 1C).

**Group 3:** Ranitidine group.

The picture (Fig. 1D) demonstrated partial shedding of the mucosa and there is also inflammatory cell infiltration.

### 3.6 Anti-hyperglycemic Activity

The anti-hyperglycemic activity of different extracts and fractions of *P. lamerei* were tested on alloxan-induced diabetic rats [28-31]. The effects of TMEs and fractions on BGL revealed that TMEs of both MLS and SO showed significant anti-hyperglycemic activities. These extracts induced an initial increase followed by a significant reduction in BGLs after 4 hrs (****P<0.001*). The reduction in BGLs was comparable to the reference drug (glibenclamide). The most active one was TME of SO after 4 hrs. The results are summarized and illustrated in (Table 5 and Fig. 2).

## 4. DISCUSSION

### 4.1 Phytochemical Screening

From (Table 1), TMEs of both MLS and SO of *P. lamerei* contained many secondary metabolites that may play important roles in the bioactivities, such as carbohydrates and/or glycosides, unsaturated sterols and/or triterpenes, flavonoids, saponins and tannins.

### 4.2 $LD_{50}$

Results of $LD_{50}$ determination revealed that the investigated TME of MLS of *P. lamerei* did not induce toxic symptoms or death in graded single

doses up to 5 gm/kg (fifty times of the therapeutic dose 100 mg/kg) during the first 24 hrs after the administration of the extract, indicating a wide margin of safety of this extract.

### 4.3 Anti-inflammatory Activity

The effect of TME of MLS of *P. lamerei* was higher than the individual fractions, which indicated the synergistic effect of the fractions in TME. The anti-inflammatory activity may be attributed to the presence of sterols, triterpenes and glycosides from the phytochemical screening [37,38]. The anti-inflammatory activity of pet. ether fraction may be related to the presence of sterols, which were found to possess potent anti-inflammatory activity in the carrageenan-induced model [39,40].

### 4.4 Anti-pyretic Activity

The positive anti-pyretic results were expected since most anti-inflammatory drugs possess anti-pyretic activity mediated by the inhibition of prostaglandin synthetase within the hypothalamus, as well as COX-2 inhibitory mechanisms [40,41]. The anti-pyretic activity may be attributed to the high content of sterols, triterpenes and tannins from the phytochemical screening [42]. The TME of MLS and pet. ether fraction are rich in sterols, which possess potent anti-inflammatory and anti-pyretic activity [43].

### 4.5 Gastroprotective Activity

The high gastroprotective results of the extracts and fractions may be attributed to the presence of sterols, triterpenes and tannins from the phytochemical screening [24,44]. Sterols are the major component of the pet. ether fraction, TMEs of both MLS and SO. They are reported to confer potent gastric protection [45,46]. The phytochemical screening revealed the presence of tannins, which may contribute to the gastroprotective activity [47]. Many studies have confirmed the gastroprotective effect of tannins by reducing the lesion area, while promoting a larger regenerative mucosa [47,48].

### 4.6 Anti-hyperglycemic Activity

The significant reduction in BGL of TMEs of both MLS and SO may be attributed to the presence of certain phytoconstituents as revealed from the phytochemical screening of TMEs of both MLS and SO, such as sterols, triterpenes and tannins [49-52]. Tannins and sterols have been proven to have the ability to regenerate the pancreatic β-cells [49], while triterpenes act as insulin sensitizers but not releasers [53].

## 5. CONCLUSION

The TMEs of both MLS and SO of *P. lamerei* Drake, (family Apocynaceae, cultivated in Egypt) showed many biological activities viz., anti-inflammatory, anti-pyretic, gastroprotective and anti-hyperglycemic activities. This may lead to the discovery of new natural drugs, with fewer side effects than the traditional ones, since it displayed a wide safety margin.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

Experiments were conducted in accordance with the international ethical guidelines for animal care of the United States Naval Medical Research Centre, Unit No. 3, Abbaseya, Cairo, Egypt, accredited by the Association for Assessment and Accreditation of Laboratory Animal Care international (AAALAC international). The adopted guidelines are in accordance with "Principles of Laboratory Animals Care" (NIH publication No. 85-23, revised 1985). The study protocol was approved by members of "The Research Ethics Committee" and by the Pharmacology Department, Faculty of Pharmacy, Minia University, Minia, Egypt 2012.

