# A study of phytochemical screening and anticonvulsant activities of Phoenix dactylifera L. leaflets in animal models

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## ABSTRACT

The Phoenix dactylifera L (date palm) belonging to family Arecaceae, called 'Nakhl' is endowed with various medicinal properties; however, utilization of leaf as a source of drugs has not yet been explored. The aims of the present study were to evaluate its anticonvulsant effects using PTZ and INH-induced seizures models. The fresh and dried leaflets of Taboni and El-Ammi cultivars were extracted in aqueous, ethanol and methanol. Phytochemical screening of extracts revealed the presence of alkaloids, flavonoids, saponins, steroids and other bioactive compounds.

Anticonvulsant activity of palm leaf methanolic extract PLME, Taboni cultivar (100, 200, 400 mg/kg ip) once daily for five successive days was evaluated against PTZ (100mg/kg, i.p.)-induced convulsions in rats (n=6). Diazepam was used as standard drug. Behavioral response of the animal to PTZ administration were evaluated using these criteria: onset of clonic convulsion, incidence, mortality and, protection percent. Intraperitoneal administration of PLME surprisingly caused 100% protection against clonic-tonic convulsion and mortality induced by PTZ, while, acute administration of PLME (200 mg/kg) in a single dose produced significant($P< 0.0001$) increase in generalized clonic-tonic seizure latency, the percent delay of convulsion was 75%, and produced complete protection against mortality following PTZ administration.

On the other hand intraperitoneal administration of PLAE, and PLEE, El-Ammi cultivar of PD, in different doses in a single or five successive days significantly ($P< 0.0001$) delayed the onset of convulsions against PTZ (80 mg/kg) and INH (300mg/kg)-induced seizures in mice respectively. Despite that both extracts did not completely protect the mice against clonic-tonic convulsions and mortality. The anticonvulsant activity of date palm leaflets extracts may be due to GABA-ergic stimulation.

In conclusion, constituents of methanolic, ethanolic and aqueous extracts of P. dactylifera leaf can cross blood brain barrier because it has CNS effect such as anticonvulsant activity. The anticonvulsant activity of methanolic extract is superior to diazepam. These activities are related to the presence of alkaloids, flavonoids, and other biogenic compounds which may be act in synergistic manner. Further investigation is required.

***Keywords***-Phoenix dactylifera, PLME, Anticonvulsant, Diazepam, PTZ, and INH.

## 1- Introduction:

Epilepsy is chronic, progressive, curable neurological disorders, affect both gender at any age and characterized by recurrent sudden convulsive and non convulsive seizures. The seizure is arise when a focal or generalized abnormal electrical discharge occur in a group of neuron in the brain. The disease is treated with drugs known as antiepileptics. The problem of these agents arise from their insufficiency to control seizure efficiently and to their adverse effects[1]. Many people living in developing countries still use herbal medicine for control of epilepsy. The clinical efficacy, minimal adverse effect profile and relatively low priced of herbal medicine are the reason for their various application in traditional medicine[2]. Hence, search for antiepileptic agents with more selective activity and lower toxicity should continue to develop newer drugs for treatment of epilepsy. Medicinal plants used for the treatment of epilepsy in traditional medicine have been shown to possess promising anticonvulsant activities in animal models and can be invaluable sources of new antiepileptic compound.

Convulsion is defined as involuntary movement of the voluntary muscles, while, the convulsive agents are substances which produce convulsive movements. They are widely used in the pharmacology laboratories as a tool to study and screen anti-epileptic drugs. Various experimental models used to assess anticonvulsant potential of drugs[3]: chemically induced convulsions (Pentylenetetrazole (PTZ), Lithium-pilocarpine induced status epilepticus, Lithium-methomyl induced seizure in rats, Isoniazid induced seizures, Systemic penicillin test), Electrically induced convulsion which include (maximal electroshock MES and minimal electroshock)[4], and Genetic induced seizures (sensory stimuli induced epilepsy) employed in genetic animals by applying high degree of sound (auidiogenic seizure) or light (photic seizure), convulsions produced are clonic tonic convulsions[5].

