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# Evaluation of Antioxidant and Hepatoprotective Potential of *Premna Tomentosa* L. In Albino Wistar Rats.

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**ABSTRACT**

In the present study the antioxidant activity of alcoholic extract of *Premna tomentosa* L. root (AT) and hepatoprotective activity of alcoholic extract of aerial parts *Premna tomentosa* L. by inducing hepatic injury with D-galactosamine hydrochloride in rats was carried out. The lyophilized alcoholic extract of *Premna tomentosa* ranging from concentration of 1000µg/ml to 10µg/ml was used to assess the antioxidant activity by various methods. For hepatoprotective activity, the alcoholic extract of aerial parts of *Premna tomentosa* at the dose of 300 mg/kg and 500 mg/kg were administered orally for 10 days. Silymarin (100 mg/kg), a known hepatoprotective drug was used as a standard drug for comparison. The blood samples were estimated for Alanine aminotransaminase (ALT) and Aspartate aminotransaminase (AST), Alkaline Phosphatase (ALP), Total Bilirubin (TB), Total Protein (TP) and Albumin. The liver samples were collected, weighed and processed for histopathology. The results showed good antioxidant activity and significant ($P<0.001$) reduction in the biochemical parameters in rats treated with *Premna tomentosa* . Histo-pathological section showed similar trend in all the groups. The alcoholic extract of aerial parts of *Premna tomentosa* L. exerts hepatoprotective activity and it can be attributed to the antioxidant principles which scavenge the free radicals responsible for pathological severity.

**Keywords:** *Premna tomentosa*, Antioxidant, D-galactosamine hydrochloride, Hepatoprotective, Silymarin etc

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## INTRODUCTION

Liver is the major organ participating in the metabolic activity of the body. It is involved with almost all the pathways related to growth. It supplies energy and fight against diseases. Liver is often abused by environmental toxins, alcohol, and over the counter drugs, that damages the liver leading to various health problems such as hepatitis, cirrhosis and alcoholic liver disease. Liver diseases remain one of the serious health problems [1].

Various medicinal plants such as *Eclipta prostrata, Emblic myrobalan, Phyllanthus niruri, Erythrina indica* etc., have shown good hepatoprotective property [2]. One such plant *Premna tomentosa.* L. (Verbinaceae), commonly called as *Pidanganari* in Tamil is used extensively in the Indian traditional system of medicine against liver, spleen and stomach disorders. *P. tomentosa* is a moderate sized deciduous tree with a tawny yellow stellate tomentum. Bark is light grayish brown [3]. Therefore, the study was undertaken to scientifically validate the antioxidant potential and influence of the alcoholic extract of *Premna tomentosa* on D-galactosamine hydrochloride induced liver damage in rats.

## MATERIALS AND METHOD

### Collection of plant materials and plant extraction

The freshly collected plants materials were dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight container. The dried and powdered material (500g) was dissolved in ethanol. The dissolved extract was then lyophilised using lyophilizer (labconco freezone 4.5) at -50$^{o}$C and 0.020mBar pressure for three days. Later, the lyophilised material (5mg/ml) was used for the preparation of the drug ranging from concentration of 1000µg/ml to 10µg/ml. This was further used to assess the antioxidant activity.

### Antioxidant activity

### Inhibition of DPPH radical

The free radical scavenging activity of plant extracts was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method of Blois (1958) [4] and Gomez-Alonso *et al.*, (2003) [5] Butylated hydroxyanisole (BHA) was used as the reference material. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. All the tests were performed in triplicate and the graph were plotted with the average of three observations.

### ABTS radical cation decolorisation assay

In this assay, the oxidant is generated by persulfate oxidation of 2, 2,-azinobis (3-ethylbenzoline-6-sulfonic acid)-($ABTS^{2-}$) as described by Re *et al.*, (1999) [6]. All the tests were performed in triplicate and the graph were plotted with the average of three observations.

### Reducing power / FRAP (Ferric reducing antioxidant potential assay)

The reducing power of plant extracts was determined according to the method of Oyaizu (1986) [7]. Butylated hydroxyl toluene (BHT) was used as the reference material. All the tests were performed in triplicate and the graph were plotted with the average of three observations.

