



In-vivo* Analgesic and *In-vitro* Cytoprotective Potential of Various Leaf Extracts of *Pongamia pinnata

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Received: 2 Nov, 2018

Revised: 26 Nov., 2018

Accepted: 27 Nov., 2018

ABSTRACT

Pongamia pinnata (L.) Pierre is a medium sized glabrous, perennial tree which grows in the littoral regions of South Eastern Asia and Australia. In the Indian Ayurvedic medicine, different parts of the plant have been used for pain relief in various disorders. The present study investigated the potential of different leaf extracts of *Pongamia pinnata* as an analgesic agent in rodents and our aim was also to study *in-vitro* cytoprotective effects of the various extracts from leaves of the plant. Different leaf extracts of *Pongamia pinnata* i.e. aqueous, alcoholic, acetone and chloroform were investigated for analgesic activity at the dose rate of 50 mg/kg and 100 mg/kg in Wistar rats. For the assessment of analgesic activity, tail flick method was used. *In-vitro* cytoprotective activity of various leaf extracts (at concentrations of 5% and 10%) was evaluated in ATCC acquired MDBK cell lines and for this study, cytotoxicity was induced by thiomethoxam. It was observed that almost all the extracts demonstrated the dose dependent analgesic activity with maximum response in the aqueous extract group @ 100 mg/kg when compared to control. For cytoprotective study, oxidative stress parameters- catalase, LPO, SOD and GPx were determined. Study on analgesic activity revealed the presence of dose dependent effect in all extracts with highest effect in aqueous extract of *Pongamia pinnata*. We believe that triterpene alkaloids and steroidal principles present in the plant products might be responsible for the analgesic effect.

Keywords: Analgesic, cytoprotective, MDBK, oxidative stress parameters, *Pongamia pinnata*

From the time immemorial, plants have been widely used as curative agents for variety of ailments. Herbal medicines also referred to as botanical medicines or phytomedicines include herbs, herbal materials, herbal preparations and finished herbal products that contain parts of plants or other plant materials as active ingredients. However, the popular knowledge should be scientifically validated through clinical and experimental studies to prove the efficacy and safety of these species (Trivellato Grassi *et al.*, 2013).

The 'Pongam Tree' is known as one of the richest and brightest trees of India. The tree is named as '*Pongamia pinnata*', belonging to Leguminosae family (sub family-Papilionaceae). In the Tamil, this is generally known as 'Ponga', 'Dalkaramacha', 'Pongam' and 'Punku'. In both the languages of Hindi and Bengali, the people named

it as 'Karanj' or 'Papar' or 'Kanji'. It is called 'Karum Tree' or 'Poonga Oil Tree' in English, Karach in Bengali, Naktamala in Sanskrit and Sukhchain in Urdu.

The major active constituent of *Pongamia pinnata* is karanjin. Chemically karanjin is a furanoflavonoid (Maurya and Yadav, 2005). All parts of the plant have been used as crude drug for the treatment of tumors, piles, skin diseases, wounds and ulcers. Ayurvedic medicine described the root and bark as alexipharmic, anthelmintic and useful in abdominal enlargement, ascites, biliousness, diseases of the eye, piles, splenomegaly, tumors, ulcers and wounds; the sprouts as alexiteric, anthelmintic, aperitif and stomachic; the leaves as anthelmintic, digestive and laxative, anti-inflammatory and useful in treatment of piles. Flowers are used for diabetes, bark for beriberi, juice of the root for cleansing foul ulcers and closing fistulous

sores. Unani system uses the ash to strengthen the teeth, the seed, carminative and depurative, for chest complaints, chronic fevers, earache, hydrocele and lumbago; the oil is used as fuel for cooking and lamps. The oil of *Pongamia pinnata* is also used as a lubricant, water-paint binder, pesticide, in soap-making and tanning industries and is known to have value in folk medicine for the treatment of rheumatism, as well as human and animal skin diseases. It is effective in enhancing the pigmentation of skin affected by leucoderma or scabies. Plant phenolics, in particular phenolic acid, tannins and flavonoids are known to be potent antioxidant and occur in fruits, nuts, seeds, roots, barks and leaves (Okuda, 2005).

