

EVALUATION OF *IN VITRO* HEPATIC TOXICITY OF LEAVES OF *PTEROSPERMUM ACERIFOLIUM* (L.) WILLD.RANA DATTA¹, SANKHADIP BOSE², SUDIP KUMAR MANDAL^{3*}

¹Department of Pharmacology, Gupta College of Technological Sciences, Asansol, West Bengal, India. ²Department of Pharmacognosy, Bengal School of Technology, Hooghly, West Bengal, India. ³Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Dr. B. C. Roy College of Pharmacy and Allied Health Sciences, Bidhannagar, West Bengal, India. Email: gotosudip79@gmail.com

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ABSTRACT

Objective: The objective of the study was to determine the *in vitro* hepatic toxicity profile of methanolic extract of leaves of *Pterospermum acerifolium* (L.) Willd. (MEPA) using a mammalian hepatic cell line (HepG2).

Methods: To assess its *in vitro* hepatic toxicity, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay using MEPA at a concentration of 25 µg, 50 µg, 100 µg, 200 µg, and 300 µg was carried out. Sorafenib tosylate was used as the standard agent to assess cytotoxicity.

Results: The inhibitory concentration 50 (IC₅₀) value for HepG2 cell lines was determined after 24 h. Thereafter the cytotoxicity study was again performed with the ½ IC₅₀, IC₅₀, and 2IC₅₀ doses of MEPA. Experimentally, the IC₅₀ was found to be 150.42 µg/ml for HepG2 using MEPA. A dose-dependent cytotoxicity and reduction in optical density were also observed with incremental MEPA administration.

Conclusion: The cytotoxic potential of MEPA was found to be much less than that of sorafenib tosylate. Thus, MEPA shows *in vitro* cytotoxicity in mammalian hepatic cells (HepG2) at a concentration as low as 100 µg.

Keywords: *Pterospermum acerifolium*, Cytotoxicity, *In vitro*, Cell line, Hepatocarcinoma.

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INTRODUCTION

Demand for herbal medicine has increased tremendously in recent years; however, there many issues regarding their safety. Very less (<10%) marketed herbal products are actually standardized. Furthermore, quality control measures are not followed on many occasions [1]. Even in the literature, very little toxicological information is available. Since some plants produce toxic constituents for defense purposes, it becomes absolutely necessary to explore the safety profile of herbal drugs. The plant *Pterospermum acerifolium* (L.) Willd., belonging to the family Sterculiaceae, is used by the tribals of Chotanagpur, Konkan, and Arunachal Pradesh region of India, for the treatment of different diseases such as wound healing and hemostatic activities [2,3].

However, no toxicological data are available for the plant. Preliminary phytochemical studies showed the presence of alkaloids, flavonoids, and glycosides in the methanolic extract of *P. acerifolium* (L.) Willd. (MEPA). Conventionally, the presence of alkaloids has been implicated to cytotoxicity; thus, it is worth to elucidate the toxicological profile of MEPA both from therapeutic and toxicological standpoint. Herbal medicines are normally considered safe, but chronic administration may lead to cumulative toxicity. Thus, it is equally important to evaluate *P. acerifolium* (L.) Willd. for its toxicological profile.

In vitro toxicity testing is frequently done on mammalian hepatic cell line HepG2 [4]. HepG2 through a cancer cell line is frequently used to assess *in vitro* toxicity. It was first derived from a 15-year-old Caucasian American male. *In vitro* studies offer additional advantages over the traditional animal models on many aspects. It allows a species-specific, simpler, and more detailed analysis [5]. *In vitro* studies, if designed well, can very well replace whole animal studies in days to come.

The present study was undertaken to elucidate the *in vitro* toxicological implications (if any) on HepG2 cell lines, to establish the safety profile of MEPA.

METHODS

Preparation of extracts

The leaves of *P. acerifolium* were collected from Asansol, West Bengal, in the month of September 2013 and 2014 at 11 a.m. The plant was identified and authenticated as *P. acerifolium* (L.) Willd. by the Director, Acharya Jagadish Chandra Bose Indian Botanic Garden, Shibpur, Howrah, India. The leaves of *P. acerifolium* were dried in the shade of about 30°C and crushed into a coarse powder.