### *In vitro* anti-lipid peroxidation assay using TBARS

Thiobarbituric acid reactive species (TBARS) assay was performed as described by Smail Aazza *et al.,* (2011) [8]. Egg yolk homogenates were used as a lipid-rich medium obtained as described elsewhere [9]. Vitamin E in ethanol (10 - 1000 µg/ ml) was used as Standard control. The antioxidant capacity was determined from three replicates and the graph was plotted with the average of three observations. The percentage antioxidant index was plotted against the concentrations of samples and $IC_{50}$ values were determined. Same amount of deionized water was used as the control.

**Total Phenolic Content (TPC)**

Total phenolic content (TPC) from extracts were quantified using Folin - Ciocalteu's method adapted to 96-well microtitre plate with minor modifications [10]. The assay was repeated twice and Total phenolic contents (average of two) were expressed as BHT equivalent per gram of lyophilized extract.

**Acute toxicity and Hepatoprotective activity**

**Animals**

Adults albino wistar rats (weighing 160-200 gm), bred in the animal house of Central Research Institute for Siddha, chennai were used for the study. Animals were housed under a standard 12 h:12 h light/ dark cycle and were provided with food and water *ad libitum* The experiments were performed between 10.00 and 12.hrs.All the experimental protocols were approved by Institutional Animal Ethics Committee and the study was conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

**Drugs and chemicals**

The drugs and chemicals used were Silymarin (Sigma) and D-galactosamine hydrochloride (Sisco Research Laboratories Pvt. Ltd., Mumbai).The kit for all biochemical estimations were purchased from Bayer Pvt. Ltd., Mumbai. The solvents and other reagents were of analytical grade.

**Acute toxicity**

Acute oral toxicity of *Premna tomentosa* was determined using Swiss albino mice (25-30g) of either sex as per the OECD 423 guidelines. The animals were selected and equally divided into two groups each comprising of six animals (Three male and three females) and were fasted 4 hours prior to the test. One group was treated with alcoholic extract of *Premna tomentosa* at the dose of 3000 mg/kg using Tween 80 as the vehicle and the other group was administered 0.2 ml of Tween 80. Animals were observed for 24 hours for mortality and thereafter kept under observation for 14 days.

**Hepatoprotective activity**

In the present study alcoholic extract of aerial parts of *Premna tomentosa* against hepatic injury was evaluated by using D-galactosamine hydrochloride induced liver damage in rats as a model. Thirty rats were equally divided into five groups. The Group I and II were served as toxic and normal control respectively, which were administered with 0.2 ml of Tween 80 for 10 days. Group III, IV and V were treated with standard drug Silymarin (100 mg/kg), and test drug *Premna tomentosa* 300 mg/kg and 500 mg/kg respectively for 10 days. All the groups were administered with hepatotoxicant D-galactosamine hydrochloride intraperitonially @ 400 mg/kg [11] on 9$^{th}$ day except in Group II which was untreated control. On 11$^{th}$ day of the experiment the blood samples were collected by retro-orbital sinus and were estimated for biochemical parameters such as AST, ALT, ALP, TB, TP, Albumin and Globulin by using a semi autoanalyser. After blood collection, all the animals were euthanized under ether anesthesia. All rats were subjected for gross lesion on liver and the liver was collected, weighed and preserved in neutral buffered 10% (V/V) formalin for histopathology. These then processed for paraffin embedding using ethyl alcohol as dehydrant and xylene as clearing agent. Paraffin sections of liver, about 4-5 µm thickness, were stained with haematoxylin and eosin. These sections were examined for histopathological changes and the cellular alterations were scored as described by (Hegde et al., 1982) [12].

**Statistical analysis**

The data were subjected to statistical analysis by Dunnet't' test.

