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# General neuropharmacological studies of piper betel leaves on mice

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***Abstract*---**The *Piper betel*plant is an evergreen and perennial creeper which is used in several traditional medicines to cure various diseases. The liquid extract of the plant has been used traditionally in curing inflammationand infection of the respiratory tract, cough, dyspnoea, indigestion,diphtheria, hysteria as well as general and sexual debility. The Indian traditional system ofmedicine has identified the *Piper betel*leaves with digestive and pancreatic lipasestimulant activities. In the present study, 14-day subacute toxicity assays were carried out.The crude extract did not produce toxic symptoms in mice in dose up to 2000mg/kg. This was also contirmed by hematological and histopathology examination.*Piper betel*showed hypotensive, cardiac, and respiratory depressant effectssmooth and skeletal muscles relaxant actions, antimicrobial, fungicidal,and nematocidal activity.Piper betel leaves extract contains large number of bioactive molecule like polyphenol, alkaloids, steroids, saponin, and tannin. Alkaloid is the main constituent which is present in the extract has been detected by the Rf. values which were found from the TLC chromatography (HPTLC) has been performed.HPTLC also has been performed.

***Keywords*---***Piper betel*, HPTLC, Acute Toxicity.

## Introduction

*Piper betel* Linn. A member of the piperaceae family is an edible plant with leaves that have been traditionally used in India, China, and Thailand. The betel plant is an evergreen and perennial, creeper, with glossy heart shaped and white catkin. The plant kingdom representsa rich source of organic components, many of which have been used for medicinal & other purposes. Herbal medicines remain the major source of health care for the world's population (Mohammad Y, Mohammad I,2010). Currently, there is a growing interest in plant based or herbal medicines even in the western world. In many respects, the mechanism of action of the herbal drugs differs from that of thesynthetic drugs or pure compounds (Jitesh S, et al, Chattopadhyay S, et al,2006). In one of the study of the World Health Organization it is estimated that 80% of the population of developing countries relies on traditional plant based medicines for their health requirements (Patra A, Jha S, Murthy P.N,2009.). *Piper betel Linn.*(Betel vine) is a tropical plant closely related to the common pepper and belongs to the family Piperaceae (Gunther E, 1952). It is extensively grown in India, Srilanka, Malaysia, Thailand, Taiwan and other Southeast Asian countries andhas a long history of over 2000 yrs (Guha P.2006). Ayurveda an Indian system of medicine has been an integral part of Indian culture and materiamedica. From the rich Indian biodiversity, it has identified various plants/herbs that have been associated with a number of potential therapeuticefficacies (Jitesh S, et al, Chattopadhyay S, et al,2006). The use of *P. betel*L. leaves to lighten melasma may produce leukomelanosis, which manifests asconfetti-like skin depigmentation (Bour-Jr et al,2008) also the roots (8-12g) are used in treatingrheumatism (Dan N.V,1990). The leaf produces an aromatic volatile oil containing a phenolcalled chavicol which has powerful antiseptic properties. *Piper betel*inflorescencecontains high levels of safrole (15mg/g wet weight) and chewing betel quid containing *Piper betel*inflorescence generates a high concentration of safrole (420 µM) (Hwang L.S, et al,1992: Wang C.K, Hwang L.S,1993). The plant possesses cardiotonic (Deshpande, S.M, et al,1970)wound healing (Santhanam G, Nagarajan S, 1990), chemopreventive (Azuine M.A, et al,1991) and reversible antifertility (Sarkar M, et al,2000).The liquid extract of the plant has been used traditionally in curing inflammationand infection of the respiratory tract, cough, dyspnoea, indigestion,diphtheria, hysteria as well as general and sexual debility (Gilani A.H, et al,2000).Betel leaf has been described from ancient times as an aromatic, stimulocarminative(katu), astringent and aphrodisiac (kamagnisandipanam) (Chopra R.N, Chopra I C, 1958).Theleaves are credited with wound healing(Mula S, et al,2008:Santhanam G, Nagarajan S,1990.) The Indian traditional system ofmedicine has identified the *Piper betel*leaves with digestive and pancreatic lipasestimulant activities (Chatterjee A, Pakrashi S.C,1995:-Rawat,AKS, et al,1989) *Betel* leaf is traditionally known to be useful for the treatmentof various diseases like bad breath, boils and abscesses, conjunctivitis, constipation,headache, hysteria, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea,ringworm, swelling of gum, rheumatism, abrasion, cuts and injuriesetc as folk medicine (Guha P.2006)Traditionally *Piper betel*leaf extractis reported to inhibit male reproductive competence (Ratnasooriya W.D, et al, 1990:-Ratnasooriya W.D, Premakumara G.A.S,1997) *Piper betel*leaves are shown to posseshepatoprotective activity (Saravanan, R, et al,2002) In folk medicine root is known for its female contraceptive effects (Guha P.2006). The rootsare used as a contraceptive and are chewed by singers to improve their voice

