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EFFECT OF ETHANOLIC EXTRACT OF MIRABILIS JALAPA ROOTS AGAINST PARACETAMOL INDUCED HEPATOTOXICITY

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ABSTRACT
The present study was carried out to evaluate the effect of ethanolic extract of *Mirabilis Jalapa* roots against Paracetamol (2.0 g/kg body weight, orally) induced hepatotoxicity. Various biochemical parameters and histopathological profile of liver tissue were considered to evaluate the hepatoprotective activity of ethanolic extract. In serum total bilirubin, total protein, aspartate transaminase, alanine transaminase and alkaline phosphatase were determined to assess the effect of the extract on the Paracetamol induced hepatic damage. Results of this study revealed that the serum markers in the animals treated with Paracetamol were significantly increased indicating severe hepatic damage by Paracetamol, whereas the blood samples from the animals treated with ethanolic extract of *Mirabilis Jalapa* roots(250mg/kg body weight, orally) showed significant reduction in the serum markers indicating the effect of the plant extract in restoring the normal functional ability of the hepatocytes. The present study reveals that the ethanolic extract of *Mirabilis Jalapa* roots could afford a significant protection against paracetamol-induced hepatocellular injury.

KEYWORDS: *Mirabilis Jalapa roots,* Paracetamol, Hepatoprotective activity.

INTRODUCTION
Chronic hepatic diseases stand as one of the foremost health troubles globally, with liver cirrhosis and drug induced liver injury accounting ninth leading cause of death in western and developing countries.[1] Herbal based therapeutics for hepatic disorders has been in use in India for a long time and has been popularized world over by leading pharmaceuticals. A large number of plants and formulations have been claimed to possess hepatoprotective activity. Nearly 160 phytoconstituents from 101 plants have been claimed to protect liver against various toxic chemicals (certain anti-biotic, chemotherapeutic agents, Non-steroidal anti-inflammatory drugs, carbon tetrachloride, Thioacetamide etc.), excessive alcohol consumption and microbes. Inspite of the tremendous advances made no noteworthy and safe hepatoprotective agents exist in modern therapeutics. Therefore, due importance has been given globally to develop plant based hepatoprotective drugs effective against a variety of hepatic disorders.[2]

Mirabilis Jalapa. L is a perennial herbal medicinal plant belonging to the family Nyctaginaceae grown as *a* popular ornamental plant worldwide for the beauty of its flowers. Phytochemical screening of the roots shows the presence of alkaloids, glycosides, carbohydrates, terpenoids and phytosterols. The main phytoconstituents found in the plant are rotenoids (mirabijalones A-D, boeravinones C and F), phenolic compounds, alanine, alpha-amyrine, arabinose, beta-amyrins, oleanolic acid, trophan, stigmasterols, betasistosterol, beta-D – glucoside, urolic acid, mirablisoic acid, mirabalisol, trigonellin, antiviral protein, Cmethylabronisoflavone, tartaric acid, betanin, brassicasterol and betalanic acid.[3] The different parts of plants are traditionally used as Purgative and emetic, for treatment of many gastrointestinal disorders, including dysentery, diarrhea, vermifuge, muscle pain, abdominal colic and wound healer.In Ayurveda this plant is used for the management of boils, inflammations, constipation, diabetes and urinary disorders. It is also used a remedy for the kidney stones and gall bladder, chyluria.[4] In-vitro and in-vivo studies has proven *Mirabilis Jalapa* to possess Antidiabetic[5], Anti-inflammatory[6], Antinociceptive[7], Cytotoxicity[8], antioxidant[8], Antispasmodic[9] Anthelmintic[10], Anti-microbial[11], Antibacterial[12], Antifungal[13] and Antiviral.[14] The present study was undertaken to study the possible hepatoprotective role of ethanolic extract of *Mirabilis Jalapa* roots against Paracetamol induced liver injury.

MATERIALS AND METHODS

Collection of Plant Material

The roots of *Mirabilis Jalapa* were collected in the month of August and September from the local area around Narsampet, Warangal and the identification and the authentication of this plant was done in the Department of Botany, Kakatiya University.

Drugs and Chemicals

Paracetamol and Silymarin from Ranbaxy Research Laboratories, India; Diagnostic kits from coral clinical systems were used. All other reagents used for the experiments were of high analytical grade.

Preparation of Plant Extract

The roots of *Mirabilis Jalapa* were shade dried. The dried roots were powdered in an electrical processor and then the powder was separated.50 gram of dried powder material was extracted in a soxhlet apparatus with 200 ml. of ethanol. The ethanolic extract was then distilled, evaporated and dried in vacuum. All the extracts were kept in desiccator and stored in a refrigerator for further pharmacological experiment.