## RESULTS AND DISCUSSION

### Antioxidant Activity

The extract showed good antioxidant activity in all in vitro free radical scavenging models (figure.1-5) 50% inhibition in DPPH, ABTS, TBARS and FRAP was observerd at 40 µg/ml, 60 µg/ml 500 µg/ml and 10 µg/ml respectively (Figure.1-4). Determination of total phenolic compounds showed 100 µg/ml *Premna tomentosa* extract contains 136.7736 µg BHT equivalent of total phenol. (Figure.5)

Free radicals are chemical entities that can exist separately with one or more unpaired electron. The propagation of free radicals can bring about thousands of reactions and thus may cause extensive tissue damage. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation etc [13]. Free radicals induce lipid peroxidation in poly unsaturated lipid rich areas such as liver and brain [14]. In this study *in vitro* lipid peroxidation was carried out by Thiobarbituric acid reactive species (TBARS) assay method. Alcoholic extract of *Premna tomentosa* showed dose dependent prevention towards generation of lipid peroxides (Fig.4).

**Figure 1: DPPH radical-scavenging activity of lyophilized PT extract and BHA. Decrease in mean absorbance with increase in concentration of the reaction mixture indicates the increase in oxidising power and % Radical Scavenging.**

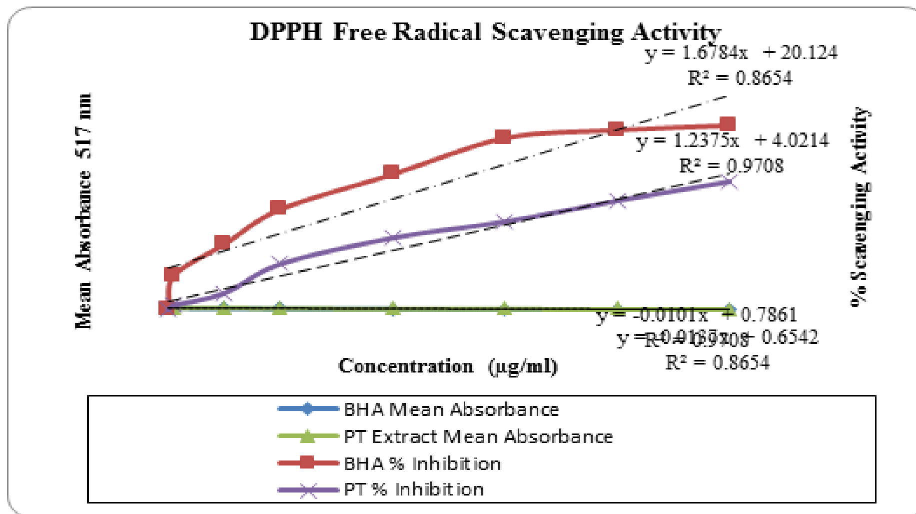



**Figure 2: ABTS radical-scavenging activity of lyophilized PT herbal preparations and Gallic acid. Decrease in mean absorbance with increase in concentration of the reaction mixture indicates the increase in oxidising power and % Inhibition.**

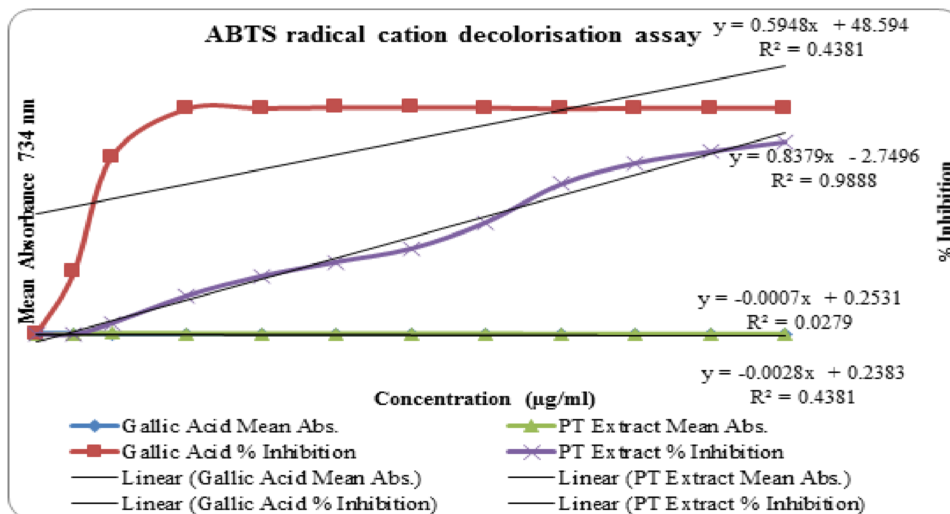


**Figure 3: Ferric reducing activity of lyophilized PT herbal and BHT- butylated hydroxytoulene. Increase in absorbance of the reaction mixture indicates the increase in reducing power.**

