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Research Article

Pharmacognostic, Phytochemical and Hepatoprotective Activity of the Leaves of *Mirabilis jalapa*

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ABSTRACT

Recent regular research on medicinal plants is producing remarkable and significant results. Thus, there is lot of scope to find new outcomes from plants like *Mirabilis jalapa* - a plant which need more investigation in the area of medicine and medical science. The present study deals with the establishment of organoleptic microscopic and phytochemical profiling of *Mirabilis jalapa* leaves, along with hepatoprotective activity on leaf extract against CCL₄. The total phenolic content was found to be 1.36 ± 0.02 mg/gm of dried leaf extract equivalent to Gallic acid and flavonoids content of the extract was found to be 4.41 ± 0.02 mg/gm. The successively extracted extract showed the IC₅₀ value against the DPPH free radical. Methanolic extract of leaves shows very significant hepatoprotective activity at 200mg & 400mg when compared with standard. Based on the result, the pharmacognostic profiling of the *Mirabilis jalapa* leaves was successfully established. Present finding also suggested that it could be a source of potent antioxidant drugs as leaves are also hepatoprotective in nature.

Keywords: *Mirabilis jalapa*, Nyctaginaceae, phenolic content, flavonoids content, Antioxidant.

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INTRODUCTION

Written shreds of evidence are found from the time of ancient Chinese, Indian, and North African civilizations for the use of natural sources for curing the various ailment. About 25% of medicines used today are derived from natural products. Natural products have evolved for self-defence. Ancient civilizations conducted various experiments using various plant and animal parts to find out what biological activity it had. Extracts of herbal medicines could lead to the discovery of other active ingredients, which could ultimately lead to the development of chemically pure drugs where would have beneficial effects. All-natural products discovery must start with a mixture of different compounds from natural sources, isolation and refinement. Medicinal plants are used as herbal medicine in traditional medicine for various types of diseases. They are used in the manufacture of modern pharmaceuticals or as a source of raw materials. It is alkaloids, saponins, flavonoids, tannins, terpenoids, phenolic compounds, steroid glycosides in bioactive phytochemical components. These chemical components form the backbone of the drugs.

Nowadays, natural products are obtained from various species of plants, birds, insects, bacteria, yeasts, and animals. *Mirabilis jalapa* Linn. (Family Nyctaginaceae), commonly known as 4'o clock, is also found in India, South America, the Philippines, and France. It is a popular ornamental houseplant that grows worldwide due to the beauty of flowers that can have a white, red, pink, purple, or multicolored, sweet scent, known as 'anthi-mandhaari' in Tamil, Gulbakshi in Gujrati, Naalumanipoovu in Malayalam, Beauty of night, 4 O'clock,

'Marvel of Peru' in English, 'Chandrakantha' in Telugu. The plant is a tall herbaceous plant, with opposite leaves, large showy flowers, curvaceous obovoid fruits, and prominent tuberous roots, and is planted as an ornamental household plant throughout the country.

Mirabilis jalapa is used by people in various countries as traditional medicine to treat diarrhea, dysentery, conjunctivitis, myalgia, swelling, inflammation, abdominal pain and muscle aches, the extract of which also has antibacterial, antiviral, and antifungal functions [2, 3,4].

MATERIALS AND METHODS

Plant material

Mirabilis jalapa leaves were collected from Sam Higginbottom Agricultural, Technology and Science University Pharmacy Herbal Garden in Prayagraj, in October 2015. The plant has been identified and authenticated by the National Botanical Institute (NBRI), Lucknow, Uttar

Pradesh, India (LWG-058) and the specimen is deposited and can be referenced in the future in department.

Macroscopy

For the development of the macroscopical monograph, we have scrutinized the size and shape, colour, surfaces, venation, apex, margin, base, lamina, texture, odour, and taste of fresh leaves.

Qualitative investigation

Microscopic evaluation was done by taking transverse sections of fresh leave cleared in chloral hydrate (removal of chlorophyll), mounted with glycerin, and observed under a compound microscope at a projection of 40x (40 times). The presence/ absence of epidermal cells (lower and upper), covering trichomes, xylem, phloem, stomata (type and distribution), and collenchyma were estimated, respectively. Lamina and midrib and a little quantity of the powder of leaves were also cleared, mounted, and observed [4,5].

Quantitative investigation

Quantitative microscopy of the leaf was performed to determine the vein islet number, vein termination number, stomata number, and stomatal index on epidermal strips. [6,7].