Animals Used

Wistar rats of either sex, weighing about 180-200 grams were used in experiments. Animals were housed in polypropylene cages with not more than three animals per cage and maintained under standard condition (12 hours light / dark cycle; temperature 25± 3^0C; relative humidity 55 ± 5%) and had free access to standard pellet feed (Hindustan Lever Ltd., India) and water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. The experiments on animals were conducted in accordance with CPCSEA and our protocols were duly approved by the Institutional Ethical Committee.

Acute toxicity studies

The acute toxicity study to carry out the gross behavioral effects and safety effects of the ethanolic extract of roots of *Mirabilis Jalapa* was carried on mice weighing about 20-25gm as per OECD 423 guidelines.[15] Overnight fasted mice received the test extract at a dose of 5 mg/kg bodyweight orally and mortality was observed for first 24 hours, with special attention for the first 4 hours and daily then, for a total of 14 days. If no mortality was observed for any rats, then the procedure was repeated again with doses of 50, 300 and 2000 mg/kg orally. The extract was well tolerated by the rats without any explicit signs of toxicity.

Assessment of hepatoprotective activity,[16]

The rats were divided into four groups containing six rats in each group.

- Group 1was treated as control and administered with normal saline for 14 consecutive days.
- Group 2 was treated with paracetamol (2.0 g/kg body weight, orally) for 14 consecutive days.
- Group 3 was treated with ethanolic extract of *Mirabilis Jalapa* roots (250mg/kg body weight, orally) for 14 consecutive daysalong with paracetamol(2.0 g/kg body weight, orally).
- Group 4 was treated with standard drug silymarin (100mg/kg body weight, orally) for 14 consecutive days along with paracetamol(2.0 g/kg body weight, orally).

On 15th day, rats were sacrificed by cervical dislocation, blood was collected and serum was separated. Serum was analyzed for the changes on liver enzyme markers such as aspartate amino transferase (AST), alanine amino transferase(ALT), alkaline phosphatase (ALP) and total bilirubin (TB) levels using commercially available kits according to the manufacturer's instructions.

Isolated liver tissue was washed with icecoldnormal saline, and a small cross-section of the liverwas separated out. It was then fixed with 10%neutral-buffered formalin and embedded in paraffin. After tissue fixation, staining was done with hematoxylin and eosin. The stained sections of slides were studied under a light microscope for any histological damage/protection.

Statistical Analysis

Values were expressed as Mean ± Standard Deviation. The Significance of differences among the group was assessed using one way analysis of variance (ANOVA). The test followed by Dunnett's multiple comparisons test of significance. p values less than 0.05 were considered as statistically significant.[17]

RESULTS

Effect of ethanolic extract of *Mirabilis Jalapa* roots against Paracetamol induced hepatotoxicity in rats with reference to biochemical changes in serum are given in Table 1. Paracetamol administration produced significant elevations of serum ALT, AST, ALP and Total bilirubin, but the total protein level decreased reflecting the liver injury caused by Paracetamol compared to that of the control group. Treatment of rats with ethanolic extract of *Mirabilis Jalapa* roots offered substantial hepatic protection confirmed by significant decrease in the levels of serum markers and significant increase in the total protein to the near normal value which can be compared as that of the ascertained hepatoprotective silymarin.

Table 1: Effect of ethanolic extract of *Mirabilis Jalapa* roots againstparacetamol induced hepatotoxicity in rats on serum Biochemical Parameters.

Treatments Serum Biochemical Parameters	Control (Normal Saline)	Positive Control (Paracetamol)	Ethanolic extract of *Mirabilis Jalapa* & Paracetamol)	Standard (Siymarin) & Paracetamol)
ALT (IU/ml)	53.21±1.92	173.94±6.32*	99.42±4.27#	62.67±5.84#
AST(IU/ml)	102.33±6.32	180.85±5.21*	153.27±8.46#	114.19±7.56#
ALP (IU/ml)	75.66±2.34	151.5±5.27*	104.67±5.19#	86.33±4.65#
Total Bilirubin (mg/dl)	1.61±0.23	5.07±0.42*	3.19±0.21#	2.29±0.74#
TotalProtein(mg/dl)	8.53±0.66	4.91±0.81*	7.61±0.12#	6.28±0.13#
Values are mean ± SD (n=6) One way ANOVA followed by Dunnet's test. *P < 0.05 when compared to control#P < 0.05 when compared toPositive Control				

Histological profile of animals is depicted in Figure 1. Histological outline of control rats showed normal cellular architecture. The section of the liver of Paracetamol treated positive control group showed the disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm with severe intense congestion and fatty degeneration. The liver section of animals treated with standard hepatoprotetivesilymarin exhibited normal hepatic architectural with few fatty globules whereas the liver section of rats treated with ethanolic extract of *Mirabilis Jalapa* roots showed normal hepatic cells and absence of severe congestion and occasional necrosis indicating noticeable protection of hepatocytes by Paracetamol induced hepatic damage.