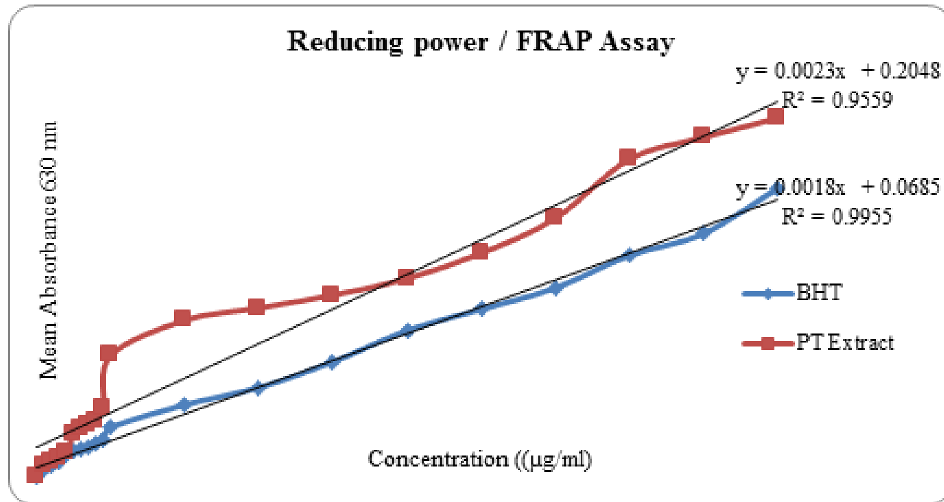



**Figure 4: In vitro anti-lipid peroxidation assay using TBARS assay of PT extract and vit. E**

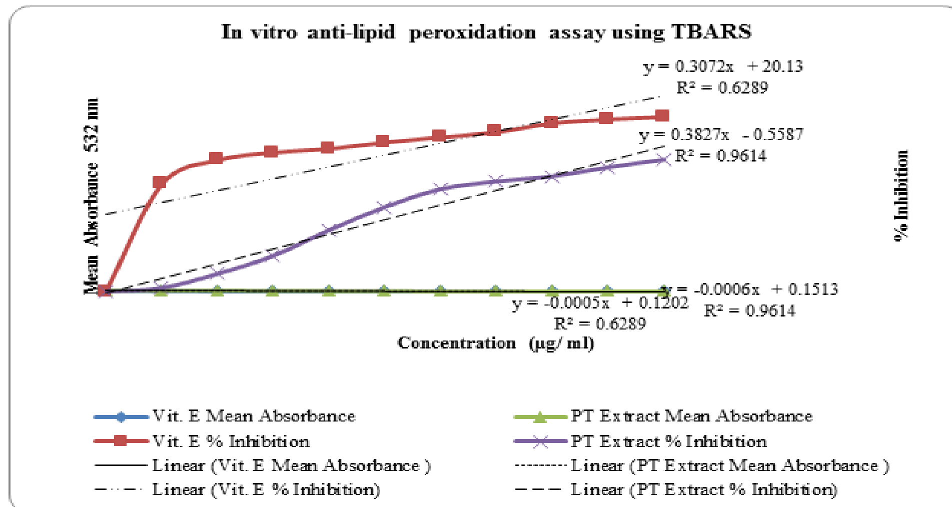
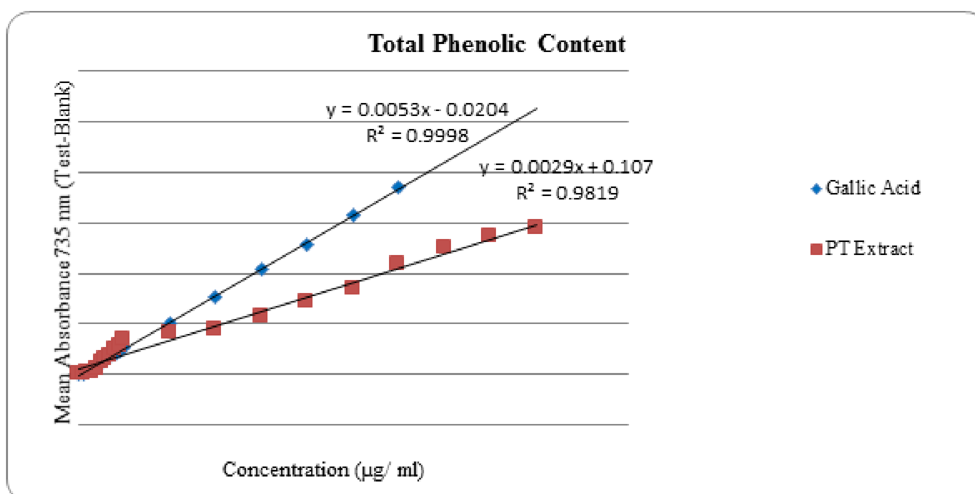