Physicochemical investigation

The number of physicochemical parameters of leaf powder such as acid insoluble ash, total ash, and moisture content was found out using the method reported by Sailor et al. with minor modification [8,9,10].

Preliminary phytochemical screening

Preliminary phytochemical screening was performed to determining the presence of Phyto-constituents in the different extracts. For the successive extraction, accurately weigh and extracted the plant material with hexane, ethyl acetate, chloroform, methanol, and ethanol.

After successive extraction of solvent, each extract is vacuum dried, weighed, and subjected to different qualitative chemical tests to establish the presence of a mixture of various phytoconstituents i.e. alkaloids, glycosides, carbohydrates, tannins, steroids, proteins, flavonoids, and saponins in the particular extract.

All extracts are observed for organoleptic properties and extractive values are calculated. [11]

Total Phenol Contents (TPC)

Total phenol content of extracts was calculated using the Folin-Ciocalteu method, which is predicated on the oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids. After oxidation, the green-blue complex was estimated at 750 nm. Briefly, in a test tube, 1 ml of the extract (1 mg/ml) was added to 0.5 ml of Folin-Ciocalteu reagent and 1.5 ml of sodium carbonate was added. After shaking, it was incubated for 30 minutes. Using gallic acid monohydrate, the standard curve was prepared and linearity was obtained in the range of 10-50 µg/ml. Using the standard curve for the estimation of total phenol content in

the extract was and values are expressed as gallic acid equivalent in mg/g of extract. TPC was expressed as mg acid equivalents (GAE) per gram extract. Values presented were the typical of three measurements.

Total Flavonoid Contents (TFC)

The flavonoid content was calculated using a reported method of Kumar et al., with minor modification, which is based upon the formation of a flavonoid-aluminum complex. One milliliter of the extract with a concentration of 1mg/ml was taken in a 10ml volumetric flask and distilled water was added to make up the volume to 5 ml. At zero time, At zero time, 0.3 ml of 5% (w/v) sodium nitrite was added to the flask. After 5 min, 0.6 ml of 10% AlCl₃ was added, and then at 6 min, 2 ml of 1M NaOH was also added to the flask, followed by volume makeup to 10ml using distilled water. Absorbance at 510 nm was read immediately. Quercetin was taken as a standard, and the total flavonoids level were determined in triplicate and expressed as quercetin equivalents in mg/100g of dry weight [12,13].

An *In-vitro* anti-oxidant assay using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) model

The ability to donate hydrogen atoms or electrons of the corresponding extract was metered from the bleaching of purple-colored methanol solution of DPPH. The ability of the extracts to scavenge the DPPH free radical was assessed according to the reported method of Kumar et al., with minor modification.

Briefly, 0.1mM solution of DPPH in 100% MeOH was added and 4 ml of sample solution was added in 40% MeOH at different concentrations (1-160 µg/mL). The mixture was shaken vigorously and kept for 30 min to incubate in the dark at room temperature until stable absorption values were obtained. The reduction of the DPPH radical was measured by continuously monitoring the decrease in absorption at 517 nm.

In the control, the substitution of 40% MeOH was made for samples. The lower absorbances of the reaction mixture indicated higher radical scavenging activity. The DPPH radical scavenging activity was calculated by the following given equation:

$$\text{Scavenging effect(\%)} = (1 - A_{\text{samples 517}} / A_{\text{control517}}) \times 100$$

Where a control is the absorbance of the control reaction that contains all reagents except the test compound) and A sample is the absorbance of the test compound. Quercetin was taken as standard. The IC₅₀ value is the concentration of the sample required to scavenge 50% of the DPPH free radical.[14,15,16]

IN-VIVO Hepatoprotective Activity

Carbon tetrachloride-induced liver toxicity

Wistar rats (160–200 g) of either sex were procured and were kept beneath controlled conditions of temperature (27 ± 20) °C and ratio 44%–56%, light/dark cycles of 12 h severally for one week before and through the experiments. Animals were provided with a standard rodent pellet diet (Amrut, India) and the food was withdrawn 18–24 h before the experiment though water was allowed *ad libitum*. The protocol for this study has been approved by the Institutional Animal Ethics Committee as per the guidance of committee for the purpose of control and supervision of Experiments on Animals CPCSEA, New Delhi, with number IEAC/ UIP/ March-2019/107

Experimental Procedure

Animals were divided into five groups ($n = 6$). Group I served as a vehicle control, which received liquid paraffin. Groups II-V were treated with CCl₄ in liquid paraffin (1:2) at the dose of 1 mL/kg body weight intraperitoneal once in every 72 h for 14 days [5]. Groups IV and V were treated with Ethanolic extract of *Mirabilis jalapa* leaf at the doses of 200 and 400 mg/kg body weight, respectively, and Group V was administered with silymarin at a dose of 25 mg/kg body weight orally. Ethanolic extract of *Mirabilis jalapa* leaf treatment was started 10 days prior to CCl₄ administration and continued until the end of the experiment.