*Phoenix dactylifera Linn* (date palm) is called "Nakhl and Nakhil" under the Quranic names and tree of life by the Arabs. It is a member of the monocotyledone[6] plant. The genus phoenix includes 17 species, all Phoenix species are dioecious. *Phoenix dactylifera* (PD) is a species of magnoliophyta plant belonging to family Arecaceae or Palm family. Nakhlha or date palm is native to North Africa and Middle East of Asia[7]. The most producer of *Phoenix dactylifera* in the world are Iraq, Saudi Arabia, Egypt, Tunisia, Algeria, UAE, Oman, Libya, Pakistan, Sudan, Europe, and USA[8]. There are almost 400 cultivars of PD in Libya[9], among these are El-Ammi and Taponi cultivars. The taxonomical classification of *PD* is shown in (Table 1).

Table [1] The taxonomical Position of *Phoenix dactylifera*[10]

| **Rank** | **Scientific name - common name** |
|---|---|
| Kingdome | Plantae-plants |
| Subkingdom | Tracheobionta-Vascular plants |
| Super division | Spermatophyta- Seed plants |
| Division | Magnoliophyta- Flowering plants |
| Class | Liliopsida- Monocotyledones |
| Subclass | Arecidae |
| Order | Arecales |
| Family | Arecaceae- Palm family |
| Genus | Phoenix- Date palm |
| Species | Phoenix dactylifera L.- Date palm |
| Binomial name | Phoenix dactylifera Linn |

Date palm leaves are known as fronds too. Regarding their shape they are divided into two types: pinnate (feather shaped) and palmate (fan shaped). Pinnate leaves are huge in size, their length range from 240-370 cm with a stiff rachis which end in a single leaflet, about 150 narrow smooth stiff gray-green leaflets, arranged on each side of the rachis. The leaflets are compressed and gradually turn into pointed thorn at the end of the leaf. The leaflets are 30cm long and 2cm wide. The lowermost leaflets are modified into sharp, thin spines on the short petiole. PD leaves have a normal life of 3-4 years[11]. As reported previously in our study PD leaflets Hammory cultivar have important bioactive constituents; flavonoids, alkaloids, saponins, steroids, terpenoids, tannins and polyphenolic compounds[12]. The palm tree was mentioned on the holy Quran since over 1439 years ago in 17 sura from original 114 sura in 21 verses. For example, it was mentioned in the following sura: Al-Bakara, verse 266 , El-Rad, verse 4, Al-Esra, verse 91, AL-Nahl, verse 11and 67, and in Marium, verse 25.

Biological activities of date palms fruits, leaves, pollens, and pits were mentioned in many literatures; and include antihypertensive, anti diabetic, anti-ulcerative[11], anti-inflammatory[13], analgesic[14, 15], anxiolytic and antipsychotic in mice[15], anti-hyperlipidemic [16], anti-diarrheal activity in rats[17]. Aqueous extract of date palm leaves demonstrated excellent antimicrobial activity against various pathogens responsible for wide variety of infections[18, 19]. Recently Salem et al. (2018) reported that P. dactylifera methanolic and aqueous extracts of leaflets exerts antioxidant activity against paracetamol-induced hepatotoxicity through scavenging free radicals and restoring hepatic antioxidant enzymes[12].

Upon significant literature survey it was no work has been performed on the PD leaflets, Taboni and El-Ammi cultivars to investigate their anticonvulsant activity. Hence the present research was taken up to investigate anticonvulsant effects and to find out the phytochemical constituents present in the leaflets.

## MATERIALS AND METHODS

### Collection of plant material:

The fresh leaflets of *Phoenix dactylifera* Linn, (El-Ammi and Taboni cultivars) were collected from Misurata- Libya, washed thoroughly with running tap water, air dried under shade at room temperature for 40 days to avoid sun constituents degradation. The plant leaflets have been classified by the Department of Botany, Faculty of Science at the University of Misurata as (*Phoenix dactylifera*) leaflets.