**Figure 5: Total Phenolic Content (TPC)**



A substance may act as antioxidant due to its ability to reduce reactive oxygen species by donating hydrogen ion [15] [16]. The reducing property of alcoholic extract of *Premna tomentosa* implies that it is capable of donating hydrogen atom in a dose dependent manner. The high content of phenolic compounds in the extract may be a contributing factor towards antioxidant activity as the phenolic compounds are known to have direct antioxidant property due to the presence of hydroxyl groups, which can function as hydrogen donor [17] [18]

**Acute toxicity**

No mortality, morbidity, weight loss or abnormal behavior was recorded after single exposure of the test compound @ 3000 mg/kg body weight during 14 days experimental period in Swiss Albino mice. This indicates that *Premna tomentosa* is safe up to a dose of 3000 mg/kg body weight.

**Hepatoprotective activity**

Effect of alcoholic extracts of aerial parts of *Premna tomentosa* D-galactosamine hydrochloride induced liver damage in rats with reference to biochemical changes in serum is shown in Table 1. The toxic control group treated with Tween 80 showed a marked increase in serum TB, AST, ALT and ALP and a decrease in TP , albumin, and globulin indicating the liver injury caused by D-galactosamine hydrochloride. Whereas, Group V animals exhibited a significant ($P < 0.01$) decrease in AST, ALT, TB, and ALP, and significant ($P < 0.01$) increase in serum albumin and increase TP and globulin level. Animals treated with 300 mg/kg of the extract exhibited a significant ($P < 0.01$) decrease in serum AST, ALT, TB, and ALP and nonsignificant increase in TP, albumin, and globulin. Reduction in the liver enzyme and increase in serum protein in test groups were in comparison with standard drug Silymarin which showed a significant decrease in serum liver enzymes and significant increase in TP and albumin and increase in globulin level.

**Table 1. Biochemical parameters of alcoholic extracts of *Premna tomentosa* in D-galactosamine hydrochloride induced liver damage in rats.**

| Group | AST | ALT | ALP | Total Bilirubin | Total protein | Albumin | Globulin |
|---|---|---|---|---|---|---|---|
| **Toxic control** | 388.16 ± 25..90 | 416.83 ± 31.18 | 223.16 ± 5.98 | 0.476 ± 0.019 | 5.55 ± 0.11 | 3.1 ± 0.16 | 2.50 ± 0.27 |
| **Normal control** | 153.66* ± 1.40 | 66.66* ± 2.43 | 146.83* ± 4.19 | 0.293* ± 0.022 | 6.38* ± 0.34 | 3.35* ± 0.14 | 3.03 ± 0.22 |
| **Standard** | 259.50* ± 4.40 | 82.83* ± 3.14 | 133.000* ± 8.69 | 0.331* ± 0.010 | 7.13* ± 0.17 | 3.95* ± 0.07 | 3.18 ± 0.101 |
| ***P.tomentosa* (300mg/kg)** | 266.33* ± 14.24 | 165.66* ± 10.93 | 184.83* ± 8.32 | 0.371* ± 0.02 | 5.86 ± 0.15 | 2.85 ± 0.19 | 3.01 ± 0.074 |
| ***P.tomentosa* (500mg/kg)** | 179.00* ± 12.07 | 91.83* ± 6.28 | 173.66* ± 6.22 | 0.373* ± 0.024 | 6.31 ± 0.11 | 3.33* ± 0.14 | 2.98 ± 0.205 |