Group I: CCl₄ control received liquid paraffin;

Group II: CCl₄ as natural recovery group (1 mL/kg, *i.p.*);

Group III: CCl₄ + treated with a daily dose of Silymarin (25 mg/kg body weight);

Group IV: CCl₄ + treated with a daily dose of Ethanolic extract of *Mirabilis jalapa* leaf (200 mg/kg, *p.o.*);

Group V: CCl₄ + treated with a daily dose of Ethanolic extract of *Mirabilis jalapa* leaf (400mg/kg, *p.o.*).[5]

Data Analysis

The results were analyzed statistically using two way analysis of variance (ANOVA) followed by Bonferroni post-tests to calculate the level of significance. Values are expressed as mean \pm SEM (Number of animals, $n=6$); significantly different at * $p<0.05$, ** $p<0.01$, *** $p<0.001$ when compared with the control group.

Evaluation of liver damage

At the end of the experiment, blood was collected from retroorbital plexus from the overnight fasted animals, after being anesthetized with 100 mg/kg ketamine, *i.p.* (23) The blood samples were taken with 20 μ l EDTA (5%) in each Eppendorf and centrifuged at 5000 rpm for 20 min (Sigma 3K30, UK).

The supernatant (serum) was separated using a micropipette and placed in new Eppendorf with well labeled and stored at -80°C for further analysis.

The homogenate was subjected to centrifugation at 4°C for 5 min at 3000 rpm and the supernatant was used for estimation of various oxidative stress markers. The opposite liver tissue specimens were used for histopathological examination.

RESULTS AND DISCUSSION

Macroscopic character

The macroscopic characters were useful in the rapid identification of plant material and also helped as a significant standardization parameter (26). Based on macroscopically, we can conclude, that the leaf of *Mirabilis jalapa* is alternate, entire, and having the symmetrical base. It is also having the serrulate margin, obtuse apex, pinnate type of venation, green upper epidermis, lower epidermis lighter in color with an aromatic odour and pungent taste.

Microscopic character

The microscopic character of leaves of *Mirabilis jalapa* were served as the diagnostic character and was helpful in the differentiation of species as well as identification of particular herbs. The transverse section passing through the midrib shows single-layered upper epidermis, lower epidermis, followed by single-layered palisade cells in the lamina portion. Vascular bundles were present in the midrib, the lower portion of the midrib is occupied by collenchymatous cells, covering trichomes, xylem, and phloem under the light microscope. Macroscopical and microscopical characters will help in the identification of the right variety and search for adulterants. The morphological characters of different plant parts can be served as diagnostic parameters. Microscopic evaluation is one of the simplest and cheapest methods for establishing the correct identification of the source of the materials ^{18,19,20,21}.



Figure 1: Transverse section of leaf at 10x



Figure 2: Veins at 40x

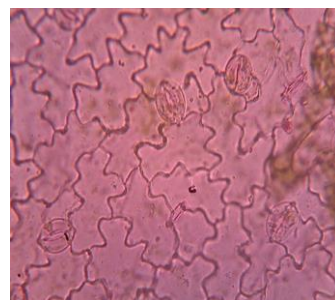


Figure 3: Stomata at 40x

Quantitative investigation

Table-1 demonstrated the different microscopical features of *Mirabilis jalapa* leaf. It showed the 46-50 stomata present in the lower surface of the leaf and 52-58 stomata present in the upper surface of the leaf.

Another feature of the leaf is the stomatal index, which was found in the lower epidermis 1.66-2.13 and upper epidermis 1.3-1.9.

The vein islet present in the leaf is 4-6 and vein termination found in the leaf is 6-10. S. No. Parameter Range
1. Vein islet number 4-6/mm²
2. Vein termination number 6-10/mm²
3. Stomata no. upper surface 46-50/mm²
4. Stomata no. lower surface 52-58/mm²
5.