### Extraction of plant materials:

Air dried leaflets of PD, El-Ammi and Taponi cultivars, were cut in small pieces ground into a coarse powder in a suitable grinder and divided into four portions for preparation

of the extracts as below. All the dried extracts were kept at a low temperature (4- 8 °C) in air tight container for further uses. The percentage yield was calculated using the formula:

% yield = ( weight of extracted material ÷ weight of original plant material used) X 100

**Preparation of aqueous extract (Palm Leaflets Aqueous Extract PLAE):**

Two hundred fifty grams of the prepared powder was boiled for 30 minutes in a can using tap water (1:4 w/v). The extract was filtered and concentrated by evaporating the water using dehydrator machine at 70 °C for 72 hours. The dried brownish-black extract weighted 27.80 gm and represented 11.12 % of raw powder.

**Preparation of methanolic extract (Palm Leaflets Methanolic Extract PLME)**

Two hundred fifty grams of the powder was extracted at room temperature, away of light for 48 hrs by macerated method using 700 ml of 99.8% methanol. The extract was concentrated by a rotary evaporator apparatus at temperature not exceeding 60 °C. The extract was dried in oven drier at (45 °C). The dried greenish-black extract weighted 28.5gm and represented 11.40 % of raw powder.

**Preparation of ethanolic extract (Palm Leaflets Ethanolic Extract PLEE)**

Two hundred grams of the powder was extracted at room temperature, away of light for 48 hrs by macerated method using 700 ml ethanol 99.8% . The extract was concentrated by a rotary evaporator apparatus at temperature not exceeding 60 °C. The extract was dried in oven drier at (45 °C). The viscid greenish-black extract weighted 22.48 gm and represented 11.24% of raw powder.

**Preparation of methanolic extract (Palm Leaflets Methanolic Extract PLME) using Soxhlet apparatus**

Air dried leaflets of PD, Taboni cultivar, were cut in small pieces and ground into a coarse powder in a suitable mixer grinder, Four hundred grams of the prepared powder was extracted with a Soxhlet apparatus for 12 hours using 1125 ml of methanol 99.8% v/v as a solvent. The resultant extract was filtered and concentrated by distilling off the solvent using vacuum drier rotary evaporator at (60 °C). The extract was dried in oven drier at (45 °C). The solid greenish-black extract weighted 43.30 gm and represented 10.82 % of raw powder.

**Chemicals and drugs**

Chemicals for extraction: methanol and ethanol (99.8%). Chemicals for phytochemical screening: acetic acid, ammonia, concentrated sulphuric acid, chloroform, ethanol, ferric chloride , iodole-potassium iodide , mercuric chloride, ninhydrine , olive oil , petroleium ethyl ether, sodium hydroxide (0.1N) , and - α-naphthol. Chemical for convulsion studies: pentylenetetrazol from Sigma-Aldrich, diazepam and isonicotinic acid hydrazide (INH) from Hoffmann-La Roche and Co., LTD Bazel, Switzerland. All solutions were prepared immediately before use and the chemicals were of A.R. grade.

**Equipments:**

Dehydrator machine (Hummer, Germany), Soxhlet apparatus, analytical balance, oven, rotary evaporator, stainless-steel blender and mixer.

**Animal:**

Adult healthy Swiss albino mice (n= 78, weighting 25±5 g ) and Sprague Dawley rats (n= 36) weighting 150-200g of either sex were used for fulfillment the aims of this study, (for evaluation of anticonvulsant activities). All animals were housed in polypropylene cages having autoclaved wooden shaving beddings, in a room controlled conditions: temperature 24 °C ± 2, relative humidity of 55 ± 5 and 12h light/ dark cycles. All animals were fed with laboratory chow and had free access to drinking water. All animals were obtained from the animals house of faculty of pharmacy and faculty of medical technology Misurata, Libya.

**Ethics statement**

All treatments were in accordance with the animal care guidelines of the Institutional Animal Ethics Committee, Misurata University, Faculty of Pharmacy. All efforts were made to minimize suffering.