In the Ayurvedic literature of India, different parts of the plant have been recommended as a remedy for various ailments and used in traditional medicine for bronchitis, whooping cough and rheumatic joints. A hot infusion of leaves is used as a medicated bath for relieving pains and for cleaning ulcers in gonorrhoea and scrofulous enlargement. Although stem bark extracts of the plant have shown analgesic (Sagar *et al.*, 2010) and antioxidant activities (Badole *et al.*, 2011), there is paucity of data for studies on leaf extract of the plant. In the light of above, the present study investigated the potential of different leaf extracts of *Pongamia pinnata* as an analgesic agent in rodents and our aim was also to study *in-vitro* cytoprotective effects of the various extracts from leaves of the plant.

MATERIALS AND METHODS

Plant material

Based on ethno pharmacological information, leaves were collected from campus of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab and were authenticated by the botanist of Collaborative Ayurveda Research Centre, GADVASU, Ludhiana. Immediately after collection leaves were washed and dried under sunlight. The dried leaves were finely grounded into powder, weighed and kept for further analysis.

Extraction

Different types of extracts of the plant were prepared using various menstruum viz water, ethanol, acetone and chloroform by maceration technique. 100 grams

of powdered leaves was soaked in 1 litre menstruum at room temperature for 48-72 hours and stirred at frequent time intervals. After maceration, the extract was initially filtered using muslin cloth and then re-filtered again using Whatman filter paper No 1. The filtrate obtained was evaporated in oven at a temperature of 40°C. The residue obtained was lyophilized and kept at 4°C in air tight bottles until used. Percent yield for different solvents viz. water, ethanol, acetone and chloroform was found to be 8%, 3.8%, 3% and 2.8% respectively.

Animals

The present investigation was conducted on 50 rats aged 3-4 months, weighing 180-200 grams, at Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The animals were purchased from small animal colony, GADVASU. The animals were acclimatized to the environment for ten days before starting experiment and kept in cages under standard laboratory conditions of temperature (20 - 26°C), with a 12-h light cycle. All animals were fed commercial rat pellets procured from Ashirwad industries, Mohali (Punjab). Feed and water were provided ad libitum to the animals. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) vide reference no VMC/13/1786-1806 dated 4/4/13 and was conducted in accordance with ethical committee guidelines. Animals were divided into ten groups of five animals each. Group I served as control, group II as positive control and remaining groups served as test groups. Group III and IV were administered with aqueous extract of concentration 50 mg/kg and 100 mg/kg orally, respectively. Group V and VI were administered with alcoholic extract at concentration of 50 mg/kg and 100 mg/kg, respectively. Similarly group VII and VIII with acetone extract and group IX and X with chloroform extract having concentrations of 50 mg/kg and 100 mg/kg, respectively.

Analgesic activity

In this experiment group I served as control. In Group II meloxicam @ 1 mg/kg orally was given as standard drug. Rest of the groups were treated as described above. The tail flick method by D'Amour and Smith (1941) was used to calculate analgesic activity. A radiant heat automatic analgesiometer was used to induce analgesia and tail flick

reflex time was measured. The cut-off time of 10 seconds was used to avoid tail injury by heat. Reaction time was measured at duration of 0.5 h, 1 h, 1.5 h, 2 h, 3 h and 4 h, respectively.

***In-vitro* cytoprotective activity**

The experimentation was done on Madin-Darby Bovine Kidney (MDBK) cell line procured from ATCC by the School of Animal Biotechnology, GADVASU, Ludhiana and maintained and sub-cultured in the Department of Pharmacology and Toxicology.