Stomata index upper surface 1.66- 2.13
6. Stomata index lower surface 1.3- 1.9

Table 1: Quantitative leaf microscopy of *Mirabilis jalapa*

S. No.	Parameter	Range
1.	Vein islet no.	4-6/mm ²
2.	Vein termination no.	6-10/mm ²
3.	Stomata no. upper surface	46-50/mm ²
4.	Stomata no. lower surface	52-58/mm ²
5.	Stomata index upper surface	1.66- 2.13
6.	Stomata index lower surface	1.3- 1.9

Physicochemical parameters

Various physicochemical parameter of the powdered drug has been investigated for the confirmation of the drug purity. (27) The moisture content of drugs might be a minimum level to dispirit the reduction of bacteria, yeast, or fungi

through storage. The 0.67 %w/w moisture is present in the leaf sample and the ash value was found 0.74 %w/w along with acid insoluble ash 0.69 %w/w (table 2).

Ash values are used to find out the quality and purity of the unsophisticated drug. It indicates the presence of a mixture of impurities like carbonate, oxalate, and silicate. The acid-insoluble ash consists mainly of silica and indicates contamination with earthy material. (28)

The water-soluble ash is used to estimate the number and amount of inorganic elements present in drugs. The extractive values are valuable to estimate the chemical constituents present in the crude drug and assist in the evaluation of definite constituents soluble in a particular solvent. (29) Sample (powder) Bulk Density Tapped Density Car's Index LOD Ash value AIA Dried leaves 0.233 0.278 16.187 0.67% 0.74% 0.69%

Table 2: Physicochemical parameters of *Mirabilis jalapa* leaf powder

Sample (powder)	Bulk Density	Tapped Density	Car's Index	LOD	Ash value	AIA
Dried leaves	0.233	0.278	16.187	0.67%	0.74%	0.69%

Preliminary phytochemical screening

Successive extraction was performed on the leaf sample.

Successive extraction was done for the confirmation of the different phytoconstituent present in the different solvents, like hexane, ethyl acetate, chloroform, methanol, and ethanol.

After successive extraction, it was found that semi-solid mass of all extracts with a different odour like hexane, ethyl acetate, chloroform showed the characteristic odour, and ethanol showed the pungent odour.

On the comparison on the taste, hexane, ethyl acetate, chloroform were found bitter in taste and ethanol extract sweet in taste. Table 3 shows colour comparison, hexane, chloroform extract found the light green in colour and rest of the extract such as ethyl acetate, ethanol showed the dark green colour extract. (30)

The extractive value of the extract was found the difference, ethanol having the higher extractive value i. e. 12%. Other extracts such as hexane, ethyl acetate and chloroform showed 4.2%, 4.7% and 5.6%, respectively.

Table 3: Color, consistency, odour, taste and extractive values of successive solvent extraction and direct extraction of *Mirabilis jalapa* leaves

Name of extracts	Consistency	Odour	Colour	Extractive value
Hexane	Semi-solid	Characteristic	Light-green	4.2%
Chloroform	Semi-solid	Characteristic	Green	4.7%
Ethyl acetate	Semi-solid	Characteristic	Green	5.6%
Ethanol	Semi-solid/ sticky	Pungent	Dark- green	12%

Based on the chemical test, we can conclude that hexane extract showed the presence of the outcome of the extractive value of the powdered drug in different solvents obtained by successive extraction reported in. (31)

All extracts subjected to qualitative chemical test and results are compiled in table 4.

The result shows that maximum constituents found an ethanolic extract of *Mirabilis jalapa* including phenols, flavonoids, and terpenoids. So it can be helpful to extract out particular constituents by solvent.

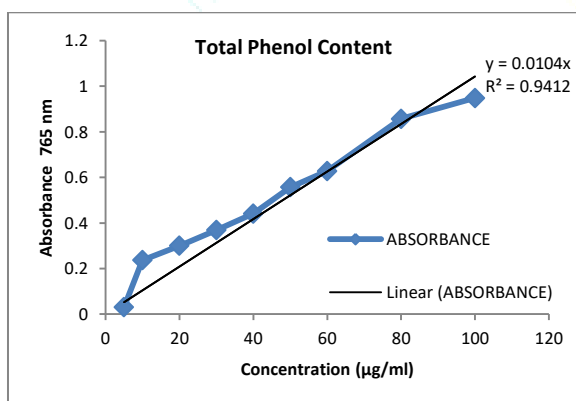
Table 4: Phytochemical screening with different reagent.