## ACKNOWLEDGEMENTS

We are appreciative to all staff members of Pharmacology Department, Faculty of Medicine, Minia University, Minia, Egypt for their contribution in the biological studies. We would also like to thank Prof. Dr. / Heba Mohamed Tawfik, Department of Pathology, Faculty of Medicine, Minia University, Minia, Egypt for her contribution in the histopathological study. Finally, we are deeply grateful to Assistant lecturer/ Al-Shaimaa Faissal, Ph.D. student, Department of Pharmacology, Minia University, Minia, Egypt for her support in the statistical analysis.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Available:http://www.theplantlist.org/browse/A/Apocynaceae/ (Retrieved 15th Jan., 2015)
2. Endress ME, Bruyns PV. A revised classification of the Apocynaceae s.l. Bot Rev. 2000;66(1):1-56.
3. Agyare C, Koffuor GA, Boakye YD, Mensah KB. Anti-microbial and anti-inflammatory properties of *Funtumia elastica*. Pharm Biol. 2013;51(4):418-425.
4. Igbe I, Ching FP, Eromon A. Anti-inflammatory activity of aqueous fruit pulp extract of *Hunteria umbellata* K. Schum in acute and chronic inflammation. Acta Pol Pharm. 2010;67(1):81-85.
5. Igbe I, Ozolua RI, Okpo SO, Obasuyi O. Anti-pyretic and analgesic effects of the aqueous extract of the fruit pulp of *Hunteria umbellata* K. Schum (Apocynaceae). Trop J Pharm Res. 2009; 8(4):331-336.
6. Arulmozhi S, Mazumder PM, Sathiyanarayanan L, Thakurdesai PA. Analgesic, anti-inflammatory and anti-ulcerogenic activities of fractions from *Alstonia scholaris*. Pharmacologia. 2012; 3(5):132-137.
7. Iweala EEJ, Okeke CU. Comparative study of the hypoglycemic and biochemical effects of *Catharanthus roseus* (Linn.) g. Apocynaceae (Madagascar periwinkle) and chlorpropamide (diabenese) on alloxan-induced diabetic rats. Biokemistri. 2005;17(2):149-156.
8. El-Kashef DF, Hamed ANE, Khalil HE, Kamel MS. Investigation of the unsaponifiable and saponifiable matters of *Pachypodium lamerei* Drake leaves and stems by GC/MS. J Pharmacogn Phytochem. 2014;3(1):128-132.
9. El-Kashef DF, Hamed ANE, Khalil HE, Kamel MS. Botanical studies of the leaf of *Pachypodium lamerei* Drake, family Apocynaceae, cultivated in Egypt. J Pharmacogn Phytochem. 2015;3(5):40-45.
10. El-Kashef DF, Hamed ANE, Khalil HE, Kamel MS. Morphological and anatomical studies of the stem of *Pachypodium lamerei* Drake, family Apocynaceae, cultivated in Egypt. J Pharmacogn Phytochem. 2015;3(5):25-31.
11. Clause EPL, Henry Krimpton. Pharmacognosy. London 4th Edition III. 1961;121.
12. Harborne JB. Phytochemical methods. Chapman and Hall Ltd., London. 1973;49-188.
13. Trease GE, Evans WC. Pharmacognosy. 14th ed. London. W.B. Sanders Company. 1985.
14. Sofowora A. Medicinal plants and traditional medicines in Africa spectrum book Ltd. Ibadan, Nigeria. 1993;289.
15. Mirghazanfari SM, Hosseinzadeh L, Shokoohinia Y, Aslany M, Kamali-Nejad M. Acute and subchronic toxicological evaluation of *Echinophora platyloba* DC. (Apiaceae) total extract in wistar rats. Clinics. 2012;67(5):497-502.
16. Said A, Hawas UW, El-Shenawy S, Nofal SM, Rashed K. Flavonoids and some biological activities of *Ailanthus excelsa* leaves. IUFS J Biol. 2010;69(1):41-55.
17. Arthur FKN, Woode E, Terlabi EO, Larbie C. Evaluation of acute and subchronic toxicity of *Annona muricata* (Linn.) aqueous extract in animals. Eur J Exp Biol. 2011;1(4):115-124.
18. Petrovic SD, Dobric S, Bokonjic D, Niketic M, Garcia-Piñeres A, Merfort I. Evaluation of *Tanacetum larvatum* for an anti-inflammatory activity and for the protection against indomethacin-induced ulcero-genesis in rats. J Ethnopharmacol. 2003;87(1):109-113.
19. Sawadogo WR, Boly R, Lompo M, Some N, Lamien CE, Guissou IP, et al. Anti-inflammatory, analgesic and anti-pyretic activities of *Dicliptera verticillata*. Int J Pharmacol. 2006;2(4):435-438.
20. Tanko Y, Kamba B, Saleh MIA, Musa KY, Mohammed A. Anti-nociceptive and anti-inflammatory activities of ethanolic flower extract of *Newbouldia laevis* in mice and rats. Int J Appl Res Nat Prod. 2008;1(3): 13-19.
21. Boakye-Gyasi E, Woode E, Ainooson GK, Obiri DD, Ansah C, Duwejua M, et al. Anti-inflammatory and anti-pyretic effects of an ethanolic extract of *Palisota hirsuta* K. Schum roots. Afr J Pharm Pharmacol. 2008;2(9):191-199.
22. Devi PAV, Priya SD. Anti-pyretic activity of ethanol and aqueous extract of root of *Asparagus racemosus* in yeast induced pyrexia. Asian J Pharm Clin Res. 2013;6(3):190-193.
23. Mishra A, Arora S, Gupta R, Manvi, Punia RK, Sharma AK. Effect of *Feronia elephantum* (Corr.) fruit pulp extract on indomethacin-induced gastric ulcer in