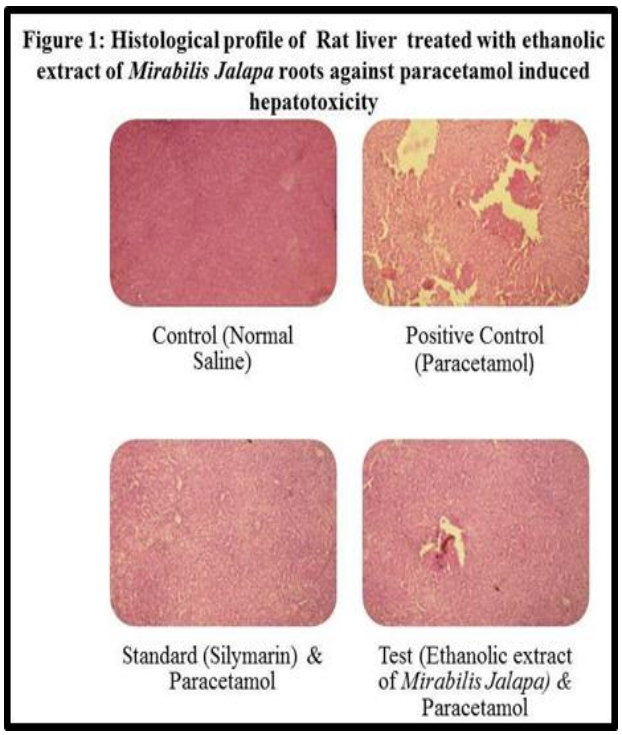

Figure 1: Histological profile of Rat liver treated with ethanolic extract of *Mirabilis Jalapa* roots against paracetamol induced hepatotoxicity

DISCUSSION

This study has been carried out to assess the effect of ethanolic extract of *Mirabilis Jalapa* roots againstParacetamol induced hepatotoxicity. Paracetamol (Acetaminophen; *N*-acetyl-*p*-aminophenol) is widely used analgesic-antipyretic agent. Paracetamol undergoes cytochrome P450 -mediated *N*-hydroxylation to form *N*-acetyl-*p*-benzoquinoneimine (NAPQI), a highly reactive intermediate. This metabolite normally reacts with sulfhydryl groups in glutathione and thereby is rendered harmless. But after ingestion of large dose of paracetamol the metabolite is formed in amounts sufficient to deplete hepatic glutathione and reacts with cellular membrane molecules, resulting in widespread hepatocyte damage and death, leading to acute liver necrosis.[18]

In the present study Paracetamol was used to induce hepatotoxicity in rats. Silymarin a potent hepatoprotective agent which enhances hepatic glutathioneinhibit the free radicals that are produced from the metabolism of toxic substances such as ethanol, acetaminophen, and carbon tetrachloride is used as a Standard.[19] Paracetamol increased the levels of serum markers in the blood, among these, aspartate transminase, alanine transaminase which indicates cellular leakage and loss of functional integrity of the liver cell membranes implying hepatocellular damage whereas changes in Serum total protein and bilirubin levels on the other hand are related to the function of the liver cells revealing the functional status of the hepatic cell which was disturbed by Paracetamol administration. It was confirmed from the present study that Hepatocellular necrosis which occurred due to Paracetamol administration was reversed by ethanolic extract of *Mirabilis Jalapa* roots as the extract reversed the increased ALT, AST, ALP and Total bilirubin levels towards normal. Biochemical studies and Histopathological profile of liver tissue proved the hepatoprotective effect of Mirabilis *Jalapa* roots against Paracetamol induced liver injury.

CONCLUSION

The present observations provide evidence that ethanolic extract of *Mirabilis Jalapa* roots exert significant hepatoprotection against paracetamol induced liver injury by normalizing biochemical parameters and histological profile of rats which can be comparable to that of standard drug silymarin. The exact mode of action is not clear; however the presence of phenolic compounds that offer antioxidant effect may be possibly responsible for hepatopotective effect.