Chemicals and reagents

Dulbecco's modified Eagle's medium (DMEM as the cell culture media), Fetal bovine serum (FBS), Dulbecco's Phosphate Buffered Saline and Antibiotic–Antimycotic solutions were purchased from HiMedia Laboratories Pvt. Ltd. (India). Trypsin-EDTA, trypan blue (for the cell viability and cell count) was procured from Sigma Chemical Co. (St Louis, MO, USA). Dimethylsulphoxide (DMSO) and ethanol (96%) were purchased from Merck (Darmstadt, Germany). Sterile culture flasks and culture plates were procured from Nest Biotech Co. Ltd, China. All other chemicals used were of analytical grade and purchased from standard manufacturers. All buffers and reagents were prepared in autoclaved Milli-Q water and filtered through membrane filters (0.22 µm) purchased from Millipore (Molsheim, France).

Standard preparation

Stock solution of thiamethoxam (Sigma-aldrich) was prepared (100 mg/mL) in DMSO and further working standards were prepared in DMEM supplemented with 10% of FBS. The inhibitory concentration to be used in the study was taken from the IC₂₅ values of thiamethoxam (307.7µM) already standardized in our laboratory.

Stock medium and working medium

Appropriate quantity of powder DMEM was dissolved in 700 ml of autoclaved triple distilled water; 3.7 g of NaHCO₃ was added and then sterilized by passing through 0.22 µm membrane filter as per the manufacturer protocol. The filtered stock was incubated at 37°C for 48 hr to check sterility and stored at 4°C till further use.

Cell culture

A frozen MDBK (Madin-Darby Bovine Kidney) cell line was obtained from the School of Animal Biotechnology, GADVASU, Ludhiana. Frozen vial was quickly thawed in a 37°C water bath, resuspended in 5 ml of culture medium-DMEM and centrifuged at 1000 rpm for 5 minutes and cells were removed from the 10% DMSO solution in which they were frozen. Final cell pellet was resuspended in appropriate quantity of complete culture medium (DMEM with 10% FBS, 1% antibiotic-anti-mycotic solution) and plated on 25 cm³ and 75 cm³ culture flask. Cell flasks were kept for cell growth in CO₂ incubator having a humidified atmosphere with 5% CO₂ at 37 °C. Flask was routinely monitored (every 12 hrs) for microbial contamination and cell growth pattern under inverted microscope and media was replaced when change in pH of medium was noticed.

Cell viability

Diluted cell suspension of 20 µl was taken in a PCR tube and to this 180 µl of 0.1% trypan blue dye in PBS was added and immediately charged in the hemocytometer. It was allowed to stand for 1 min. The slide was examined under light microscope at 10x magnification to count the live (bright) and dead (blue) cells in 64 secondary squares (4 large corners squares). The following calculations were then made:

1. Average number of cell = Total count/4
2. Total viable cell/ml = (Average number of viable cells) × 10⁴ × 10 (Dilution Factor)

The cell concentration was adjusted 1×10⁵ cells/mL of culture medium. Cells were grown in culture flasks using DMEM supplemented with 10% FBS, 1% antibiotic–antimycotic solution in a humidified atmosphere with 5% CO₂ at 37 °C. When the cell culture had more than 80% confluency, cells were dispersed with 0.25% trypsin–0.02% EDTA and were subcultured. Before starting the experiment, the cells were trypsinized and single cell suspension was prepared. Cell number and cell viability was determined by the trypan blue dye exclusion method. Cell suspensions with viability of more than 95% were used in the experiments.

Experimental design

In the study, thiamethoxam was used to induce cytotoxicity

in the MDBK cells. For this the experiment was divided into eleven cohorts. Cohort I served as control and Cohort II served as the DMSO control. Cohort III was exposed to IC₂₅ concentration of thiamethoxam for 24 hours. For this purpose, MDBK cells were plated at a concentration of 2 × 10⁴ cells/mL in 24 well culture plates and incubated at 37°C in 5% CO₂ in humidified incubator for atleast 12 h before treatment. Cohorts IV, VI, VIII and X were treated with the different extracts alone — aqueous, alcoholic, acetone and chloroform respectively (at the concentrations of 50mg/ml and 100 mg/ml). To evaluate the cytoprotective effect of the leaf extracts, cohorts V, VII, IX, XI were treated with the combined concentrations of aqueous + IC₂₅, alcoholic + IC₂₅, acetone + IC₂₅ and chloroform + IC₂₅. After exposure period, culture medium was separated out and adherent cells were treated with 0.2 ml cell lysis buffer (0.5 % Triton × 100 with NET: 100 mM NaCl, 1 mM EDTA and 20 mM Tris, pH 7.5) and supernatant was collected in microcentrifuge tube and stored at -20°C till further analysis. At the end of experiment, the evaluation of cytoprotective effect of extracts on MDBK cells was done by analysis of cell morphology and oxidative stress parameters.