(Usmanghani K, et al,1997.).In ancient times flower is used as an ingredient for chewing food known as betelquid in South-East Asia (Hwang L.C, et al,1992).The plant, extract is found to have highest, phenolic content (Jamal, et al, 2010) which can contribute to antimicrobial activity. It is reported that Piper betel leaves is a CNS stimulant (Guha, 2006). Based on the results of scientific investigations, several value-added products such as betel toothpaste, mouthwash, face cream, shampoos, instant betel quid, betel pellet, antitick lotion, antitick powder and wound healing creams were developed in order to enhance the marketability of betel and improve the prospects of the industry. Clinical trial conducted using the wound healing cream on dermatitis patients revealed that treatment was significantly effective on skin rashes(Arambewela LSR, et al,2010). At present a clinical study is in progress to evaluate the antidiabetic activity of spray-dried powder of betel hot water extract.The leaf has been reported to contain methyl piper betlol, peperol-A, piperol-B and they alsohave been isolated and leaf also contains hydroxyl chavicol, eugenol piper betol and the betel oilcontains carvacrol, allyl catechol, chavicol, chavibetol, cineole, estragol, p-cymene,caryophyllene, cadinene . The antispasmodic action of betel oil on involuntary muscle tissue,inhibiting excessive peristaltic movemets of the intestines at moderate doses. The importantAyuvedic formulations of piper betel plant are Lokantha Rasa, PuspadhavaRasal, Brhatsarwajwarahara, lanha, laghusutaseknara Rasa, Brhatvisamajwarantaka Rasa we found someadditional anatomical characters such as silica bodies, pearl glands. These characters have notbeen reported in Ayurvedic pharmacopoeia so these additional diagnostic characters of piper betel Linn are recommended to make necessary inclusions in the Ayurvedic pharmacopoeia. In another study, scientists report(Chi Pang Wen, et al.) the extent of cancer risks of betel quid chewing (without tobacco added) beyond oral cancer. In addition to oral cancer, significant increases were seen among chewers for cancer of the esophagus, liver, pancreas, larynx, lung, and all cancer.

## Materials and Methods

*Plant Material*

The fresh leaves of *Piper betel* L. (500 mg ) were obtained from a piper plantation, local area of Luckow in October 2011. The plant material was identified by a botanist of Botany Section, from National Botanical Research Institute (NBRI) Ref. No: NBRI/CIF/259/2011. They were cleaned with distilled water. Only green leaves, which did not have scars or spots of disease, were selected for further extraction. The leaves were then air-dried at room temperature overnight, and further dried in hot air oven at 50 °C until dryness. The dried leaves were pulverized to coarse powder using an electric blender.

*Extraction Procedure*

The collected leaves of the plants were shade dried and powdered in a grinder mixture to get coarse powder and then passed through 40 mesh sieve. The powdered leaves (50g) were later extracted with methanol (300ml) using Soxhlet extractor separately. The extract was evaporated to dryness.

*Phytochemical Screening*

Preliminary chemical tests were done with extract for the presence of difference group of chemicals i.e. saponin, flavanoids, glycoside, steroids, alkaloids.