albino rats. Trop J Pharm Res. 2009; 8(6):509-514.
24. Inas ZAA, Hala AHK, Gehan HH. Gastroprotective effect of *Cordia myxa* L. fruit extract against indomethacin-induced gastric ulceration in rats. Life Sci J. 2011; 8(3):433-445.
25. Choudhary M, Kumar V, Singh S. Gastric anti-secretory and cytoprotective effects of hydroalcoholic extracts of *Plumeria alba* Linn. leaves in rats. J Integr Med. 2014; 12(1):42-51.
26. Raji Y, Oyeyemi WA, Shittu ST, Bolarinwa AF. Gastroprotective effect of methanol extract of *Ficus asperifolia* bark on indomethacin-induced gastric ulcer in rats. Niger J Physiol Sci. 2011;26(1):43-48.
27. Hano J, Bugajski J, Danek L. Effect of adrenergic blockade on gastric secretion altered by catecholamines in rats. Arch Immunol Ther Exp (Warsz). 1976;24(4): 507-524.
28. Swami G, Nagpal N, Rahar S, Preeti S, Porwal A, Nagpal MA, et al. Effect of aqueous leaves extract of *Carissa carandas* Linn. on blood glucose levels of normoglycemic & alloxan-induced diabetic wistar rats. Int J Curr Pharm Res. 2010; 2(3):65-67.
29. El Alfy TS, El Sawi SA, Sleem A, Moawad DM. Investigation of flavonoidal content and biological activities of *Chorisia insignis* Hbk. leaves. Aust J Basic Appl Sci. 2010; 4(6):1334-1348.
30. Eleazu CO, Okafor PN. Anti-oxidant effect of unripe plantain (*Musa paradisiacae*) on oxidative stress in alloxan-induced diabetic rabbits. Int J Med Biomed Res. 2012; 1(3):232-241.
31. Sedigheh A, Jamal MS, Mahbubeh S, Somayeh K, Mahmoud RK, Azadeh A, et al. Hypoglycaemic and hypolipidemic effects of pumpkin (*Cucurbita pepo* L.) on alloxan-induced diabetic rats. Afr J Pharm Pharmacol. 2011;5(23):2620-2626.
32. Perez RM, Perez C, Zavala MA, Perez S, Hernandez H, Lagunes F. Hypoglycemic effects of lactucin-8-O-methylacrylate of *Parmentiera edulis* fruit. J Ethnopharmacol. 2000;71(3):391-394.
33. Manohar VS, Jayasree T, Kishore KK, Rupa LM, Dixit R, Chandrasekhar N. Evaluation of hypoglycemic and anti-hyperglycemic activity of freshly prepared aqueous extract of *Moringa oleifera* leaves in normal and diabetic rabbits. J Chem Pharm Res. 2012;4(1):249-253.
34. Das S, Bhattacharya S, Prasanna A, Kumar RBS, Pramanik G, Haldar PK. Preclinical evaluation of anti-hyperglycemic activity of *Clerodendronin fortunatum* leaf against streptozotocin-induced diabetic rats. Diabetes Ther. 2011;2(2):92-100.
35. Rahman MM, Rokeya B, Shahjahan M, Ahmed T, Roy SK, Ali L. Hypoglycemic effect of *Nyctanthes arbortristis* Linn. extracts in normal and streptozotocin-induced diabetic rats. Malays J Pharm Sci. 2013;11(1):21-31.
36. Kamel MS, Ohtani K, Kurokawa T, Assaf MH, El-Shanawany MA, Ali AA, et al. Studies on *Balanites aegyptiaca* fruits, an anti-diabetic egyptian folk medicine. Chem Pharm Bull. 1991;39(5):1229-1233.
37. Lalitha P, Gayathiri P. In *vitro* anti-inflammatory and phytochemical properties of crude ethyl acetate extract of *Baliospermum montanum* leaf (Muell,-Arg.). Afr J Biotechnol. 2013;12(39):5743-5748.
38. Perez G. Anti-inflammatory activity of compounds isolated from plants. Sci World J. 2001;1:713-784.
39. Gupta MB, Nath R, Srivastava N, Shanker K, Kishor K, Bhargava KP. Anti-inflammatory and anti-pyretic activities of β-sitosterol. Planta Med. 1980;39(6):157-163.
40. Niazi J, Gupta V, Chakarborty P, Kumar P. Anti-inflammatory and anti-pyretic activity of *Aleuritis moluccana* leaves. Asian J Pharm Clin Res. 2010;3(1):35-37.
41. Hegde K, Joshi AB. Preliminary phytochemical screening and anti-pyretic activity of *Carissa Spinarum* root extract. Der Pharmacia Lettre. 2010;2(3):255-260.
42. Hossain E, Mandal SC, Gupta J. Phytochemical screening and In-*vivo* anti-pyretic activity of the methanol leaf-extract of *Bombax malabaricum* DC. (Bombacaceae). Trop J Pharm Res. 2011; 10(1):55-60.
43. Begum TN, Ilyas MHM, Anand AV. Anti-pyretic activity of *Azima tetracantha* in experimental animals. Int J Curr Biomed Pharm Res. 2011;1(2):41-44.
44. Hemamalini K, Ashok P, Sunny G, Kumar Reddy S, Ganesh G, Santoshini K, et al. Gastroprotective activity of *Gymnosporiae merginata*, *Solanum pubescence* and *Anogessius accuminata* leaf extract against ethanol-induced gastric mucosal injury in rats. Int J Pharm Biomed Res. 2011;2(1):38-42.