**Experimental design**

**Preliminary phytochemical detection**

Aqueous, ethanolic and methanolic extracts of date palm leaflets, El-Ammi and Taboni cultivars were subjected to phytochemical screening to detect different chemical groups of compounds using standard procedures([20, 21]).

**Detection of alkaloids:** 0.5g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5ml of the filtrate was added 2ml of dilute ammonia. 5ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion. The formation of a cream indicated the presence of alkaloids.

**Detection of flavonoids:** Diluted ammonia (5ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1ml) was added. A yellow coloration that disappear on standing indicate the presence of flavonoids.

**Detection of saponins:** 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil then shaken vigorously after which it was observed for the formation of an emulsion.

**Detection of tannins:** About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

**Detection of terpenoids (Salkowski test):** 0.5g of the extract was added 2 ml of chloroform and concentrated sulphuric acid $H_2SO_4$ (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicate the presence of terpenoid.

**Detection of anthraquinones:** 0.5 g of the extract was boiled with 10 ml of sulphuric acid ($H_2SO_4$) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for color changes [10].

**Detection of steroids:** 2 ml of acetic acid was added to 0.2g of extract. The solution was cooled in ice, and then concentrated sulphuric acid ($H_2SO_4$) was carefully added .color development from violet to bluish-green indicated the presence of a steroidal ring.

**Detection of gum:** 5ml solution of the extract was taken and 1ml of Molisch's reagent and 1ml $H_2SO_4$were added, red violet ring produced at the junction of two liquids indicated the presence of gum.

**Detection of phenol:** 2ml of extract (prepared 0.5g extract added 5ml water) . added 1ml ferric chloride (dilute 135.5g of $FeCl_3.6H_2O$ added in water containing 20ml HCl diluted to 1 liter) greenish- black color indicates the presence of phenols.

**Detection of iridoids:** One ml of concentrated HCI was added to extract and heat it the presence of black precipitate indicates the presence of iridoids.

**Detection of free quinines:** A few drops of sodium hydroxide 0.1 N were added to the petroleum ether extract. Yellow coloration indicated the presence of free quinine.

**Detection of amino acids:** A few drops of ninhydrine solution was added to the extract the presence of amino acids was indicated by a red color.

**Anticonvulsant tests**

**Anticonvulsant effect of PLAE, El-Ammi cultivar of PD on PTZ-induced convulsions in mice:**

Forty two animals were divided into 7 groups each of 6 mine, and intraperitonealy administered with PLAE (25, 100, 200 mg/kg) in a single dose only, diazepam (4 mg/kg, positive control), sterile distilled water (DW) (0.25ml, negative control), 30 min later were injected with PTZ (80 mg /kg). The sixth and seventh groups were injected with PLAE (25 and 100 mg/kg) respectively for five successive days. In these two groups the convulsive agent PTZ was given 30 minutes after the last dose of PLAE. Each mouse was placed individually into a transparent plastic cage for observation lasting 30 min and 24 h. for mortality after PTZ administration. Seizures were assessed in term of onset of convulsions, incidence (number of mice showing convulsing), onset delay percent, protection percent and mortality percent[22, 23].

**Anticonvulsant effect of PLEE, El-Ammi cultivar of PD on INH-induced convulsions in mice:**

Thirty six mice of either sex (6 per group) were divided in to VI groups. Group I received sterile distilled water (0.25ml), group II was allotted for standard agent (diazepam 4mg/kg, i.p.). Group III was received palm leaf ethanolic extract PLEE, (300 mg/kg, i.p.) in a single dose. Group IV, V and VI were received PLEE (50, 100 and 300 mg/kg) respectively for five successive days. All animals were injected with INH (300 mg/kg, i.p)[24], 30 min later the last treatment. Immediately following INH administration, each mouse was placed individually into a transparent plastic cage for observation lasting 90 min and 24 h. for mortality. Seizures were assessed in term of onset of convulsions, incidence (number of mice showing convulsing), onset delay percent, protection percent and mortality percent[25].