Oxidative stress parameters

Oxidative stress parameters- glutathione peroxidase (GPx) and lipid peroxidation (LPO) were evaluated by using commercial kits (Bioxytech) while catalase (CAT) and superoxide dismutase (SOD) were evaluated by

Bergmeyer (1983) and Madesh and Balasubramanian (1998) respectively.

Statistical Analysis

Statistical analysis was done by one-way ANOVA at 5% level of significance using SPSS software.

RESULTS AND DISCUSSION

Analgesic activity

The results depicting the analgesic activity of various leaf extracts of *Pongamia pinnata* on wistar rats are given in the Table 1. Group I served as control and group II served as standard. In the standard group meloxicam @ 2 mg/kg b.wt was administered orally. In groups III, V VII and IX the aqueous, alcoholic, acetone and chloroform extracts were given respectively, @ 50 mg/kg whereas in group IV, VI VIII and X respectively these extracts were given @ 100 mg/kg orally. In group II, the effect started at 30 min., and achieving peak at 90 min. whereas in the control group, there was no increase in the reflex time. In the extract groups, III, IV, V, VI the activity started at 60 min., reaching its maximum at 90 min. it was observed that all the extract groups demonstrated analgesic activity, but maximum activity was recorded in group IV which displayed maximum increase in the tail flick response time. Triterpene alkaloids and steroidal principles present in the plant products are reported to have analgesic activity.

Table 1: Effect of different leaf extracts of *Pongamia pinnata* on analgesic activity of rats

| Group | Dose (mg/kg) | Reflex Time (in sec) values are expressed as Mean ±S.E | | | | | | |
|-------|--------------|--|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|------------------------|
| | | 0 min | 30 min | 60 min | 90 min | 120 min | 180 min | 240 min |
| I | | 0.34±0.02 ^b | 0.34±0.02 ^b | 0.32±0.02 ^f | 0.36±0.02 ^f | 0.36±0.02 ^f | 0.34±0.02 ^c | 0.34±0.02 ^c |
| II | 2 | 0.42±0.02 ^a | 0.42±0.08 ^{ab} | 2.08±0.08 ^a | 3.24±0.11 ^a | 2.96±0.20 ^a | 1.74±0.09 ^a | 1.14±0.12 ^a |
| III | 50 | 0.34±0.02 ^b | 0.34±0.02 ^{bc} | 0.38±0.02 ^{ef} | 1.82±0.04 ^{bc} | 0.96±0.06 ^f | 0.66±0.02 ^{cd} | 0.54±0.02 ^b |
| IV | 100 | 0.34±0.02 ^b | 0.34±0.03 ^{bc} | 0.70±0.03 ^d | 1.92±0.05 ^b | 1.76±0.04 ^b | 0.84±0.05 ^{cd} | 0.57±0.02 ^b |
| V | 50 | 0.34±0.02 ^b | 0.32±0.04 ^c | 0.60±0.04 ^c | 1.82±0.03 ^{bc} | 1.50±0.08 ^{cd} | 0.69±0.03 ^d | 0.54±0.02 ^b |
| VI | 100 | 0.34±0.02 ^b | 0.46±0.08 ^a | 1.74±0.08 ^b | 1.64±0.06 ^{cd} | 1.20±0.07 ^{ef} | 0.88±0.08 ^c | 0.54±0.05 ^b |
| VII | 50 | 0.34±0.02 ^b | 0.34±0.02 ^{bc} | 0.42±0.02 ^{ef} | 1.52±0.06 ^d | 1.51±0.06 ^{cd} | 0.80±0.05 ^{cd} | 0.52±0.02 ^b |
| VIII | 100 | 0.32±0.02 ^b | 0.34±0.02 ^{bc} | 0.38±0.02 ^{ef} | 1.34±0.05 ^e | 1.28±0.06 ^{de} | 0.82±0.04 ^{cd} | 0.56±0.02 ^b |
| IX | 50 | 0.34±0.02 ^{ab} | 0.32±0.02 ^c | 0.42±0.02 ^{ef} | 1.32±0.04 ^e | 1.00±0.04 ^f | 0.78±0.03 ^{cd} | 0.60±0.03 ^b |
| X | 100 | 0.36±0.02 ^{ab} | 0.38±0.04 ^{abc} | 0.50±0.04 ^e | 1.64±0.05 ^{cd} | 1.56±0.04 ^{bc} | 1.14±0.12 ^b | 0.66±0.02 ^b |

