

# Polyherbal Formulation for Kidney and Liver Protection

S J Kabilan, R Baskar, G Poorani

**Abstract:** The objectives of the study were to assess the potential of herbal formulation made up of *Wedeliachinensis* leaves and *Boerhaaviadiffusa* roots in possessing activities like nephroprotectivity and hepatoprotectivity. Nephroprotective and hepatoprotective activity was evaluated using MTT cytotoxicity assay using mammalian cell culture. The results of the hepatoprotective and nephroprotective activities showed that the formulation mixture of herbs *Wedeliachinensis* and *Boerhaaviadiffusa* roots is an excellent source of organ stimulator with high therapeutic importance. The hepatoprotective and nephroprotective properties make this formulation a unique one focusing on liver and kidney diseases.

**Keywords:** Hepatoprotectivity, Nephroprotectivity, Polyherbal formulation

## I. INTRODUCTION

Nephroprotectivity is an activity of any compound protecting the kidney cells and functions [2]. Likewise, hepatoprotectivity was to protect the liver cells from harmful substances [7]. The above mentioned herbal plants contain few phytoconstituents that possess the ability to act as a nephroprotectant and hepatoprotectant [10]. They play a major role in maintenance of these organs and preventing them from getting damaged [19]. They also boost up their performance and have a cleansing activity on them [20]. They prevent aging of cells present in those organs keeping them healthy for longer time [21].

*Wedeliachinensis* (Manjalkarisalai in Tamil), Asteraceae is a well reputed herbal medicine in Siddha, Ayurvedha and Unani system of traditional medicine. Recent studies show the presence of diterpenes, flavanoids, triterpenes, phytosteroids and saponins. It is also reported to possess anti-inflammatory, antioxidant, analgesic, hepatoprotective, antimicrobial, CNS depressant, wound healing, antistress and anticancer activity [5].

*Boerhaaviadiffusa* (Mookirattaikeerai in Tamil) is one of the well known medicinal plants that are used to treat variety of human diseased conditions as mentioned in Ayurveda, Charaka Samhita, and Sushrita Samhita. Huge variety of phytochemicals like flavonoids, alkaloids, glycosides, rotenoids, steroids, triterpenoids, lipids, lignans, carbohydrates, proteins, and glycoproteins etc have been reported from the herb. The promising therapeutic effects of this plant include diuretic, hepatoprotective, anti-inflammatory, anti-cancer, anti-fibrinolytic,

immuno-modulatory, anti-diabetic, immuno-suppressive, analgesic, anti-lymphoproliferative and used for the treatment of TB [11].

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The aim of this study is to develop an herbal formulation that possesses nephroprotective and hepatoprotective activity.

## II. MATERIALS AND METHODS

### A. Chemicals

DMEM medium, Fetal Bovine Serum (FBS), Trypsin, Saline, H<sub>2</sub>O<sub>2</sub>.

### B. Sample Collection and Extraction

#### Sample Collection

*Wedeliachinensis* leaves and *Boerhaaviadiffusa* root powder were collected from an FSSAI approved herbal powder manufacturer from Coimbatore, Tamil Nadu. All herbs were stored in air-tight, light resistant container for further use. The samples were labeled as the *Wedeliachinensis* leaves (WC), *Boerhaaviadiffusa* (BD) and Formulation mix.

#### Sample extraction

About 20g of powdered mix of these herbs was successively extracted with 150 ml of distilled water. Then it is allowed to evaporate in open air to obtain aqueous extracts. The extracts were filtered using membrane filter.

### C. Determination of Hepatoprotective activity

Human liver HepG2 cells were exposed to a medium containing H<sub>2</sub>O<sub>2</sub>(1mM) along with /without various concentrations of the formulation (100, 200, 300, 400 and 500 mg/ml). Then cytotoxicity was assessed by estimating the viability of HepG2 cells by MTT reduction assay. HepG2 cells were grown in DMEM culture medium and made into single-cell suspension and seeded into a 96-well flat bottom plate with 1 × 10<sup>4</sup> cells per well. After 48 hr incubation, 100 μL of 1mM H<sub>2</sub>O<sub>2</sub> was added to each well followed by the addition of 100 μL diluted extract at varying concentrations to the appropriate wells and the plates were incubated for further 48 hr at 37°C in a humidified incubator with 5% CO<sub>2</sub>. Supernatant was removed from each well, and 100 μL of MTT (0.5 mg/mL) was added. MTT enters the cell's mitochondria, where it is reduced to an insoluble, colored (dark purple) formazan product. The cells were then solubilized with 100 μL of an organic solvent DMSO and the released, solubilized formazan reagent is measured spectrophotometrically.

Hepatoprotective activities of the formulation extracts are measured against the toxicity caused by H<sub>2</sub>O<sub>2</sub> on the liver cells and the readings were obtained at 540 nm (Surendran et al., 2011).