*Acute Toxicity Studies*

The acute toxicity study was carried out by guidelines set by OECD 420 guidelines. Albino male and female mice (25-35g) maintained under standard laboratory condition was used. A total number of six animals were used per group which received a single dose (2000 mg/kg b.wt (p.o.) of herbal drug. Animals were kept overnight fasting prior to drug administration. After the administration of polyherbal drug, the food was withheld for 3-4 hours. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24h (with special attention during the first 4 hours) and daily thereafter for a period of 14 days. Daily cage side observation included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory, autonomic changes was observed (OECD, 1990)

*Hematological Analysis*

On the necropsy day, blood was withdrawn through cutting of the tail end of mice. The blood was placed into EDTA bottles for hematological assay and in plain bottle for clinical biochemistry determination. The blood for hematological assay was immediately analyzed using a hematological analyzer (KX-21NSysmex Cooperation, Japan). The parameters measured were white blood cell (WBC), hemoglobin (HGB), red blood cell (RBC), hematocrit (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration(MCHC), lymphocyte %, lymph no (lymphocytes number) and platelets (PLT).

*Histopathological Analysis*

Dose of the extract was administered orally to six groups of mice on daily basis for 14 days. Mice in group 1 served as control. The animals in group 2000mg/kg body weight (Thomas, 1987). At the end of 14-day extract exposure, the animals were sacrificed by cervical dislocation under ether anesthesia. The livers, kidneys, hearts, spleen and brain of the mice were harvested and fixed in 10% formol saline for 48hours and processed for paraffin wax embedding with an automatic tissue processor by dehydrating through 70%, 90%, 95% and two changes of absolute ethanol for 90 minutes each. Clearing was achieved through two changes of xylene for 2hours each; and infiltrating with two changes of paraffin wax for 2hours. Sections were cut at 5µm with a rotary microtome. The sections were stained by haematoxylin and eosin (H&E) method (Oduola T, et al, 2009) examined and photographed using a light microscope.

**Results & Discussion**

*Chemical Tests*

The preliminary phytochemical analysis of *Piper betel* showed that the plant contains alkaloids, flavanoids, glycosides, tannins, reducing sugar, steroids are present but saponin are absent. The constituents of which are present or absent are summerised in table1.

Table 1 List of the constituents present in extract of *Piper betel.*

| S.NO. | Secondary metabolites | Results |
|---|---|---|
| 1. | Alkaloids | ++ |
| 2. | Flavanoids | ++ |
| 3. | Glycosides | ++ |
| 4. | Tannins | ++ |
| 5. | Reducing sugar | ++ |
| 6. | Steroids | ++ |
| 7. | Saponins | -- |

+ + Present, - - Absent

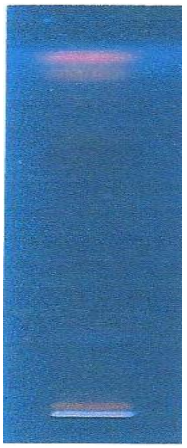
*Thin Layer Chromatography*

Thin layer chromatography (TLC) has been performed and the platewas then kept in iodine vapor saturated tank left for few hours and obtained spots the retention factor (Rf.) 0.22, 0.24, are found.

*Separation of active compounds by HPTLC*

Separation of active compounds from methanol extract of the selected leaves were detected by Thin layer chromatographic plates using various solvent systems and analyzed in HPTLC *Piper betel* methanol extract showed five compounds having an Rf value of 0.02, 0.17, 0.74, 0.75 and 0.85 and λ max at 366, and 254nm. The compound five appeared to be the major compound with 65.63% area.