**Anticonvulsant effect of PLME, Taboni cultivar of PD on PTZ-induced convulsions in rats:**

Thirty six rats of either sex (6 per group) were divided in to VI groups, received PLME ( 100, 200, 400 mg/kg, i.p.) once daily for five successive days, diazepam (4 mg/kg, i.p. positive control) and vehicle (sterile distilled water 0.25 ml, negative control). The last group received PLME (200 mg/kg, i.p.) in a single dose only. 30 min later, PTZ ( 100mg /kg, i.p.) was injected to each animal. All groups were kept under observation during experiment time for behavioral changes, immediately following PTZ administration, each rat was observed separately for next 30 minutes and 24 h. for mortality using these criteria: onset of clonic convulsion, incidence (number of rats showing convulsion), mortality percent, and protection percent. The induction of PTZ-induced convulsion was carried out according to previously described methods[26, 27].

**Statistical analysis**

All observations are expressed as the mean ± S.E.M. Differences between group means were analyzed with one-way analysis of variance (ANOVA) followed by Dunnet's test to assess the significance of differences between individual groups and accepted as significant when $P< 0.05$.

## RESULTS

### Phytochemical screening

Preliminary phytochemical screening of aqueous, ethanolic and methanolic extracts of PD leaflets of Taboni and El-Ammi cultivars revealed the presence of important phytochemical constituents such as flavonoids, alkaloids, steroids, terpenoids and other various groups. However, the tests show that PLME do not contain amino acid. (Table 2).

Table (2): Phytochemical analysis of the PLAE, PLME and PLEE.

| Chemical groups | Results | Chemical groups | Results |
|---|---|---|---|
| Alkaloids | + | Steroids | + |
| Flavonoids | + | Gum | + |
| Saponins | + | Phenolic compounds | + |
| Tannins | + | Iridoids | + |
| Terpenoids | + | Free quinolones | + |
| Anthraquinones | + | Amino acid | _ |

Positive sign (+) indicates presence, negative sign (-) indicates absence.

### Anticonvulsant activity

### Anticonvulsant effect of PLAE, El-Ammi cultivar of PD on PTZ-induced convulsions in mice

Intraperitoneal administration of PTZ (80 mg/kg) rapidly produced convulsion in all the mice used. All extracts groups compared with the DW group (control group). Mice pretreated with PLAE at the single doses of 25, 100 and 200 mg/kg, i.p. showed significant delay the onset of convulsion ($P < 0.0001$). The percentage delay were 17.38%, 39.63%, and 40.12% respectively. whilst the standard antiepileptic drug diazepam (4 mg/kg, i.p.) completely abolish the convulsions and mortality. As well as, administration of PLAE (25 and 100 mg/kg) for five successive days significantly ($P < 0.0001$) delayed the onset of convulsion too, and the percentage delay were 57.19% and 52.46% respectively. The results are represented in Table 3.

Table ( 3 ) : Acute and chronic effect of PLAE of PD El-Ammi cultivar on PTZ-induce convulsions in mice

| Treatment | Dose mg/kg, | Mean onset of convulsion (sec) | No of convulsed/ used | % delay of convulsion onset | Protection % | Mortality % |
|---|---|---|---|---|---|---|
| PTZ+DW | 80 | 159.17±6.82 | 6/6 | - | 0 | 2/6 (33.3%) |
| Diaz | 4 | 00.00 | 0/6 | - | 100 | 0/6 (0%) |
| PLAE | 25 | 192.67±10.29** | 6/6 | 17.38 | 0 | 1/6 (16.6%) |
| PLAE | 100 | 263.00±7.77*** | 6/6 | 39.63 | 0 | 0/6 (0%) |
| PLAE | 200 | 265.83±7.71*** | 6/6 | 40.12 | 0 | 0/6 (0%) |
| **Chronic (5 days) administration of PLAE** | | | | | | |
| PLAE | 25 | 371.83±15.95*** | 6/6 | 57.19 | 0 | 2/6 (33.3%) |
| PLAE | 100 | 334.83±17.34*** | 6/6 | 52.46 | 0 | 3/6 (50%) |

Values are expressed as mean ± SEM (Standard Error Mean); n=6 mice. , ***P< 0.0001 and significant different from control group. DW- distilled water , Diaz= diazepam, PTZ= pentylenetetrazole, PALE= palm leaf aqueous extract, No= number.