45. Tovey FI, Capanoglu D, Langley GJ, Herniman JM, Bor S, Ozutemiz O, et al. Dietary phytosterols protective against peptic ulceration. Gastroenterol Res. 2011;4(4):149-156.
46. Subhadhirasakul S, Pechpongs P. A terpenoid and two steroids from the flowers of *Mammea siamensis*. Songklanakarin J Sci Technol. 2005;27(2):555-561.
47. Jesus NZT, Falcão HS, Gomes IF, Leite TJA, Lima GRM, Barbosa-Filho JM, et al. Tannins, peptic ulcers and related mechanisms. Int J Mol Sci. 2012;13(3): 3203-3228.
48. Dekanski D, Janicljevic-Hudomal S, Tadic V, Markovic G, Arsic I, Mitrovic DM. Phytochemical analysis and gastro-protective activity of an olive leaf extract. J Serb Chem Soc. 2009;74(4):367-377.
49. Mohan Y, Jesuthankaraj GN, Thangavelu NR. Anti-diabetic and anti-oxidant properties of *Triticum aestivum* in streptozotocin-induced diabetic rats. Adv Pharmacol Sci. 2013;2013:1-9.
50. Bhushan MS, Rao CHV, Ojha SK, Vijayakumar M, Verma A. An analytical review of plants for anti-diabetic activity with their phytoconstituents & mechanism of action. Int J Pharm Sci Res. 2010; 1(1):29-46.
51. Li WL, Zheng HC, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. J Ethnopharmacol. 2004; 92(1):1-21.
52. Perez GR, Vargas SR. Triterpenes from *Agarista mexicana* as potential anti-diabetic agents. Phytother Res. 2002; 16(1):55-58.
53. Li TH, Hou CC, Chang CLT, Yanga WC. Anti-hyperglycemic properties of crude extract and triterpenes from *Poria cocos*. Evidence-Based Compl Alt Med. 2011; 2011;1-8.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://www.sciencedomain.org/review-history.php?iid=1190&id=13&aid=9452*