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## *In vitro* hepatoprotective activity of *Indigofera barberi* gamble against d-galactosamine induced toxicity

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### Abstract

The present study is aimed to detect the *In vitro* hepatoprotective activity of isolated compound from *Indigofera barberi* Gamble belonging to the family Fabaceae. *In vitro* hepatoprotective potential of isolated compound from *Indigofera barberi* was evaluated using HepG2 cells. Isolated compound (10, 100, 1000 µg/ml) was assessed for hepatoprotective potential against D-Galactosamine induced toxicity in HepG2 cell line by monitoring cell viability, aspartate amino transferase (AST), alanine amino transferase (ALT), Alkaline Phosphatase (ALP), Gamma Glutamyl Transpeptidase (γGTP), lipid peroxidation (LPO) and glutathione level (GSH). The results indicated that D-Galactosamine treatment caused a significant decrease in cell viability. HepG2 cells showed significant dose depended increase in percentage of cell viability at the dose 10, 100 and 1000 µg/ml of isolated compound compared to D-Galactosamine exposed HepG2 cells. The percentage of cell viability of HepG2 cells after incubation with D-Galactosamine was  $18.75 \pm 0.97\%$ , whereas the isolated compound IB-4 (1000 µg/ml) restored the viability to  $79.23 \pm 1.47\%$ . Isolated compound from *Indigofera barberi* significantly prevented the increase in LPO and GSH level which was brought to near normal. The effect of isolated compound was comparable with that of standard drug Silymarin. The D-Galactosamine induced changes in the HepG2 cells were significantly ameliorated by treatment of isolated compound from *Indigofera barberi* and also strongly revealed that compound isolated from *Indigofera barberi* having good hepatoprotective potential.

**Keywords:** *Indigofera barberi*, D-Galactosamine, Human liver hepatoma cells, cell viability, Ethonolic extract, flavonoids

### 1. Introduction

In the traditional system of medicine, medicinal plants play the major role in cure of various diseases. The use of natural remedies for the treatment of liver diseases is followed since ancient times starting with Ayurvedic treatment in India is well known as per the old literatures available. Nowadays many herbal products for liver diseases are formulated to strengthen the traditional system of medicine. Standardization of herbal products also performed to support safety and clinical efficacy of the formulation. Biological screening is necessary to provide a scientific evidence for validating the traditional utilization of medicinal plants. A great number of screening programs are going on worldwide to standardize the new plant based bioactive molecules. The plant selected for the present study is *Indigofera barberi* Gamble belongs to the family Fabaceae used traditionally to treat various skin diseases, renal disease and also as a liver tonic. The present study was carried out to check the hepatoprotective efficiency of the isolated compound from *Indigofera barberi* by *in vitro*.

### 2. Materials and Methods

#### 2.1 Collection and Identification of *Indigofera barberi*

The aerial parts of *Indigofera barberi* Gamble were collected from Thalakona (Nelakona regions) of Chittoor District of Andhra Pradesh, India in the month of November 2010. The plant material was taxonomically identified by Prof. P. Jayaraman, Plant Anatomy Research Centre, Chennai, Tamil Nadu and India. The voucher herbarium specimen (PARC/2012/1246) has been preserved in our laboratory for further reference. The aerial parts of *Indigofera barberi* were subjected to shade drying for about two and half months, then segregated, pulverized by a mechanical grinder and passed through a 22 mesh sieve. The coarse powdered plant materials were kept in an airtight container for further analysis.

#### 2.2 Preparation of ethanolic extracts from *Indigofera barberi*

The aerial parts of *Indigofera barberi* were dried and powdered. The powdered materials were successfully extracted with 95% ethanol (80°C) by hot continuous percolation method in

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Soxhlet apparatus for 24 hours<sup>[1]</sup>. The extracts were filtered and concentrated using rotary flash evaporator and residues were dried in desiccators over sodium sulphide below 60 °C.

$$\left. \begin{array}{l} \text{Percentage yield with} \\ \text{reference to crude plant} \\ \text{material} \end{array} \right\} \frac{\text{Weight in grams of extracts obtained}}{\text{Weight in grams of plant material taken}} \times 100$$

## 2.3 Isolation of Compounds by column chromatography

### Preparation of Admixture

The ethanolic extract of *Indigofera barberi* selected for isolation of compounds. Since phytochemical screening of *Indigofera barberi* shows many classes of phytochemicals, so ethanolic extract was selected for isolation of secondary metabolites.