**Anticonvulsant effect of PLEE, El-Ammi cultivar of PD on INH-induced convulsion in mice**

The results are given in Table (4). Isoniazid (300 mg/kg, i.p.) slowly produced different behavioral responses in vehicle group of mice, including, increased activity, vocalization, irritability, fighting, and tail tremor, followed by wild running, recurrent episode of clonic and tonic convulsions, and death. The mean onset time of the first clonic spasm was 41.50±2.99 min. None of these manifestations were occurred in group II which received (diazepam 4 mg/kg, i.p.). PLEE of PD in a single dose (300mg/kg, i.p.), induced sedation, reduced activity of the mice and significantly (P< 0.0001) delayed the onset of convulsion to 70.00±3.61 min. without protection against mortality as compared to vehicle group. Additionally, administration of PLEE (50, 100 and 300 mg/kg) for five successive days, were significantly (P <0.0001) delayed the onset of convulsions and slightly induced protection and mortality against INH-induced convulsions in mice. The delayed percent were 64.37%, 29.45% and 38.56% respectively. The best results were occurred with the lowest dose (50 mg/kg) of PLEE of PD where, it's protection against convulsion was 66.60% and the reduced mortality percent to 33.33%.

Table 4: Acute and chronic anticonvulsant effect of PLEE, El-Ammi cultivar of PD on INH-induced convulsion in mice.

| Treatment | Dose mg/kg i.p. | Mean onset of clonic convulsion (min) | No. of convulsed / used | % delay | Protection % | No. of death/ used | Mortality % |
|---|---|---|---|---|---|---|---|
| INH | 300 | 41.50±2.99 | 6/6 | 0.0% | 0.0% | 6/6 | 100% |
| Diaz | 4 | - | 0/6 | - | 100% | 0/6 | 0.00% |
| PLEE | 300 | 70.00±3.61*** | 6/6 | 40.71% | 0.0% | 6/6 | 100% |
| **Chronic (5 days) administration of PLEE** | | | | | | | |
| PLEE | 50 | 116.50±6.48*** | 4/6 | 64.37% | 33.33% | 2/6 | 33.33% |
| PLEE | 100 | 58.83±4.08** | 6/6 | 29.45% | 0.0 % | 3/6/ | 50.00% |
| PLEE | 300 | 65.67±3.63*** | 6/6 | 38.56% | 0.0 % | 5/6 | 83.33% |

Values are expressed as mean ± SEM (Standard Error Mean); n=6 mice. , ***P< 0.0001 and significant different from control group. INH= isonidazid , Diaz= diazepam, PLEE= palm leaf ethanolic extract. No = number.

**Anticonvulsant effect of PLME, Taboni cultivar of PD on PTZ-induced convulsions in rats**

In control rats group, intraperitoneal administration of PTZ (100 mg/kg) rapidly caused clonic-tonic convulsion (140±7.17sec) as well as lethality was occurred in all rats used after tonic seizure. On the contrary to diazepam, sterile distilled water (0.25 ml, i.p.) did not show any protection against PTZ-incident of convulsion and mortality. In addition pretreatment with a single dose of PLME, (200 mg/kg, i.p.) 30 minutes prior injection of PTZ, produced complete protection against mortality and prevents clonic tonic convulsions in 50% of the animals while, the other animals appeared only clonic convulsions with significant (P< 0.0001) delayed in onset time (75%) as compared to DW group. Whereas, standard drug diazepam (4mg/kg, i.p.) completely abolished the convulsion and mortality.

