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In- Vitro Evaluation of Hepatoprotective Potential of *Diplocyclos Palmatus* (L) C. Jeffrey Leaf Against Paracetamol Induced Liver Toxicity using HEPG2 Cell Line

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KEYWORDS

Paracetamol, HepG2, Diplocyclos Palmatus, DMEM, shivlingi

ABSTRACT:

Aim:

To evaluate In-vitro hepatoprotective activity of Hydro alcoholic extract of Diplocyclos palmatus (HADP) leaf extract against paracetamol induced liver toxicity in HepG2 Cell line.

Methods:

HepG2 cells were plated using 96 well microplate and incubated at 37 °C for 24 h in 5% CO2.After incubation the cells were exposed to 14 Mm paracetamol to induce hepatotoxicity. Cells are then treated with different concentrations of Plant extract (200µg/ml and 400µg/ml and incubated for 24 h in CO2 incubator and silymarin as standard (300µg/ml). The morphological cells were observed under digital inverted microscope (20X magnification) after 24 h and photographed. The cells were then washed with phosphate-buffer saline (PBS, pH-7.4) and 20 µL of (MTT) solution (5 mg/mL in PBS) was added to each well. The plates were then stand at 37ºC in the dark for 4 h. The formazan crystals were dissolved in 100µL DMSO and the absorbance was read spectrophotometrically at 570 nm.

Results:

The results indicated that paracetamol induced causes a significant decrease in cell viability about (18.5%).HADP at 200µg &400 µg showed increase in cell viability (34.1%,70.8%).Silymarin showed increase in cell viability (88.1%).

Conclusion

Our study revealed that HADP leaf extract has potent hepatoprotective effect against paracetamol induced liver toxicity in HepG2 cell line and which may be attributed to decrease in paracetamol induced reactive oxygen species levels and resultant oxidative stress.

Introduction:

The liver performs over 5,000 vital biological functions, including aiding in blood clotting, detoxifying the blood, converting food into essential nutrients, regulating hormone levels, preventing infections and diseases, promoting healing from injuries, and metabolizing cholesterol, glucose, and iron[1-3].Liver dysfunction, also known as liver damage or hepatic toxicity, is frequently brought on by exposure to toxins taking too many medications,

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or using certain therapeutic agents[4]. NSAIDs cause harm to the kidney, liver, and entire gastrointestinal tract. Analgesic and well-known antipyretic, paracetamol causes liver necrosis when taken in excess. Usually, it is removed mostly as glucuronide and sulfate. In harmful quantities, Cytochrome-P450 enzymes oxidize a larger proportion of paracetamol molecules to the highly reactive NAPQI as a result of the sulfation and glucuronidation pathways becoming saturated. By reducing NAPQI by one electron, semiquinone radicals are produced. These radicals can covalently attach to cellular membrane macromolecules and cause lipid peroxidation, which damages tissue. Higher dosages of NAPQI and paracetamol can oxidize and alkylate intracellular GSH, which lowers the liver's GSH pool and has subsequent[7-9.] Liver toxicity induced by acetaminophen is common cause of acute liver failure across countries. Synthetic agents often causes adverse effects. The HepG2 cell line it is a continuous cell line most commonly used in the evaluation of chemicals and drugs for their cytotoxicity, antitumor activity, and for various other morbidities. These cell lines are frequently used as an *in vitro* alternative to primary human hepatocytes. These Cell lines are are well known for their unlimited life span, stable phenotype, high availability, and easy handling[10]. Hence plant based medicine is highly impactful in treating liver diseases.