Values lacking a common superscript in given column differ significantly from each other (p<0.05).

Also similar results were obtained by Sagar *et al.* (2010) in the study for evaluation of analgesic activity of methanolic extract of the stem bark of the plant. In agreement to the present study, Rao *et al.* (2007) reported the analgesic activity of leaf extracts of *Pongamia pinnata* in albino mice. In the study conducted by Singh *et al.*, 2017, analgesic activity was observed in the aqueous and alcoholic leaf extracts of *Kigelia africana*. Also Marzouk *et al.* (2011) reported the peripheral analgesic activity of seed and fruit extracts of *Citrullus colocynthis*.

In-vitro cytoprotective activity

Table 2 reveals the effects of the different leaf extracts (at concentrations 5% and 10%) of the plant on catalase levels. It was seen that the value of catalase decreased significantly to 8.47 ± 0.01 nM H₂O₂ utilized/min/mg protein in the thiamethoxam treated group as compared to the value in control group (21.15 ± 0.01). Oxidative stress induced by neonicotinoid insecticide thiamethoxam was the reason behind the decrease in catalase values. No significant changes were obtained in the value of catalase enzyme in the cohorts IV, VI, VIII and X, in comparison to the control group, which were exposed to the different concentrations of leaf extracts – aqueous, alcoholic, acetone and chloroform respectively revealing that no toxicity is induced by the extracts.

Table 2: Effect of different leaf extracts of *Pongamia pinnata* on catalase levels (nM H₂O₂ utilized/min/mg protein) in thiomethoxam- induced oxidative stress in MDBK cell lines

| COHORTS | Catalase values (5% plant extract conc.) | Catalase values (10% plant extract conc.) |
|---------|--|---|
| CONTROL | 21.15 ± 0.01 ^{ab} | 21.15 ± 0.01 ^{ab} |
| DMSO | 20.48 ± 0.01 ^c | 20.48 ± 0.01 ^c |
| IC 25 | 8.47 ± 0.01 ^d | 8.47 ± 0.01 ^d |
| AQ | 21.13 ± 0.01 ^b | 21.16 ± 0.01 ^a |
| AQ+IC | 8.45 ± 0.01 ^d | 8.44 ± 0.02 ^d |
| ALC | 21.13 ± 0.01 ^b | 21.12 ± 0.01 ^b |
| ALC+IC | 8.46 ± 0.02 ^d | 8.45 ± 0.01 ^d |
| ACE | 21.18 ± 0.01 ^a | 21.17 ± 0.01 ^a |
| ACE+IC | 8.45 ± 0.01 ^d | 8.44 ± 0.01 ^d |
| CHL | 21.15 ± 0.01 ^b | 21.14 ± 0.01 ^{ab} |
| CHL+IC | 8.46 ± 0.01 ^d | 8.47 ± 0.02 ^d |

Values lacking a common superscript in given column differ significantly from each other (p<0.05).