### D. Determination of in-vitro nephroprotective activity

Fresh African green monkey normal kidney cells (Vero) were grown in DMEM culture medium and made into

single-cell suspension and seeded into a 96-well flat bottom plate with  $1 \times 10^4$  cells per well. After 24 hr incubation, 100  $\mu\text{L}$  of 1mM  $\text{H}_2\text{O}_2$  was added to each well followed by the addition of 100  $\mu\text{L}$  diluted extract at varying concentrations to the appropriate wells and the plates were incubated for further 24 hr at  $37^\circ\text{C}$  in a humidified incubator with 5%  $\text{CO}_2$ . Supernatant was removed from each well, and 100  $\mu\text{L}$  of MTT (0.5 mg/mL) was added. MTT enters the cell's mitochondria, where it is reduced to an insoluble, colored (dark purple) formazan product. The cells are then solubilized with 100  $\mu\text{L}$  of an organic solvent DMSO and the released, solubilized formazan reagent is measured spectrophotometrically. Nephroprotective activities of the formulation extracts are measured against the toxicity caused by  $\text{H}_2\text{O}_2$  on the liver cells and the readings were obtained at 540 nm (Srinivasan et al., 2015).

### III. RESULTS AND DISCUSSION

#### A. Determination of Hepatoprotective activity

Hepatotoxicity was induced by  $\text{H}_2\text{O}_2$  in HepG2 cell lines. Formulation extract has been evaluated for its hepatoprotectiveness. The presence of phytoconstituents in formulation mix extract has inhibited the induced hepatotoxicity.

Table I shows the % of cell death occurred due to toxicity induced by  $\text{H}_2\text{O}_2$  with or without different concentration of Formulation extracts.

**Table I: Percentage cell death upon  $\text{H}_2\text{O}_2$  toxicity in HepG2 cell lines**

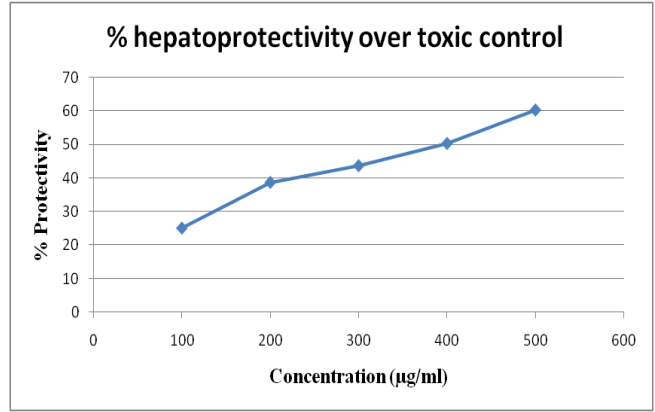
S.No	Test Concentration ( $\mu\text{g}/\text{ml}$ )	% of cell death in presence of formulation + $\text{H}_2\text{O}_2$
1	100	$66.27 \pm 2.68$
2	200	$54.25 \pm 2.63$
3	300	$49.85 \pm 3.51$
4	400	$43.98 \pm 5.3$
5	500	$35.19 \pm 5.85$
$\text{H}_2\text{O}_2$ toxic control		$88.56 \pm 1.75$

The above data shows that with increasing concentration of formulation, the cell death has been reduced, which shows the protective activity of the formulation.

**Table II: Hepatoprotective activity of formulation mix over toxicant in HepG2 cell line**

S.No	Test Concentration ( $\mu\text{g}/\text{ml}$ )	% Protection offered over toxicant control
1	100	$25.16 \pm 3.03$
2	200	$38.74 \pm 2.98$
3	300	$43.70 \pm 3.97$
4	400	$50.33 \pm 5.98$
5	500	$60.26 \pm 6.61$

**Figure 1: Percentage hepatoprotectivity of formulation mix over toxic control**



The PC50 (Protective Concentration 50%) is  $381.48 \mu\text{g}/\text{ml}$  which results in better hepatoprotective activity over the  $\text{H}_2\text{O}_2$  toxicity in HepG2 cells.

It has been revealed from Table II, that effective protection of about 50 % over toxicant control has been attained at  $381.48 \mu\text{g}/\text{ml}$  concentration (Srinivasan et al., 2015). Both the *Boerhaaviadiffusa* and *Wedeliachinensis* possess hepatoprotective activity and contains phytoconstituents having hepatoprotective activity.

#### B. Determination of nephroprotective activity

Nephrotoxicity was induced by  $\text{H}_2\text{O}_2$  in Vero cell lines. Formulation mix extract has been evaluated for its nephroprotectiveness. The presence of various phytoconstituents and minerals in formulation mix extract has inhibited the induced nephrototoxicity (Ahmed et al., 2010).

Table III shows the % of cell death occurred due to toxicity induced by  $\text{H}_2\text{O}_2$  with or without different concentration of formulation mix extracts.