Plant Profile:

Diplocyclos palmatus, belongs to the family of Cucurbitaceae also known as shivalingi or lollipop plant, is one such plant that is commonly overlooked despite having significant medicinal benefits. Seed is similar to ‘shivling ‘icon of lord shiva. Flowering period of plant is generally in the month of August and September and fruits in August and October. The fruit is especially well-known for its use in reproductive medicine, where it treats impotence, female infertility, and vaginal discharge. It has expectorant and laxative properties[11-13]. Known for its aphrodisiac, antipyretic, and anti-inflammatory qualities, the seeds are utilized to help with conception. Skin disorders, rheumatic pains, asthma, coughs, flatulence, and even snake bites can all be treated with the herb. Plant is well known for their gynecological, detoxifying, antispasmodic, antibacterial, analgesic, and anti-arthritic properties. Infertility has been a prevalent problem throughout history and still affects a large number of couples globally.The plant's leaves are utilized as an element in a specific nutritional preparation of the tribal people of Chhattisgarh as a tonic, together with Bengal gram flour[14-16]

Synonym- *Bryonopsis laciniosa var walkeri Chakrav, Bryonia palmata Linn.* Hence this research article highlights properties of *Diplocyclos palmatus* plant against paracetamol induced liver toxicity.

Fig No: 1 Leaves of *Diplocyclos palmatus*

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Materials and Methods:

Collection and authentication of plant material

Diplocyclos palmatus (L) C Jeffrey leaves was collected from a nearby garden, road sides Tamil Nadu. The leaf was identified and authenticated by SIDDHA CENTRAL RESEARCH INSTITUTE , Anna Govt. Hospital Campus, Arumbakkam, Chennai – 600106.

Code:D20122401P

Extraction Method:

Freshly collected leaf materials of *Diplocyclos palmatus (L) C Jeffrey* was thoroughly washed under running water to remove adherent impurities. Leaves was dried under shade drying at room temperature. 250 grams of shade dried leaves was placed in Soxhlet apparatus using 500 ml of 1:1 ratio of water and ethanol. Extraction procedure was continued until the siphon tube become colourless. The obtained extract was weighed and stored at low temperature for future analysis. The percentage yield was calculated .

Fig No :2 Extraction using Soxhlet apparatus

PRELIMINARY PHYTOCONSTITUENTS TEST [17-19] :

Tests for alkaloids :Dragendorff's test :By adding 1 mL of Dragendorff"s reagent to 2 mL of extract, an orange red precipitate was formed, indicating the presence of alkaloids.

Tests for flavonoids :Shinod's test :Ten drops of dilute HCL and a piece of magnesium were added to 1 mL of extract, the resulting deep pink colour indicating the presence of flavonoids.

Test for tannins :1 ml of extract was taken and few drops of 1% lead acetate were added. Formation of

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yellowish precipitate indicated the presence of tannins.

Tests for Cardiac glycosides :Keller Killiani test:A solution of 0.5 mL, containing glacial acetic acid and 2-3 drops of ferric chloride, was mixed with 2 mL of extract. Later, 1 mL of concentrated H2SO4, was added along the walls of the test tube. The appearance of deep blue colour at the junction of two liquids indicated the presence of cardiac glycosides.

Test for Steroids:Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to 3ml of acetic anhydride, and 1 to 2 drops of concentrated sulfuric acid were added. Appearance of dark green color showed the presence of steroids.

Test for phenols:Ferric chloride test : Take to 2ml of extract, treated with 3-4 drops of Ferric chloride solution formation of bluish black color indicates presence of phenols.

Test for protein :The extract was dissolved in 10ml of distilled water and filtered through Whatman no. 1 filter paper and the filtrate is subjected to tests for proteins.

Millon's test : To 2ml of filtrate 0.5ml of millon's reagent was added. Formation of a white precipitate indicated the presence of proteins.

Test for saponins :Froth Test: Extracts was diluted with distilled water and made up to 20ml and suspension was shaken in a graduated cylinder for 15 min. Formation of foam layer of about two centimeters indicated the presence of saponins.

Test for terpenoids :The extract was dissolved in one ml of chloroform; 1ml of acetic anhydride was added followed by the addition of 2ml of concentrated H2SO4. Formation of reddish violet color indicated presence of triterpenoids.

***In-vitro*Studies :** In-Vitro studies using HepG2 cell line.

Cell Culture Maintenance [20-21]

HepG2 cells was Cells were maintained in the logarithmic phase of growth in Dulbecco& modified eagle medium (DMEM) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin. They were maintained at 37°C with 5% CO 2 in 95% air humidified incubator.