Also in comparison to the cohort III, there were no significant changes obtained in the values of catalase in the cohorts treated with the combination of thiamethoxam and different extracts-: aqueous, alcoholic, acetone and chloroform respectively at their lower and higher concentrations. So, no amelioration in the toxicity induced by thiamethoxam in MDBK cell lines was observed in the cohorts treated with different concentrations of the leaf extracts.

Table 3 depicts the effects of the different leaf extracts (at concentrations 5% and 10%) of the plant on lipid peroxidation levels. It was seen that the value of LPO increased significantly to 3.13 ± 0.01 nm of TBARS/mg of protein in the thiamethoxam treated group as compared to the value in control group (0.35 ± 0.02). No significant changes were obtained in the values of lipid peroxidation in the cohorts IV, VI, VIII and X, in comparison to the control group, which were exposed to the different concentrations of leaf extracts – aqueous, alcoholic, acetone and chloroform respectively revealing that no toxicity is induced by the extracts.

Table 3: Effect of different leaf extracts of *Pongamia pinnata* on LPO (nm of TBARS/mg of protein) levels in thiomethoxam-induced oxidative stress in MDBK cell lines

| COHORTS | LPO values (5% plant extract conc.) | LPO values (10% plant extract conc.) |
|---------|-------------------------------------|--------------------------------------|
| CONTROL | 0.35 ± 0.02 ^c | 0.35 ± 0.02 ^c |
| DMSO | 0.41 ± 0.01 ^b | 0.41 ± 0.01 ^b |
| IC 25 | 3.13 ± 0.01 ^a | 3.14 ± 0.01 ^a |
| AQ | 0.36 ± 0.005 ^c | 0.37 ± 0.005 ^c |
| AQ+IC | 3.15 ± 0.01 ^a | 3.16 ± 0.01 ^a |
| ALC | 0.33 ± 0.02 ^c | 0.35 ± 0.02 ^c |
| ALC+IC | 3.13 ± 0.01 ^a | 3.14 ± 0.01 ^a |
| ACE | 0.35 ± 0.02 ^c | 0.35 ± 0.02 ^c |
| ACE+IC | 3.14 ± 0.01 ^a | 3.15 ± 0.01 ^a |
| CHL | 0.36 ± 0.02 ^c | 0.36 ± 0.01 ^c |
| CHL+IC | 3.14 ± 0.01 ^a | 3.15 ± 0.01 ^a |

Values lacking a common superscript in given column differ significantly from each other (p<0.05).

Also in comparison to the cohort III there were no significant changes obtained in lipid peroxidation levels in the cohorts treated with the combination of thiamethoxam and different extracts- aqueous, alcoholic, acetone

and chloroform respectively at their lower and higher concentrations. So, no amelioration in the toxicity induced by thiamethoxam in MDBK cell lines was observed in the cohorts treated with different concentrations of the leaf extracts.

The results of the effects of the different leaf extracts (at concentrations 5% and 10%) of *Pongamia pinnata* on Superoxide Dismutase levels are shown in Table 4. It was seen that the value of SOD decreased significantly to 0.05 ± 0.003 U/mg protein in the thiamethoxam treated group as compared to the value in control group (0.25 ± 0.005). In comparison to the control group, no significant changes were obtained in the superoxide dismutase values in the cohorts IV, VI, VIII and X which were exposed to the different concentrations of leaf extracts – aqueous, alcoholic, acetone and chloroform respectively revealing that no toxicity is induced by the extracts when used alone. Also in comparison to the cohort III there were no significant changes obtained in the SOD levels in the cohorts V, VII, IX and XI. So, no amelioration in the toxicity induced by thiamethoxam in MDBK cell lines was observed in the cohorts treated with different concentrations of the leaf extracts.