**Table III: Percentage cell death upon  $\text{H}_2\text{O}_2$  toxicity in Vero cell lines**

S.No	Test Concentration ( $\mu\text{g}/\text{ml}$ )	% of cell death in presence of formulation + $\text{H}_2\text{O}_2$
1	100	$89.63 \pm 0.44$
2	200	$88.34 \pm 0.77$
3	300	$83.41 \pm 0.44$
4	400	$67.09 \pm 1.18$
5	500	$63.21 \pm 1.18$
$\text{H}_2\text{O}_2$ toxic control		$93.26 \pm 0.44$

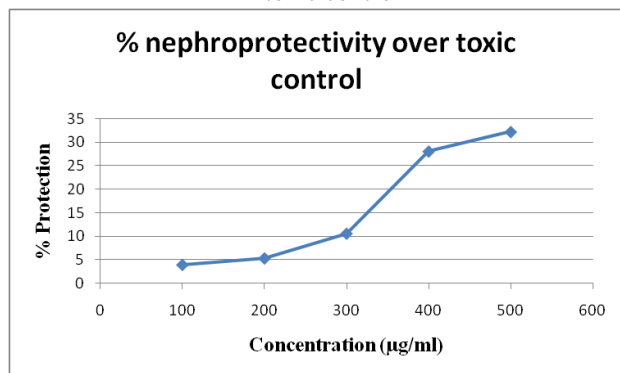
From the table, it is evident that the  $\text{H}_2\text{O}_2$  toxicity was inhibited with increasing concentration of formulation.

**Table IV: Nephroprotective activity of formulation mix over toxicant in Vero cell line**



S.No	Test Concentration (µg/ml)	% Protection offered over toxicant control
1	100	3.89 ± 0.48
2	200	5.28 ± 0.83
3	300	10.56 ± 0.48
4	400	28.06 ± 1.27
5	500	32.22 ± 1.28

Figure 2: % nephroprotectivity of formulation mix over toxic control



The PC50 (Protective Concentration 50%) is 732.06µg/ml

It has been shown from Table IV, that effective protection of about 50 % over toxicant control has been attained at 732.06µg/ml concentration (Kiruba et al., 2014). *Boerhaaviadiffusa* possess nephroprotective activity and contains phytoconstituents having nephroprotective activity which results in moderate nephroprotective activity over the H<sub>2</sub>O<sub>2</sub> toxicity in Vero cells.

#### IV. CONCLUSION

The results of hepatoprotective and nephroprotective activities showed that the formulation mixture of herbs *Wedeliachinensis* and *Boerhaaviadiffusa* roots is an excellent source of organ protector with high therapeutical importance. The hepatoprotective and nephroprotective properties make this formulation a unique one focusing on liver and kidney diseases. It may therefore be recommended for people to prevent and get rid of kidney and liver related disorders.

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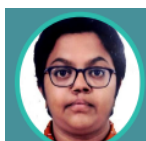
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Recent Publications:

- **Baskar,R.**, Akshaya, S.B., Akshitha, R., Dhilip Kumar, G. , Poorani, G. (2018).Evaluation of antioxidant and phytochemical activity in solvent extracts of *Delonix regia*.International Journal of Green Pharmacy, Volume 12(3), July-September issue, S607-S616.
- **Baskar, R.**, Sweatha, S., Karunambika, R., G.Ramya, R.Shanthi (2017). Influence of methionine on ergothioneine content and antioxidant activity of *Pleurotus platypus*.Asian Journal of Microbiology, Biochemistry and Environmental Sciences, Volume 19 (Supplementary issue), S34-S38.
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- PooraniGurumallesh, **Baskar Ramakrishnan** and BhaarithiDhurai (2019). A novel metalloprotease from banana peel and its biochemical characterization. International Journal of Biological Macromolecules, 134, 527- 535 (**Impact factor: 4.7**)



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Notable Publications:

INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES (2019) IF 4.78 A novel metalloprotease from banana peel and its biochemical characterization

MATERIAL SCIENCE AND ENGINEERING C (2019) IF 5.08 Biological synergy of greener gold nanoparticles by using *Coleus aromaticus* leaf extract

INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES (2019) IF 4.78 A systematic reconsideration on proteases

PROCESS BIOCHEMISTRY (2019) IF 2.88 Green synthesis of anisotropic silver nanoparticles from the aqueous leaf extract of *Dodonaea viscosa* with their Antibacterial and Anticancer activities

JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY B: BIOLOGY (2018) IF 4.06 Improved Conductivity and Antibacterial activity of poly (2- aminothiophenol) - silver nanocomposite against human pathogens

JOURNAL OF MICROENCAPSULATION (2016) IF 2.04 Formulation, characterization, in vitro and in vivo evaluation of castor oil based self-nano emulsifying levosulpiride delivery systems