**Table 2. Histopathological score of alcoholic extracts of *Premna tomentosa* in D-galactosamine hydrochloride induced liver damage in rats.**

| Group | Score for Histopathology |
|---|---|
| Hepatotxic control | 2.000 ± 0.00 |
| Normal control | 0.00 ± 0.00 |
| Standard | 1.166 ± 0.116 |
| *P.tomentosa* (300 mg/kg) | 1.166 ± 0.4014 |
| *P.tomentosa* (500mg/kg) | 0.500 ± 0.223 |

Histopathological score for all the groups are illustrated in Table 2. Histologically, Group II animals showed normal hepatic architecture (Figure.6), the Group I animals exhibited hepatocellular degenerations (Figure.7). Moderate degenerations and hypertrophy of hepatocytes (Figure.8) were observed in Group IV animals treated with 300 mg/kg alcoholic extract of Premna *tomentosa*. However, the Group V rats treated with 500 mg/kg of the extracts exhibited significant liver protection against D-galactosamine hydrochloride

induced liver damage, as evident by the presence of normal hepatic cords and mild degenerations (Figure.9). Group III animals treated with Silymarin animals showed a normal hepatic architecture (Figure.10) with mild degenerations. The present investigation indicates that both the doses of alcoholic extracts of aerial parts of *Premna tomentosa* possess significant protection against D-galactosamine hydrochloride induced hepatotoxicity in rats.

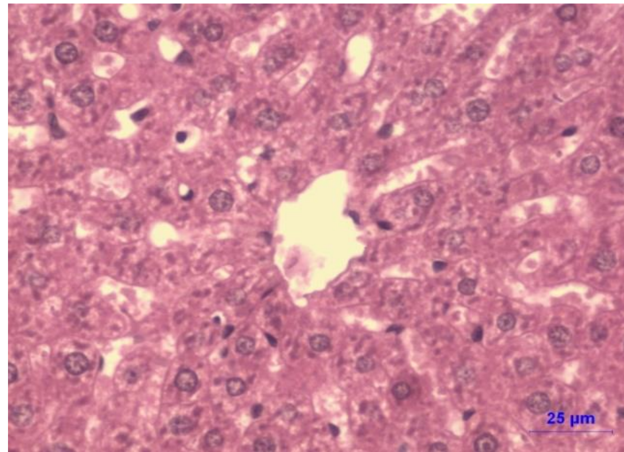



**Figure 6: Section of the liver showing normal histology in normal rats.**

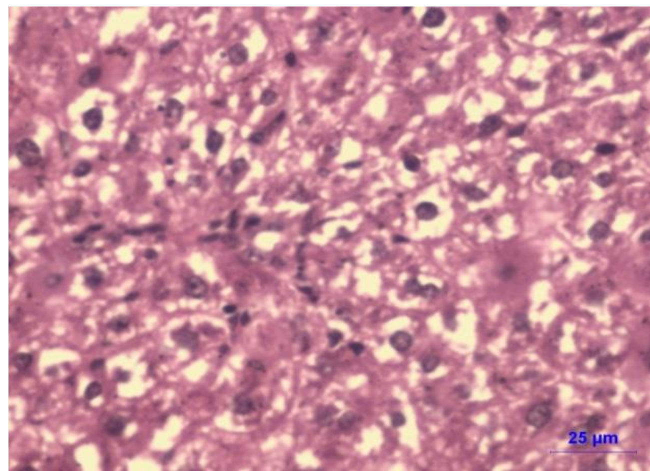



**Figure 7: Section of liver showing hepatocellular degeneration in D-galactosamine hydrochloride treated rats.**

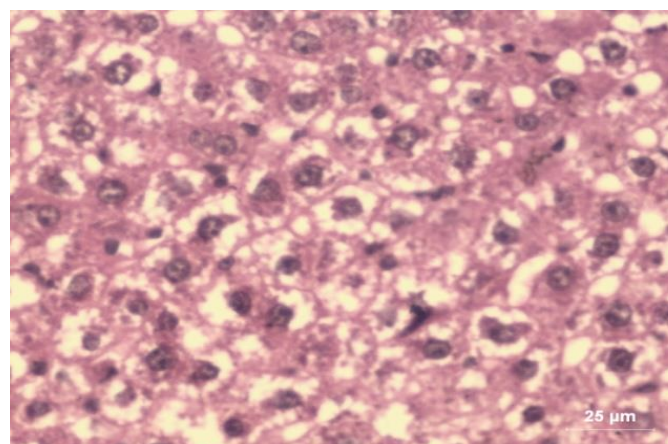



**Figure 8: Section of the liver showing Moderate degenerations and hypertrophy of hepatocytes in *P. tomentosa* (300 mg/kg) treated rats.**