The hepatoprotective activity of the sample was tested against HepG2 cell line by MTT (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The cells were seeded in 96-well microplates (1 x 10^4 cells/well) and incubated at 37°C for 24 h in 5% CO_2 incubator. After incubation, the cells were exposed to 14 mM paracetamol to induce hepatotoxicity in HepG2 cell line and allowed to incubate for 4 h in CO_2 incubator. Then, the induced cells were treated with different concentrations of sample (200µg/ml and 400µg/ml and incubated for 24 h in CO_2 incubator and silymarin as standard(300µg/ml). The morphological changes of untreated (control) and the treated cells were observed under digital inverted microscope (20X magnification) after 24 h and photographed. The cells were then washed with phosphate-buffer saline (PBS, pH-7.4) and 20 µL of (MTT) solution (5 mg/mL in PBS) was added to each well. The plates were then stand at 37ºC in the dark for 4 h. The formazan crystals were dissolved in 100µL DMSO and the absorbance was read spectrophotometrically at 570 nm.

Percentage of cell viability was calculated using the formula:

Cell viability (%) = (Absorbance of sample/Absorbance of control) X 100

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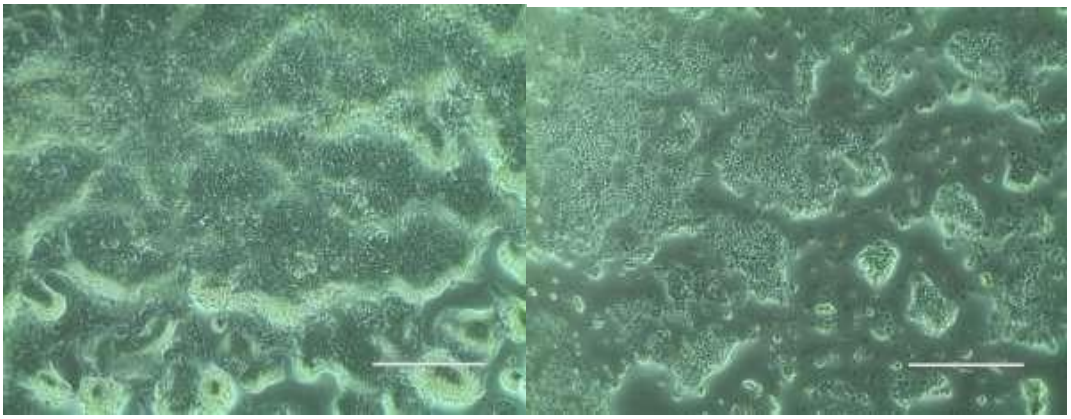
Results and Discussion:

The total percentage yield of *Diplocyclos palmatus* is 13.6% for 220 gram of dry leaf powder.

Preliminary phytoconstituents test shows the presence of 1. Alkaloids, 2. Flavonoids, 3. Tannins, 4.Phenols, 5. Terpenoids, 6.Cardiac Glycosides, 7.Steroids

Fig no: 3 Preliminary phytoconstituents test



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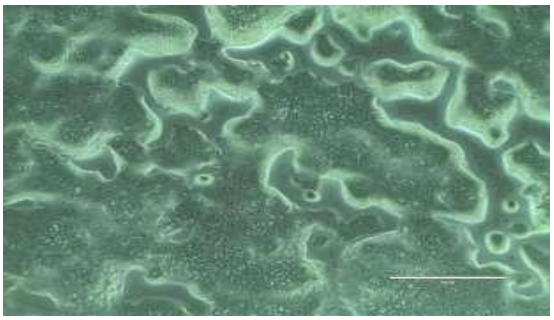
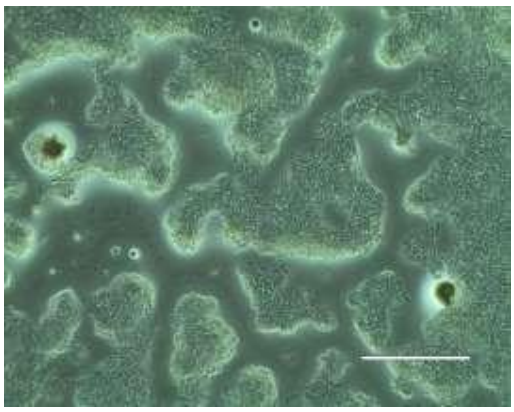
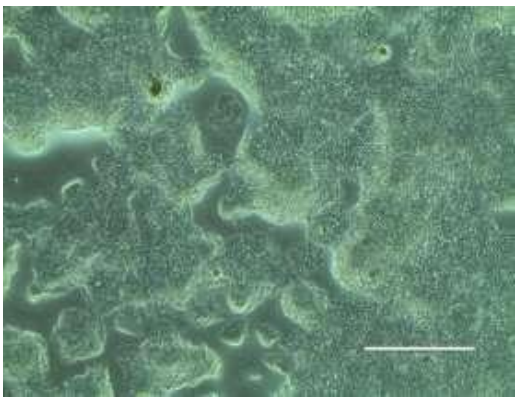
Control Group -Untreated