Cell culture

Hepatocellular carcinoma cell line HepG2 was purchased from National Facility for Animal tissue and cell culture, Pune, India, and supplied from Indian Institute of Chemical Biology for *in vitro* studies. Hepatoma cells were subcultured after every 2 days at an initial concentration of 1×10⁶ cells/ml and maintained in sterile Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum [5]. The culture was maintained at 37°C in a humidified atmosphere containing 5% CO₂ in the air [6]. During subculturing or during the use of HepG2 in experiments, this adherent property has been diminished by adding ×1 Trypsin solution in the cell [7]. In all the experiments, untreated hepatic cells were used as a control group.

Preparation and extraction of the tested plant sample

Leaves of *P. acerifolium* (L.) Willd. were air-dried in the shade. The dried leaves were extracted with methanol, were crushed, and then extracted by continuous hot extraction process for 72 h in Soxhlet apparatus, using a reflux condenser. Then, the solvent was removed by filtration. Fresh solvent was added and further extracted for 3 h. The extract was concentrated by vacuum under reduced pressure. Thereafter, the extract was lyophilized for 4 h to produce methanol free extract. It was kept in a container, sealed with parafilm and stored at 4°C in an airtight container, and was designated as MEPA. Stock solution was prepared as 1 mg/ml in phosphate buffer saline (PBS) from which desired doses were tested.

Preliminary phytochemical studies

Preliminary phytochemical studies showed the presence of alkaloids, flavonoids, and glycosides in the MEPA.

Cytotoxicity study by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay

Cytotoxicity studies after trypsinization of HepG2 cells (1×10^5), 100 μ l cell suspension per well in a log phase were seeded in 96 well tissue culture plates. They were treated with MEPA freshly prepared 1 mg/ml stock solution in a concentration of 25 μ g, 50 μ g, 100 μ g, 200 μ g, and 300 μ g for 24, 48, and 72 h at 37°C in a humidified atmosphere containing 5% CO₂ in the air. Untreated cells served as control. Sorafenib tosylate was used as a standard [8]. At the end of treatment, in the respective time, the media from the upper layer have been removed; then again, 100 μ l DMEM media was added. Then, 20 μ l of MTT (5 mg/ml in PBS) as a stock solution was added to each well and incubated for another 4 h [9]. The MTT assay is a colorimetric assay for measuring the activity of enzymes that reduce MTT to formazan dyes, giving a purple color [4]. A solubilization solution dimethyl sulfoxide 100 μ l was added to dissolve the insoluble formazan product into a colored solution [10,11]. The absorbance was taken at 570 nm by microplate manager (Reader type: Model 680 XR Bio-Rad Laboratories Inc.) inhibitory concentration 50 (IC₅₀) value for HepG2 cell line was determined after 24 h. The IC₅₀ value was determined (for the cell lines) and the cytotoxicity study was again performed using $\frac{1}{2}$ IC₅₀, IC₅₀, and 2 IC₅₀ doses.

Statistical analysis

Statistical analysis was done by Student's t-test. $p < 0.05$ was considered as statistically significant.

The percentage cell inhibition was calculated by the following formula:

$$\% \text{ Cell inhibition} = [\text{OD of control} - \text{OD of treated}] / \text{OD of control} \times 100$$

OD = Optical density

The percentage cell viability was calculated by the formula:

$$\text{Viable cells (\%)} = (\text{Total number of viable cells per ml} / \text{Total number of cells per ml}) \times 100$$

RESULTS

MEPA at concentrations of 100 μ g, 200 μ g, and 300 μ g significantly ($p < 0.05$) inhibited the growth of HepG2 cells compared with that of the control cells after 24, 48, and 72 h of treatment in a concentration-dependent manner (Fig. 1). In the MTT assay, there was a significant ($p < 0.05$) concentration-dependent reduction in the OD values after treating the HepG2 cells with the same concentrations of MEPA for 24, 48, and 72 h compared to the control cells (Fig. 2). These observations provided proof for cytotoxic nature of MEPA. Cytotoxicity of sorafenib tosylate was, however, much greater than MEPA (IC₅₀ of 2.09 μ g/ml) [12-14]. The IC₅₀ as calculated after MTT assay was 150.42 μ g/ml for HepG2 cells. Another graph of MTT assay taking $\frac{1}{2}$ IC₅₀, IC₅₀, and 2 IC₅₀ doses for 24 h showed a dose-dependent decrease in OD at 570 nm (Fig. 2).