Under 254 nm



under 366 nm

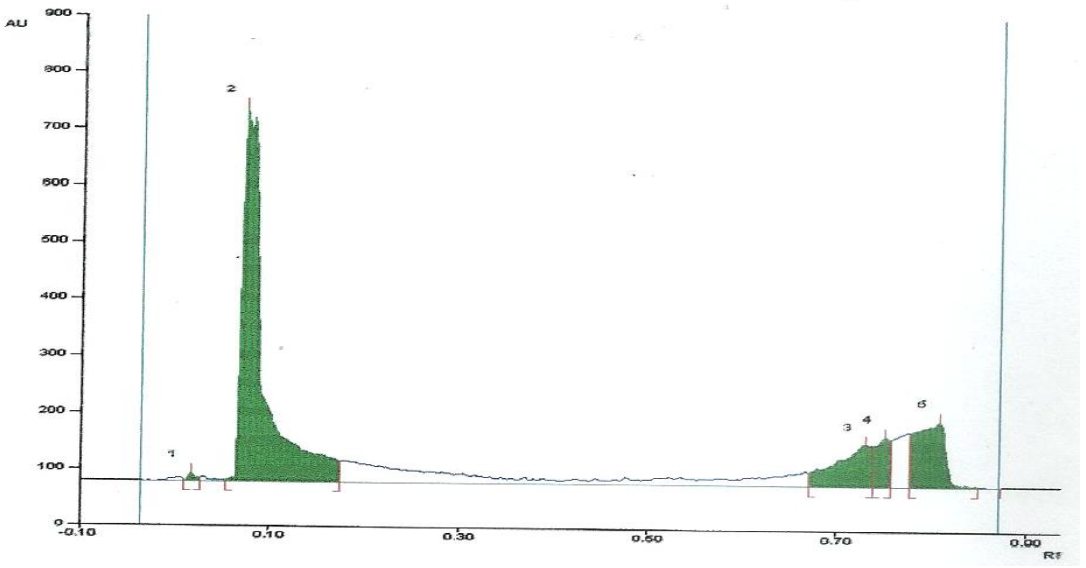



Fig 3.2.2 HPTLC Finger Print of extract
Scan wave length-366 nm

*Acute toxicity*
*Hematological Analysis*

Hematological values measured showed a significant elevation of HGB leveland RBC level in treatment group.The value of HCT was significant increased ascompared with the control group. Other hematology values, WBC, Mon, Gra, MCV, MCH, MCHC,Lymphocyte %, Lymphocyte no and PLT were not significantly different as compared to the control mice and they remained with in normal limits (control values). Hematology data are presented in Tables 2 respectively.

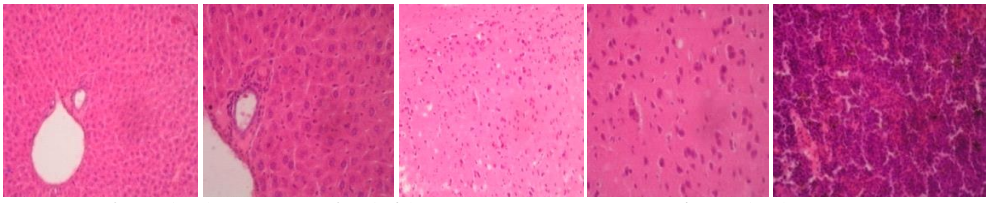
Table 2 Effect of extract of *Piper betel* on some haematological parameters.

| Serial No. | Parameters | Control | PB(2000 mg/kg) |
|---|---|---|---|
| 1 | WBC (m/mm3) | 6.83 ± 0.23 | 11.22 ± 0.16 |
| 2 | Lym ( % ) | 57.1 ± 0.23 | 68.33 ± 0.54 |
| 3 | Mon (%) | 3.40 ± 0.14 | 2.310 ± 0.11 |
| 4 | Gra (%) | 39.6 ± 0.11 | 26.98 ± 1.13 |
| 5 | MCV( fl) | 47.5 ± 0.09 | 43.66 ± 0.37 |
| 6 | Htc (%) | 41.9 ± 0.13 | 43.43 ± 0.33 |
| 7 | MCH (pg) | 14.8 ± 0.23 | 13.36 ± 0.19 |
| 8 | MCHC (g/dl) | 31.2 ± 0.16 | 29.08 ± 0.33 |
| 9 | Hgb (g/dl) | 13.1 ± 0.26 | 13.30 ± 0.28 |
| 10 | Plt (m/mm3) | 50.7 ± 0.20 | 43.20 ± 0.12 |
| 11 | MPV (fl) | 5.00 ± 0.11 | 4.960 ± 0.06 |
| 12 | Pct (%) | 0.30 ± 0.06 | 0.200 ± 0.00 |
| 13 | Mode (fl) | 4.80 ± 0.09 | 5.200 ± 0.10 |
| 14 | Medn (fl) | 6.10 ± 0.26 | 6.010 ± 0.06 |
| 15 | P | 30.0 ± 0.26 | 20.00 ± 0.57 |
| 16 | L | 57.0 ± 0.57 | 70.00 ± 0.96 |
| 17 | M | 3.00 ± 0.57 | 3.000 ± 0.12 |
| 18 | E | 9.00 ± 0.60 | 7.000 ± 0.18 |
| 19 | B | 1.00 ± 0.25 | 1.000 ± 0.15 |