Diseased Group-Paracetamol- 14 mM



Silymarin-300µg/ml





HADP-200 µg/ml

HADP-400 µg/ml

Fig no :4 Microscopic images showing cell viability

Treatments	Average Absorbance	Cell Viability (%)
Group I-Control –Untreated	0.905667	100
Group II-Induced with Paracetamol -14mM	0.167667	18.51306588
Group III-Treated with Silymarin-300 µg	0.798333	88.14869341
Group IV-HADP 200 µg	0.309333	34.15531837
Group V-HADP 400 µg	0.605667	70.87523003

Table no : 1 *In – Vitro* analysis using Hepg2 Cell line

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Decreased in cell viability was observed with paracetamol induced at dose of 14 Mm is (18.5%) . There was increase in cell viability in HADP 200 µg&400 µg is (34.1% &70.87%).

Discussion:

Analgesic and well-known antipyretic, paracetamol causes liver necrosis when taken in excess. However, it is considered safe when it is has been taken in therapeutics dosage limits. Usually, it is removed mostly as glucuronide and sulfate. In harmful quantities, Cytochrome-450 enzymes oxidize a larger proportion of paracetamol molecules to the highly reactive NAPQI as a result of the sulfation and glucuronidation pathways becoming saturated. By reducing NAPQI by one electron, semiquinone radicals are produced. These radicals can covalently attach to cellular membrane macromolecules and cause lipid peroxidation, which damages tissue. Higher dosages of NAPQI and paracetamol can oxidize and alkylate intracellular GSH, which lowers the liver's GSH pool and has subsequent[22].

Preliminary phytoconstituents study reveals the presence of Alkaloids, Flavonoids, Terpenoids, Tannins, Phenols and Cardiac Glycoside which may be responsible for the strong antioxidant and hepatoprotective potential preventing oxidative stress, cellular damage and inflammation , counteracting reactibe oxygen species.

Present study reveals that *Diplocyclos palmatus* leaf at the dose of 200 µg & 400 µg showed increase in the cell viability compared to the diseased group induced with paracetamol at the dose of 14 mM[21].It has shown a major role in preventing oxidative stress, reduced inflammation .

Conclusion

It can be concluded that HADP has potent antioxidant and hepatoprotectective activity restoring normal cells , preventing oxidative damage. It has capability of enhancing activites of hepatic enzymes. However, further In-vivo investigation should be carried out to identify the main constituents responsible for hepatoprotection.

References:

[1] Abdel-Misih, S. R.; Bloomston, M. Liver anatomy. Surg. Clin. North Am. 2010, 90, 643–653.

[2] Sibulesky, L. Normal liver anatomy. Clin. Liver Dis. 2013, 2, S1–S3.

[3] Kalra, A.; Yetiskul, E.; Wehrle, C. J.; Tuma, F. Physiology, liver. StatPearls; StatPearls Publishing: Treasure Island, FL, 2023.

[4] Guyton, A. C.; Hall, J. E. Textbook of Medical Physiology, 11th ed.; Saunders: Philadelphia, PA, 2006.