Table 4: Effect of different leaf extracts of *Pongamia pinnata* on SOD (U/mg protein) levels in thiomethoxam- induced oxidative stress in MDBK cell lines

| COHORTS | SOD values (5% plant extract conc.) | SOD values (10% plant extract conc.) |
|---------|-------------------------------------|--------------------------------------|
| CONTROL | 0.25 ± 0.005^a | 0.25 ± 0.005^a |
| DMSO | 0.22 ± 0.01^b | 0.22 ± 0.01^b |
| IC 25 | 0.05 ± 0.003^c | 0.05 ± 0.003^c |
| AQ | 0.24 ± 0.008^{ab} | 0.25 ± 0.008^a |
| AQ+IC | 0.05 ± 0.006^c | 0.05 ± 0.006^c |
| ALC | 0.25 ± 0.005^a | 0.26 ± 0.01^a |
| ALC+IC | 0.06 ± 0.005^c | 0.06 ± 0.05^c |
| ACE | 0.23 ± 0.008^{ab} | 0.24 ± 0.01^{ab} |
| ACE+IC | 0.06 ± 0.008^c | 0.06 ± 0.008^c |
| CHL | 0.24 ± 0.01^{ab} | 0.24 ± 0.01^{ab} |
| CHL+IC | 0.06 ± 0.006^c | 0.06 ± 0.006^c |

Values lacking a common superscript in given column differ significantly from each other ($p < 0.05$).

The results of the effects of the different leaf extracts (at concentrations 5% and 10%) of *Pongamia pinnata* on

Glutathione peroxidase levels are shown in Table 5. It was seen that the value of GPx decreased significantly to 4.84 ± 0.01 mU/mg protein in the thiamethoxam treated group as compared to the value in control group (10.60 ± 0.01). In comparison to the control group, no significant changes were obtained in the Glutathione peroxidase values in the cohorts IV, VI, VIII and X which were exposed to the different concentrations of leaf extracts – aqueous, alcoholic, acetone and chloroform respectively revealing that no toxicity is induced by the extracts themselves. Also in comparison to the cohort III there were no significant changes obtained in the GPx levels in the cohorts treated with the combination of thiamethoxam and different extracts- aqueous, alcoholic, acetone and chloroform respectively at their lower and higher concentrations. So, no amelioration in the toxicity induced by thiamethoxam in MDBK cell lines was observed in the cohorts treated with different concentrations of the leaf extracts.

Table 5: Effect of different leaf extracts of *Pongamia pinnata* on GPX levels (mU/mg protein) in thiomethoxam- induced oxidative stress in MDBK cell lines)

| COHORTS | GPX values (5% plant extract conc.) | GPX values (10% plant extract conc.) |
|---------|-------------------------------------|--------------------------------------|
| CONTROL | 10.60 ± 0.01^b | 10.59 ± 0.01^{bc} |
| DMSO | 11.05 ± 0.02^a | 11.06 ± 0.02^a |
| IC 25 | 4.84 ± 0.01^c | 4.84 ± 0.01^d |
| AQ | 10.59 ± 0.01^b | 10.57 ± 0.01^{bc} |
| AQ+IC | 4.80 ± 0.01^c | 4.82 ± 0.01^d |
| ALC | 10.56 ± 0.03^b | 10.57 ± 0.01^b |
| ALC+IC | 4.80 ± 0.01^c | 4.83 ± 0.01^d |
| ACE | 10.58 ± 0.01^b | 10.57 ± 0.01^{bc} |
| ACE+IC | 4.80 ± 0.01^c | 4.83 ± 0.01^d |
| CHL | 10.60 ± 0.02^b | 10.56 ± 0.003^c |
| CHL+IC | 4.82 ± 0.01^c | 4.81 ± 0.01^d |

Values lacking a common superscript in given column differ significantly from each other ($p < 0.05$).

CONCLUSION

Study on analgesic activity revealed the presence of dose dependent effect in all extracts with highest effect in aqueous extract of *Pongamia pinnata* when compared to the control group. Present study also revealed no significant differences in the activity of all the four

oxidative stress parameters as compared to standard group in which toxicity was induced which suggested absence of any ameliorative effect of the different methods of leaf extracts of the plant. Further studies can be done to separate the bioactive constituents responsible for the pain relieving effect present in the leaf extracts thereby contributing to the development of the newer analgesic drugs from plant origin which can help in curbing the adverse effects caused by the use of Steroidal and Non-Steroidal Anti-inflammatory drugs.

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