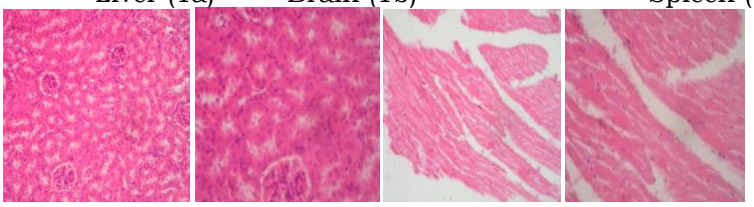
Mean values± S.E.M

*Histopathology*

No alterations were observed in the organs of the control animals as well as in animals treated with 2000mg/kg extract of *Piper betel* of male mice shows photomicrographs of the organs at different amplified levels taken in animals treated with a subacute dose of 2000mg/kg *Piper betel*.

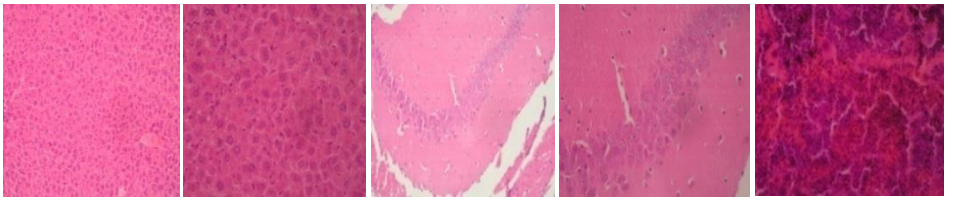


Liver (1a) Brain (1b) Spleen (1c)

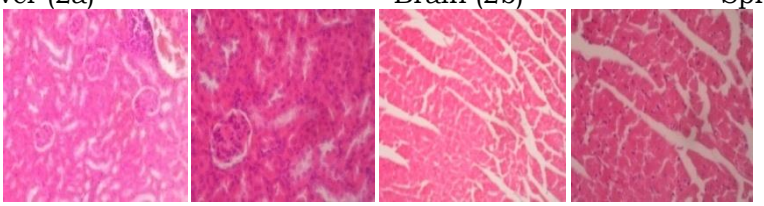


Kidney (1d)Heart (1e)

Figure 1 Plates 1a, 1b, 1c, 1d and 1e (×20 and ×40) were sections of Liver, brain, spleen, kidney and heart respectively of animals that received 2000 mg/kg of the extract and stained with H&E.



Liver (2a) Brain (2b) Spleen (2c)



Kidney (2d)Heart (2e)

Figure2 Plates 2a, 2b, 2c, 2d and 2e (×20 and ×40) were sections of liver, brain, spleen, kidney and heart respectively of control animals.

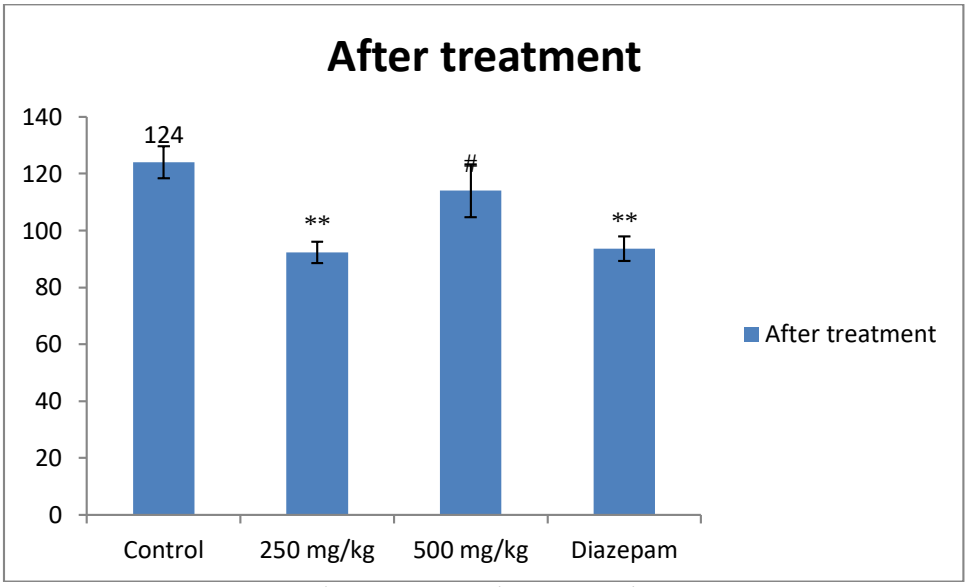
**Pharmacological Activity Assessments**