[5] Moore, K. L.; Dalley, A. F. Clinically Oriented Anatomy, 5th ed.; Lippincott Williams & Wilkins: Philadelphia, PA, 2006.

[6] Watanabe, S. U.; Phillips, M. J. Acute phalloidin toxicity in living hepatocytes: Evidence for a possible disturbance in membrane flow and for multiple functions for actin in the liver cell. Am. J. Pathol. 1986, 122, 101–111.

[7] Vermeulen, N. P. E.; Bessems, J. G. M.; Van de Straat, R. Molecular aspects of paracetamol-induced hepatotoxicity and its mechanism-based prevention. Drug Metab. Rev. 1992, 24, 367–407.

[8] Parmar, S. R.; Vashrambhai, P. H.; Kalia, K. Hepatoprotective activity of some plant extracts against paracetamol-induced hepatotoxicity in rats. J. Herb. Med. Toxicol. 2010, 4, 101–106.

[9] Lahon, K.; Das, S. Hepatoprotective activity of Ocimum sanctum alcoholic leaf extract against paracetamol-induced liver damage in albino rats. Pharmacogn. Res. 2011, 3, 13–18.

[10] Donato MT, Tolosa L, Gómez-Lechón MJ.

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Culture and functional characterization of human hepatoma HepG2 cells. InProtocols in in vitro hepatocyte research 2014 Nov 3 (pp. 77-93). New York, NY: Springer New York.

[11] Patel, S. B.; Ghane, S. G. Diplocyclos palmatus (L.) C. Jeffrey: An important medicinal striped cucumber. In Phytochemistry and Pharmacology of Medicinal Plants; 2023.

[12] Gabrielian, S. E.; Gevorgovich, A. Bryonia as a novel plant adaptogen for prevention and treatment of stress-induced disorders. Promising Res. Abstr. 1997, 5003, 1–8.

[13] Naik, J. B.; Patil, S. V.; Ghotane, R. B.; Patil, P. B. A review on Bryonia laciniosa (Shivlingi beej). World J. Pharm. Res. 2022, 11, 340–352.

[14] Dwivedi, S.; Sohani, S.; Akram, M.; Dwivedi, S. N. Diplocyclos palmatus L. Jeffrey (Shivalingi): Morphological features and ethnomedicinal importance. Int. J. Pharm. Life Sci. 2021, 12, 72–78.

[15] Pandey, M. M.; Rastogi, S.; Rawat, A. K. Indian traditional Ayurvedic system of medicine and nutritional supplementation. Evid.-Based Complementary Altern. Med. 2013, 2013, 376327.

[16] Gupta, P.; Dwivedi, S.; Wagh, R. Physicochemical evaluation and fluorescence analysis of stem and leaves of Diplocyclos palmatus (L.) Jeffrey (Shivalingi). Int. J. Drug Discov. Herb. Res. 2013, 3, 641–643.

[17] Kancherla N, Dhakshinamoothi A, Chitra K, Komaram RB. Preliminary analysis of phytoconstituents and evaluation of anthelminthic property of Cayratia auriculata (in vitro). Maedica. 2019 Dec;14(4):350.

[18] Arya V, Thakur N, Kashyap CP. Preliminary phytochemical analysis of the extracts of Psidium leaves. Journal of Pharmacognosy and Phytochemistry. 2012 May 1;1(1):1-5.

[19] Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. International journal of chemical studies. 2020 Mar 1;8(2):603-8.

[20] T. Mossmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods. 65 (1983): 55–63.

[21] Jairaman C, Yacoob SA, Venkatraman A, Nagarajan Y, Murugesan G. Propugnating effect of bark of Rhizophora mucronata against different toxicants viz carbon tetrachloride, ethanol and paracetamol on HepG2 cell lines. Journal of Pharmacopuncture. 2019 Mar 31;22(1):41

[22] Eesha BR, Mohanbabu AV, Meena KK, Vijay M, Lalit M, Rajput R. Hepatoprotective activity of Terminalia paniculata against paracetamol induced hepatocellular damage in Wistar albino rats. Asian Pacific Journal of Tropical Medicine. 2011 Jun 1;4(6):466-9.