DISCUSSION

Preliminary phytochemical studies showed the presence of an ample amount of alkaloids. The observed cytotoxicity may be due to these phytoconstituents; alkaloids have historically been found to possess such properties. Therapeutically such cytotoxicity may be a constraint in the usage of MEPA for various *in vivo* ailments such as diabetes and wound healing activity. The dosage regimen and the duration of therapy are important parameters that may guide the researchers to balance between its beneficial and potential toxic properties. Since the study was carried out using whole MEPA, further isolation and characterization of the bioactive principles may provide potent molecules for targeting cancerous cells. Furthermore, from toxicological point of view, identification and separation of such principles may enhance the benefit: Risk ratio of the herbal drug.

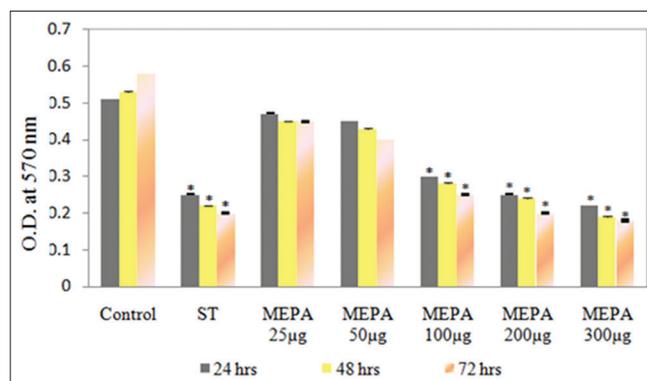


Fig. 1: Histogram shows the effect of MEPA on cell cytotoxicity by MTT assay on HepG2 cell lines after 24, 48, and 72 h. The OD at 570 nm is compared to the untreated cells and sorafenib tosylate treated cells. Data expressed as mean \pm SEM. *Denotes significant decrease in OD at 570 nm from control values $p < 0.05$

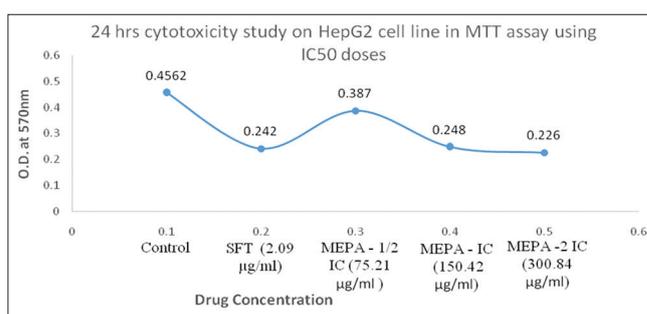


Fig. 2: The line diagram shows the effect of $\frac{1}{2}$ IC₅₀, IC₅₀, and 2 IC₅₀ doses of MEPA on cell cytotoxicity by MTT assay on HepG2 cell lines after 24 h. The OD at 570 nm is compared to the untreated cells and sorafenib tosylate treated cells. Data are expressed as mean \pm SEM

CONCLUSION

MEPA showed *in vitro* cytotoxicity in mammalian hepatic cells (HepG2) at a concentration as low as 100 μ g. The potential to produce cytotoxicity may be a basis to use MEPA for treating hepatocarcinoma. However, the selectivity of MEPA toxicity toward cancerous tissues needs to be evaluated; else, the finding may also indicate potential damage of liver tissues of treated organisms, especially on repeated administration.

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AUTHORS' CONTRIBUTIONS

Bose S (SB) conceived, supervised, and guided the project. Datta R (RD) carried out the research work, including collection, extraction, and *in vitro* studies. Mandal SK (SKM) also supervised and guided the research work and prepared the manuscript.

CONFLICTS OF INTEREST

All authors report no conflicts of interest regarding this manuscript.

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