REFERENCES

1. Jain SK, Rajvaidy S, Desai P, Singh GK, Nagori BP. Herbal Extract as Hepatoprotective- A Review. Journal of Pharmacognosy and Phytochemistry, 2013; 2(3): 170- 175.
2. Vishwanath J, Preetham GB, Anil KB, Satwik RJ. A Review on Hepatoprotective plants. International Journal of Drug Development & Research, 2012; 4(3): 1-8.
3. Sharmila S, Rajendra Y, Jaya PCR. Phytochemical and Pharmaclogical Studies Of Mirabilis Jalapa. Linn. International Journal of Pharmacy & Technology, 2012; 4(2): 2075-2084.
4. Rozina R. Pharmacological and biological activities of Mirabilis jalapaL. International Journal of Pharmacological Research, 2016; 6(5): 160-168.
5. Ji YZ, Zhou SW, Zeng Sheng-Ya, Zhou JN, Ming-JJ, Yan H. Hypoglycemic and Hypolipidemic Effects of Ethanolic Extract of Mirabilis jalapa L. Root on Normal and Diabetic Mice. *Evidence-Based* Complementary and Alternative *Medicine*, 2012; Article ID 257374, 9.
6. Singh M, Kumar V, Singh I, Gauttam V, Kalia AN. Anti-inflammatory activity of aqueous extract of Mirabilis jalapa Linn. leaves. Pharmacognosy Research, 2010; 2: 364-367.
7. Walker CB, Trevisan G, Mateus.FR, Carina F, Maria EP, Juliano F, Melania PM. Antinociceptiva activity of Mirabilis jalapain mice. Journal of Ethnopharmacology, 2008; 120(2): 69-75.
8. Nowshin NR, Mostafizur RM, Shahidulislam M, Sadia AC. Cytotoxicity and Antioxidant activity of extractives from Mirabilis jalapa.L. Journal of Pharmaceutical sciences, 2008; 1(1&2): 85-88.
9. Aoki K, Cortes AR, Ramirez MC, Gomez – Hernandez M, Franciso J, Munoz L. Pharmacological study of antispasmodic activity of Mirabilis jalapaLinn flowers. Journal of Ethnopharmacology, 2008; 116(1): 96-101.
10. Subin MZ, Jayakar B, Vidya V, Rachana VG. In vitro Anthelmintic activity of aerial parts of Mirabilis jalapa Linn. International Journal of Pharmaceutical Sciences Review and Research, 2012; 12: 107-110.
11. Vadde KK, Venuturumilli RS, Ramya S, Sahaja VR, Saritha K, Govinda RK, Naidu VN, Ravi SN. Phytochemical Screening and Antimicrobial Activity of the Leaf Extract of Mirabilis jalapa Against Pathogenic Microorganisms. International Journal of Phytomedicine, 2010; 2: 402-407.
12. Chifundera K, Kizungu B, Mpoyi WM. Antibacterial activity of Mirabilis jalapa seed powder. Journal of Ethnopharmacology, 1991; 39(2): 197-199.
13. Satish S, Mohana DC, Ranhavendra MP, Raveesha KA. Antifungal activity of some plant extracts against important seed borne pathogens of Aspergillus sp. J Agricl Technol, 2007; 3(1): 109-19.
14. Jorge MV, Maddalena Q, Luis FS. Antiviral and Antiviroid Activity of MAP-Containing Extracts from Mirabilis jalapa Roots. Plant Disease, 1999; 83(12): 1116-1121.
15. OECD/ OCDE, OECD Guideline for Testing of Chemicals, Acute Oral Toxicity- Acute Toxicity Class Method, 423. Adopted 17th December, 2001. [Cited on 21 August 2017]. Available from: https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf
16. Arpita S, Rajesh GK, Shyam SG, Satyawan S, Rawat AKS, Rao CV. Hepatoprotective Potential of Ziziphusoenoplia (L.) Mill Roots against Paracetamol-Induced Hepatotoxicity in Rats. American Journal of Phytomedicine and Clinical Therapeutics, 2015; 3(01): 64-78.
17. Indranarayana A, Satyanarayana L. Biostatisticsfor Medical, Nursing and Pharmacy Students. NewDelhi: Prientice Hall of India Private Limited; Chapter 16 General principles of Statisticaltest; 219-230, chapter 17: Statistical significanceparametric and nonparametric test, 2006; 231-244.
18. Mitchell JR., Thorgeirsson SS, Potter WZ, Jollow DJ, Keiser H, Acetaminophen-induced hepatic injury: Protective role of glutathione in man and rationale for therapy, Clinical Pharmacology & Therapeutics, *1974; 16(4):* 676–684.
19. Vargas-Mendoza N, Madrigal-Santillán E, Morales-González Á, et al. Hepatoprotective effect of silymarin. World Journal of Hepatology, 2014; 6(3): 144-149.