Surprisingly PLME, Taboni cultivar in the doses of (100, 200, and 400 mg/kg, i.p.) for five successive days produced 100% protection against clonic-tonic convulsion and mortality induced by PTZ. The results are presented in Table 5.

Table (5): Acute and chronic anticonvulsant effect of the PLME, Taponi cultivar of PD on PTZ-induced seizures in rats.

| Treatment | Dose mg/kg i.p. | mean onset of seizures(sec) | No. convulsed / used | % delay of convulsion onset | Protection % | No. of death/ used | Mortality % | P value |
|---|---|---|---|---|---|---|---|---|
| PTZ | 100 | 140±7.17 | 6/6 | - | 0.00 | 6/6 | 100% | |
| Diaz | 4 | 00.00 | 0/6 | - | 100 | 0/6 | 0% | 0.0001 |
| PLME | 200 | 560± 36.61*** | 3/6 | 75.00 | 50 | 0/6 | 0% | 0.0001 |
| | | **Chronic (5 days) administration of PLME** | | | | | | |
| PLME | 100 | 00.00 | 0/6 | - | 100 | 0/6 | 0.0% | 0.0001 |
| PLME | 200 | 00.00 | 0/6 | - | 100 | 0/6 | 0.0% | 0.0001 |
| PLME | 400 | 00.00 | 0/6 | - | 100 | 0/6 | 0.0% | 0.0001 |

Values are expressed as mean ± SEM (Standard Error Mean); n=6 mice. , ***P< 0.0001 and significant different from control group. PTZ= pentylenetetrazole, PLME= palm leaf methanolic extract of Taboni cultivar. $P < 0.0001$

## Discussion

Seizures induced by the systemic administration of chemicals like PTZ, picrotoxin, strychnine and isoniazide in laboratory animals are important models for preclinical evaluation of potential drug for epilepsy[28,29]. PTZ is an antagonist of GABA at $GABA_A$ receptor which has been widely implicated in epilepsy[30]. It is proposed that PTZ induces convulsion by either inhibiting GABA pathway in CNS[30], or by increasing the central noradrenergic activity[31]. Prevention of seizures induced by PTZ and maximal electrical shock in laboratory animals is the most commonly used preliminary screening test to characterize potential anticonvulsant drugs. PTZ test represent a valid model for human generalized myoclonic and also absence seizures[32]. Furthermore; drugs which protect animals against the generalized clonic seizure induced by PTZ are effective in protection and management of petit mal epilepsy[33].

Isoniazide is a known agent for treatment of tuberculosis, in higher dose it acts as convulsant in animal models. There was a specificity of INH on GABA, since the basal levels of glutamine, taurine, aspartate and glutamate were unchanged[34]. Convulsant dose of INH lowered the level of GABA and glutamic acid decarboxylase (GAD) enzyme which is responsible for synthesis of GABA[35]. Isoniazid binds directly with pyridoxine to form isonicotinyl hydrazine. Also, isoniazid is dehydrised to its hydrazones which block pyridoxine phosphokinase, thus preventing conversion of pyridoxine to its active from, pyridoxal 5' phosphate. Additionally INH hydrazides inactivate pyridoxal 5 phosphate, which is essential for the formation of gamma amino-butyric acid from glutamic acid. Vaishal and Agarwal 2004 reported that low level of GABA and the accumulation of glutamic acid lead to CNS excitation and convulsions in animal models[36].

Amino acids in the brain include two important categories; inhibitory as (GABA and glycine) and excitatory such as (glutamate and asartate). Reduction in GABA content in certain sites leads to local or generalized convulsions. The convulsant agent as PTZ and INH diminish GABA contents in animal models. Anticonvulsant action may be achieved through enhancing inhibitory (GABAergic) processes, and by diminishing excitatory transmission[34,37, 38].