Statistic evaluation
All results were expressed as mean ± S.E.M. for the indicated number experiments. The significance of the difference among groups was analyzed using ANOVA followed by the Student-Newman-Keuls test. A *p* value <0.001 was considered significant. All statistic analyses were made using the software In Stat for windows.

1. Effect of Locomotor activity-Piper betel in a dose of 500 mg/kg did not produce any significant change in locomotor activity (114 ± 9.22 #) as compared to control (124 ± 5.69). However next dose (250 mg/kg) produce significant reduction (92.33 ± 3.78 **) respectivily in locomotor activity. The all results are summerised in table 3.3.

| Treatment mg/kg | After Treatment (sec.) |
|---|---|
| Control (10ml/kg) | 124 ± 5.69 |
| 250mg/kg P.B. | 92.33 ± 3.78 ** |
| 500mg/kg P.B. | 114 ± 9.22 # |
| Diazepam (1mg/kg) | 93.66 ± 4.36 ** |

n =6, P**< 0.01, P#>0.05 as compared to control.

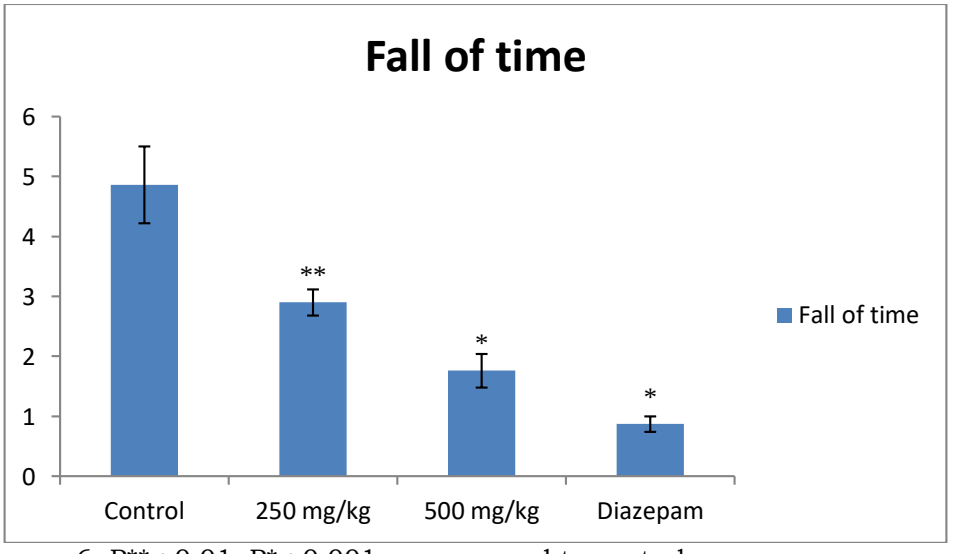



n =6, P**< 0.01, P#>0.05 as compared to control.

2. Effect of motor- co-ordination-Reduction in the mean change in fall off time was reported with PB-250 mg/kg (2.90 ± 0.22 **) and PB-500 mg/kg (1.76 ± 0.28 *) compared to control (4.86 ± 0.64)

| Treatment mg/kg | Fall of Time (Min.) |
|---|---|
| Control (10ml/kg) | 4.86 ± 0.64 |
| 250mg/kg P.B. | 2.90 ± 0.22 ** |
| 500mg/kg P.B. | 1.76 ± 0.28 * |
| Diazepam (1mg/kg) | 0.87 ± 0.13 * |

n =6, P**< 0.01, P*< 0.001 as compared to control.