S.No.	Test	Hexane	Chloroform	Ethyl acetate	Ethanol
1.	Alkaloids	+	+	+	+
2.	Protein	-	+	-	+
3.	Flavanoids	-	-	+	+
4.	Carbohydrate	-	+	-	+
5.	Glycosides	-	-	+	+
6.	Steroids	-	-	-	+
7.	Tannins	-	+	-	+
8.	Saponins	-	+	-	+

Total Phenol Content

Total Phenol Content The results (Table 5) are based on calibration curve ($y = 0.0104x$, $R^2 = 0.9412$) of gallic acid.

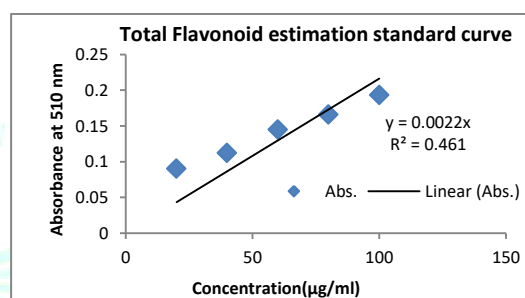
The content of phenolic compounds in ethanolic extracts was found to be 31.3($\mu\text{g}/\text{ml}$) which is the highest among all extracts. Due to the polar nature of ethanol, it shows the highest value and phenolic compounds are more soluble in polar organic solvents.



Extract	Concentration ($\mu\text{g}/\text{ml}$)
Hexane	8.8
Chloroform	16.6
Ethyl acetate	14.5
Ethanol	31.3

Table 5: Results from the quantitative estimation of total phenolic content**Total flavonoid content**

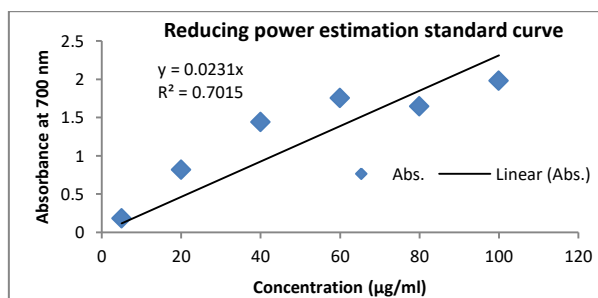
The results (table 6) were derived from the calibration curve ($y = 0.0022x$, $R^2 = 0.461$) of quercetin. The flavonoid contents was calculated and maximum concentration is found in ethanolic extract i. e. 51.1 ($\mu\text{g}/\text{ml}$).



Extract	Concentration ($\mu\text{g}/\text{ml}$)
Hexane	45.5
Chloroform	37
Ethyl acetate	39.5
Ethanol	51.5

Table 6: Results from the quantitative estimation of total flavonoid content**In-vitro anti-oxidant Result**

Ethanolic extract has the best DPPH radical scavenging potency with a minimum IC_{50} value was calculated as 17.6 $\mu\text{g}/\text{mL}$. (34). (table 7)



Extract	Concentration ($\mu\text{g}/\text{ml}$)
Hexane	7.65
Chloroform	2.2
Ethyl acetate	0.47
Ethanol	17.6

Table 7: Results from the quantitative estimation of DPPH Scavenging content

In- vivo CCl₄ induced hepatotoxicity results

Present results indicated that administration of *Mirabilis jalapa* extract, that the liver weight of *Mirabilis jalapa* treated

group showed a significant decrease in both the doses (200 and 400 mg/kg) when compared with positive control and where near to normal control (table 8).

Table 8: Effect of ethanolic extract *Mirabilis jalapa* on liver weight in CCl₄ induced hepatotoxicity.

Groups`	Dose(mg/kg)	Liver weight g /100g of body weight
Vehicle Control	NC	3.80 ± 0.044
CCl ₄	1ml/kg	6.04 ± 0.07
Silymarin + CCl ₄	25mg/kg	4.20 ± 0.062
MJ ₁ + CCl ₄	200mg/kg	5.21 ± 0.07*
MJ ₂ + CCl ₄	400mg/kg	4.40 ± 0.05**

All values are expressed as Mean±SEM (n=6) one way ANOVA. *represents significant at p<0.05 and **represents highly significant at p<0.01 when compared with control.(34)

Histopathological assessment is a direct way to assess the protective effect of a drug. Only CCl₄ was provided to the group. The loot of cells around the central vein was visible. Studies involving treatment with *Mirabilis jalapa* extract

found that the degree of damage was insignificant. The results of histopathological studies are supported and well associated with the data obtained in the assessment of enzyme parameters.