Currently there is an increasing demand for new types of antiepileptics because adverse effects from the drugs used clinically are increasing and often render treatment difficult[39]. One of the approaches to search for new antiepileptic drugs is the investigation of naturally occurring compounds, which may belong to new structural classes. Herbal medicine represent one of the most important fields of traditional medicine all over the world. Over the past 20 years, there has been an increased interest in the investigation of natural materials as sources of new drugs. Different extracts from

traditional medicinal plants have been tested to identify the source of the therapeutic effects[40,41].

The data obtained in this study demonstrated chronic (5 days) administration of PLME, Taboni cultivar of P.D entirely protect the rats against PTZ-induced convulsions and mortality furthermore, acute administration of the extract was significantly delayed onset time of seizures (75% delayed) and produced complete protection against mortality induced by PTZ (100mg/kg). While, acute and chronic administration of PLAE and PLEE of PD, El-Ammi cultivar were insufficiently protect the mice against PTZ (80mg/kg) and INH (300mg/kg)-induced clonic-tonic convulsions., despite they were significantly ($P< 0.0001$) delayed the onset, and reduced the severity of convulsions. Al-Taher 2008 reported that dimethoxy toluene, the major constituent of *phoenix dactylifera L* Spathe significantly increased the latency period and reduced the duration of seizures induced by PTZ in mice[42]. Vyawahare et al. (2009) reported that None of the doses of the methanolic extract of PD fruit (30, 100, 300 mg/kg) significantly reduced the duration of maximum electroshock(MES)-induced convulsions in mice but a nonsignificant dose-dependent reduction in duration of hind limb extension was seen, whereas the standard agent phenytoin was protect all the animals against MES manifestations [12].

In the present study administration of standard anti-epileptic drug diazepam 4mg/kg completely antagonized the convulsions, and reversed all the effects produced by PTZ and INH and provided 100% protection to rats and mice. The anticonvulsant activity of PLME of PD leaflets is superior to diazepam. Diazepam has been shown to be an effective agent in ameliorating the symptoms of generalized absence epilepsy via GABA receptor and opening of chloride channel. Since PTZ is a known GABA antagonist[38], so the anticonvulsant activity of PLME of P.D may partially act by increasing GABA concentration in the brain to sufficient levels , while PLAE and PLEE were returned the depleted GABA to insufficient concentration.

In the current investigation, anticonvulsant activities can be attributed to the presence of bioactive constituents; alkaloids, steroids, flavonoids, tannins and saponin in aqueous, ethanolic and methanolic extracts of PD leaflets, Taponi and El-Ammi cultivars. This is in line with study done by Al-Dawah and his colleague 2013 who reported that Phytochemical screening of methanol extract of PD leaflets showed the presence of alkaloids, flavonoids, phenols, carbohydrates, saponin, amino acids, trepenoids and tannins[43]. These compounds can show extensive pharmacologic and other activities. Triterpenoids and saponints showed the anticonvulsant properties[43,44,45]. It is also found that many flavonoids could act as benzodiazepine-like molecules in the CNS and modulate GABA-generated chloride currents in animal models of anxiety, sedation and convulsion[46]. Other researcher reported that, the anticonvulsant effects was attributed to alkaloids[47]. Kasture et al., 2002 mentioned that triterpene possess a wide spectrum of anticonvulsant and anxiolytic activity[48].

Therefore, taking all these data together we believe that the probable mechanisms of potent central anticonvulsant activity of leaflets extract is mediated by stimulation of GABA receptors, GABAergic transmission, and inhibition of excitatory

neurotransmitters in the brain. These effects are attributed to the presence of saponins, terpenoids and other bioactive constituents.

## 5. Conclusion

On the basis of these finding, it may be inferred that methanolic extract of *phoenix dactylifera* leaflets, Taboni cultivar has a potent anticonvulsant activities that comparable to diazepam. As well as, aqueous and ethanolic extracts of El-Ammi cultivar have a less anticonvulsant effects. These activities were related to the presence of flavonoids, alkaloids, tannins, steroids, terpinods, and other biogenic compounds. The potent activity may be due to synergistic action of the constituents.

## Recommendations

Further studies are necessary to isolate the constituents and to elucidate the mechanisms lying with these activities.

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