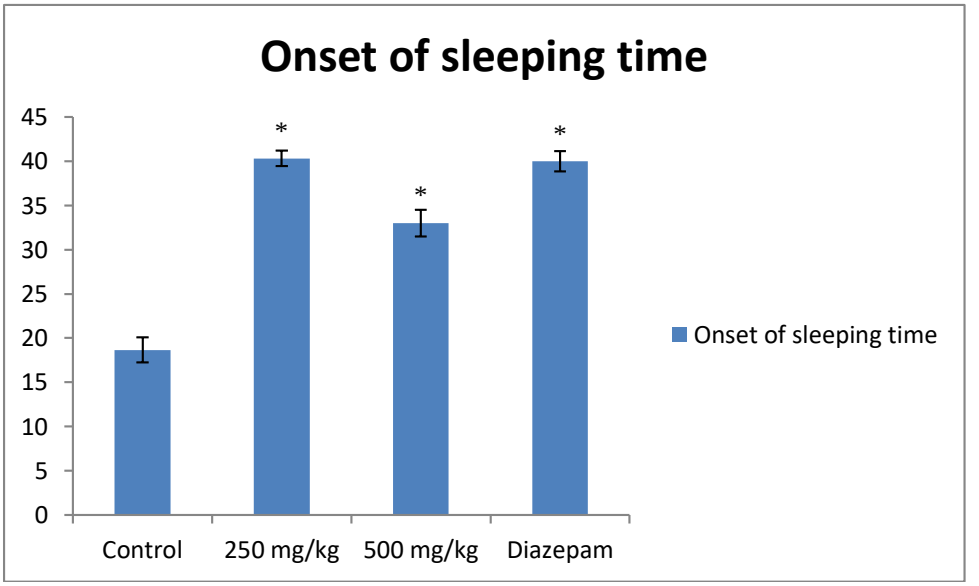



n =6, P**< 0.01, P*< 0.001 as compared to control.

3. Pentobarbital-induced sleeping time-The effect of *Piper betel* on pentobarbital induced sleeping time in mice. The total sleeping time was about( 40.33 ± 0.87 *and 33 ± 1.53 *min) at dose of 250 mg/kg and 500 mg/kg of body weight respectively where as in control group it was about (18.66 ± 1.40 min).

| Treatment (mg/kg) | Onset of sleeping time(min.) |
|---|---|
| Control (10ml/kg) | 18.66 ± 1.40 |
| 250mg/kg P.B. | 40.33 ± 0.87 * |
| 500mg/kg P.B. | 33 ± 1.53 * |
| Diazepam (1mg/kg) | 40 ± 1.15 * |

n=5, P* <0.001 as compared to control.

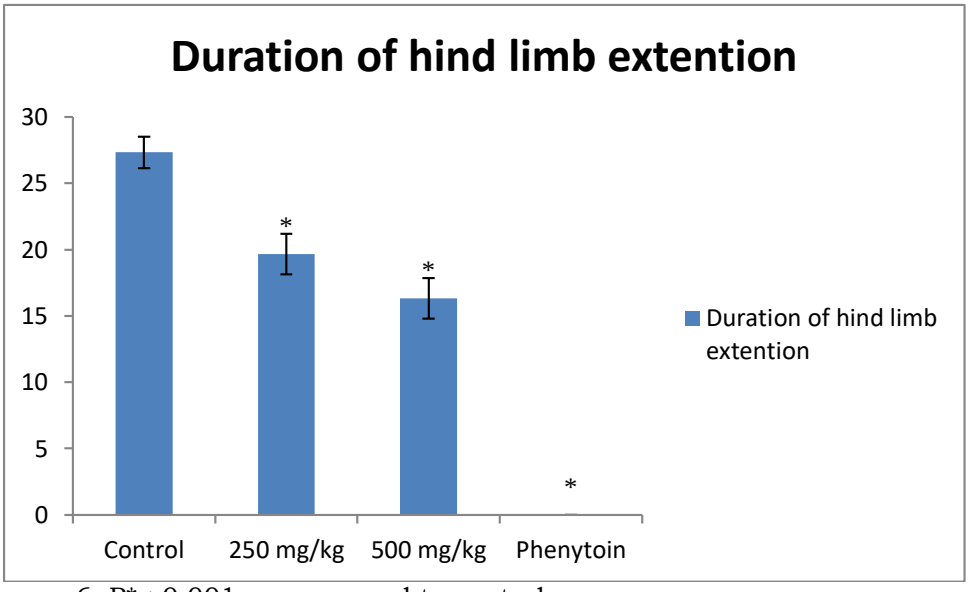



n=6, P* <0.001 as compared to control.