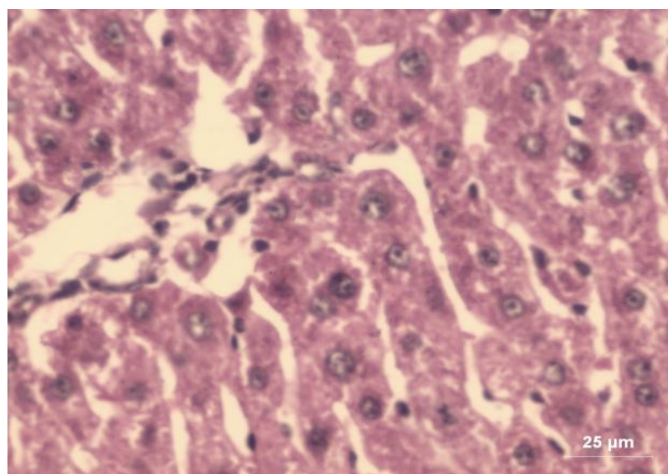



**Figure 9: Section of the liver showing normal hepatic cords and mild degenerations in *P. tomentosa* (500 mg/kg) treated rats.**

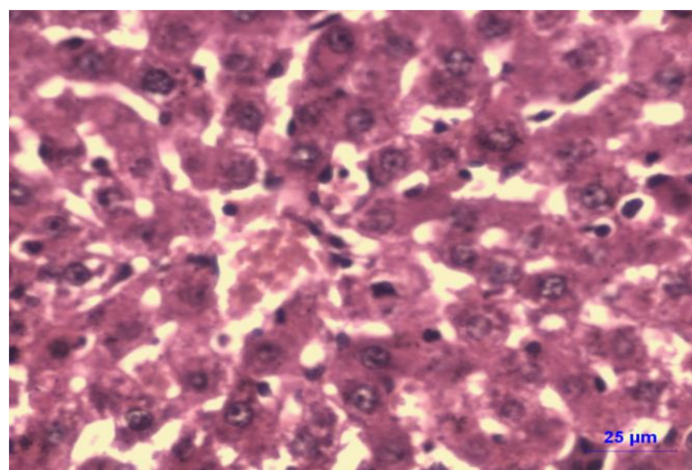



**Figure 10: Section of the liver showing normal histology with mild degeneration in Silymarin treated rats.**

The liver injury produced by the D-galactosamine hydrochloride histologically resemblance with human viral hepatitis. It causes the depletion of uridine nucleotides and thereby diminishes the synthesis of RNA and plasma membrane proteins [19]. Significant increase in the level of liver enzymes and total bilirubin, a marked reduction in the total protein in serum was observed in Group I, as an index of liver damage produced by D-galactosamine hydrochloride. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in liver [20]. Considerable decrease in the activities of liver enzymes and total bilirubin, increase in the level of total protein and improvement of normal hepatic architecture in the *Premna tomentosa* pre-treafigted rats clearly indicates the protection offered by pre-treatement with the alcoholic extract of *Premna tomentosa* and thereby, suggests a hepatoprotective property. But further studies are necessary to isolate and identify the compounds responsible for the activity.

## CONCLUSION

With the results of the above experiments, it may be concluded that alcoholic extract of aerial parts of *Premna tomentosa* possess significant scavenging effect on the free radicals and inhibits peroxidation of lipid components and thereby acts as effective antioxidant principle which can be attributed to its hepatoprotective property,Further, studies are needed not only to confirm but also to isolate and characterize the active principle responsible for the activity. In addition, it is necessary to evaluate the teratogenicity and the long term toxicological aspects of the plant.

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