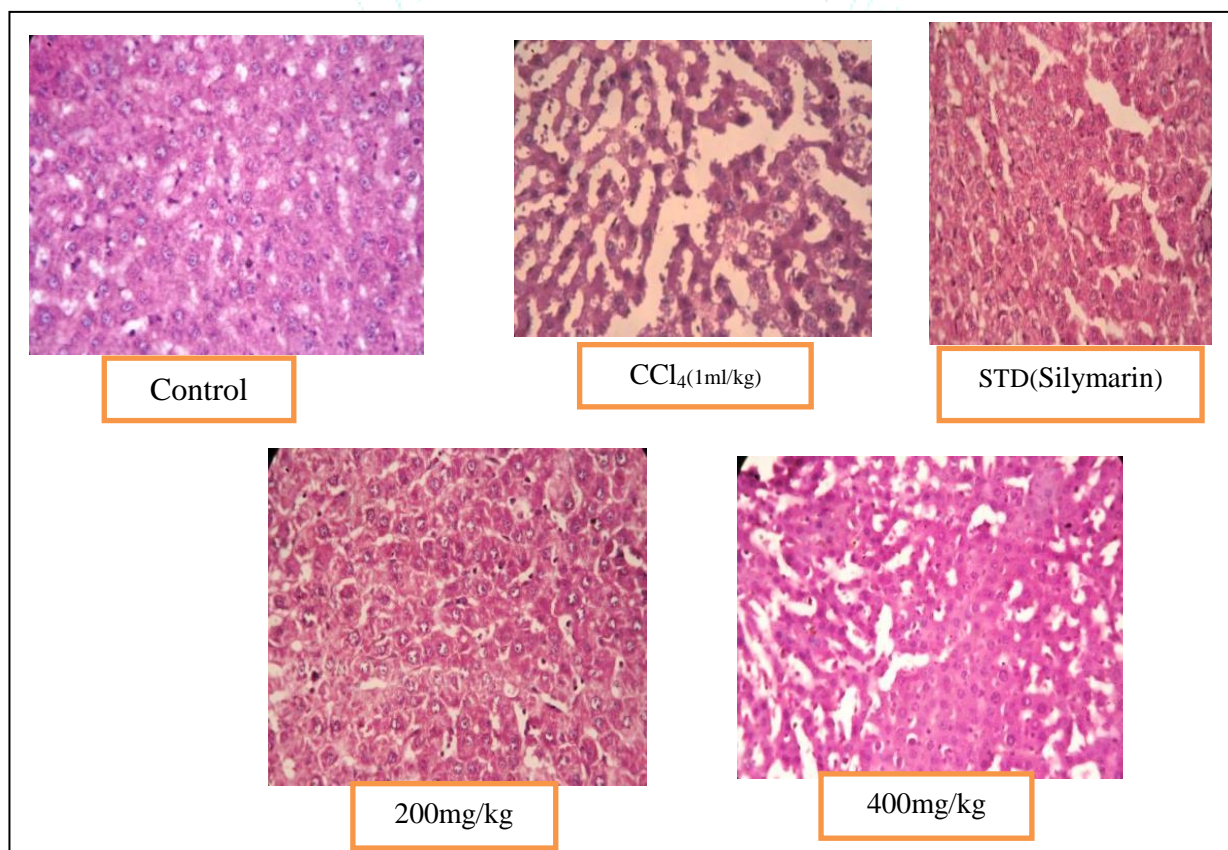


Figure 4: Effect of ethanolic extract *Mirabilis jalapa* on liver segment in CCl₄ induced hepatotoxicity.

Control: -Liver segment of normal control rats showing normal architecture;

Toxic group (CCl₄):- Liver segment of CCl₄ treated rats showing huge fatty changes, necrosis, ballooning degeneration, and severe infiltration of the lymphocytes and therefore the loss of cellular boundaries;

Standard group silymarin: - Liver section of rats treated CCl₄and 25mg/kg of silymarin showing signs of inflammatory cascade around central vein indicating a light

degree of fatty amendment, and necrosis and focal necrosis (dilatation).

MJ 200mg/kg: - Liver segment of rats treated CCl₄ and 200 mg/kg of MJ showing less inflammatory cells around central vein, absence of necrosis.

MJ400mg/kg: - Liver segment of rats treated CCl₄ and 400 mg/kg of MJ showing: minimal inflammatory cellular infiltration, large septa of connective tissue flowing together and penetrating into the parenchyma. There is regeneration of hepatocytes evident.

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