4. Maximal electroshock induced seizures (MES).-The mean duration of hind limb extension in vehicle treated group was27.33 ± 1.19 *sec which was reduced to16.33 ± 1.52 * sec with pretreatment with 500 mg/kg PB. The dose 250 mg/kg were found to be ineffective in this regard. Considering the parameter of number of mice protected, only one mouse was protected with the pretreatment of 500 mg/kg PB.

| Treatment (mg/kg) | Duration of hind limb Extension (sec.) |
|---|---|
| Control (10ml/kg) | 27.33 ± 1.19 * |
| 250mg/kg P.B. | 19.66 ± 1.52 * |
| 500mg/kg P.B. | 16.33 ± 1.52 * |
| Phenytoin (25mg/kg) | Nil |

n=6, P*< 0.001 as compared to control.

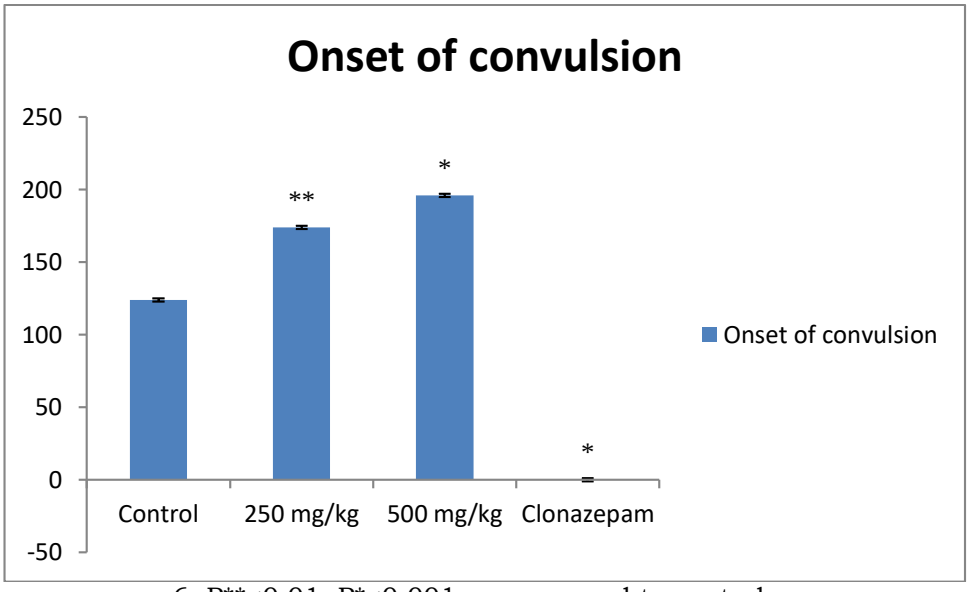



n=6, P*< 0.001 as compared to control.

5. Pentylenetetrazole induced seizure (PTZ)-In vehicle treated control mice, convulsions were produced after 124 ± 12.75 sec. The pretreatment with PB delayed this onset up to 196.66 ± 14.83 *. All the control mice died immediately after onset of convulsions while one of six was survived in each PB 250 and 500 mg/kg treated group. Standard clonazepam showed 100% protection.

| Treatment ( mg/kg) | Onset of convulsion (sec.) |
|---|---|
| Control (10ml/kg) | 124 ± 12.75 |
| P.B.(250mg/kg) | 174.33 ± 13.65 ** |
| P.B.(500mg/kg) | 196.66 ± 14.83 * |
| Clonazepam (0.1mg/kg) | Nil |

n=6, P**<0.01, P*<0.001 as compared to control.




n=6, P**<0.01, P*<0.001 as compared to control.

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