### Column Packing

The crude extract of EEIB (ethanolic extract of *Indigofera barberi*) was subjected to column chromatography for the isolation of biologically active compounds. The silica gel (column grade) was used as stationary phase and eluted with various solvent systems for the mobile phase. EEIB (20.0g) was dissolved in ethanol added with 16 gm of silica gel of mesh size 60 – 120, thoroughly mixed. The material was packed in the column and by using petroleum ether the admixture was loaded to the top of the column. Then the column was eluted with petroleum ether initially and by the order of increasing polarity of the solvents like petroleum ether, benzene, ethyl acetate and ethanol were added. Each and every tube was analyzed by TLC, with single and similar spots are pooled together. The solvents were nullified using rotary evaporator under vacuum.

Elution of the column with petroleum ether: benzene (60:40, v/v) gave a solid, designated as IB-1, and was characterized. The compound IB-1 was found to be homogeneous and tested on TLC in the following solvent systems, Petroleum ether: benzene (4:6, v/v) and Benzene: acetone (8.5:1.5, v/v) and showed very negligible quantity and was not able to weigh.

Elution of the column with benzene (100%) has resulted a semi solid, designated as IB-2 and was characterized. The compound IB-2 was found to be homogeneous and tested on TLC in the following solvent systems, Benzene: acetone (7:3, v/v) and Benzene: ethyl acetate (8:2, v/v) and showed very negligible quantity and was not able to weigh.

Elution of the column with benzene: ethyl acetate (2:8, v/v) has resulted a semi solid, designated as IB-3 and was characterized. The compound IB-3 was found to be homogeneous and tested on TLC in the following solvent systems, Benzene: chloroform (5:5, v/v) and Benzene: ethyl acetate (9:1, v/v) and showed very negligible quantity and was not able to weigh.

Elution of the column with benzene: ethyl acetate (4:6, v/v) has resulted a semi solid, designated as IB-4 (185 mg) and was characterized. The compound IB-4 was found to be homogeneous and tested on TLC in the following solvent

The percentage yield was calculated for the extracts and major compounds with reference to the crude material taken using the formula given below.

systems, Toluene: ethyl acetate: methanol (5:3:2, v/v) and Toluene: ethyl acetate: formic acid: methanol (5:2:1:1, v/v) and compound IB-4 was colorless semi solid, m.p.314 °C.

### 2.4 Cell lines and culture medium

HepG2 (Human liver hepatoma cells) was used in this study. These were obtained from National Centre for Cell Sciences, Pune.

### 2.5 *In vitro* hepatoprotective effect of the isolated compound (IB-4) from *Indigofera barberi* in freshly isolated rat hepatocytes<sup>[2]</sup>

#### Isolation and culture of hepatocytes

Liver cells were isolated by a modified procedure of Seglen. The calcium-free HEPES buffer and collagenase solutions were warmed in a water bath (37 °C). The abdomen of the rat was opened under phenobarbital sodium (35 mg/kg body weight) anaesthesia. A midline incision was made and a loosely tied ligature is placed around the portal vein approximately 5mm from the liver and the cannula was inserted up to the liver and then the ligature is tightened and heparin was injected into the femoral vein (1000 IU). The inferior vena cava was cut below the renal vein. Perfusion was performed for 20 min (37 °C) with calcium free HEPES buffer, which contained 1% bovine serum albumin fraction V at a flow rate of 30 ml/min. The liver swells during this time, slowly changing its colour from dark red to greyish white. The swollen liver was then perfused with TPVG solution (50 ml) followed by perfusion with calcium free HEPES buffer, which contained additional collagenase solution (0.075%) and calcium chloride (4mM) at a flow rate of 15 ml/min for 20 mins.

After the perfusion, the lobes were removed and transferred into a sterile petri dish containing calcium-free HEPES buffer and dispersed gently. It is transferred into a sterile conical flask and the crude cell suspension is stirred with the help of a magnetic stirrer for 5 min to release hepatocytes into the solution. The cell suspension is filtered through a nylon mesh (250µm) and the preparation is centrifuged at 1000 rpm for 15 min. The supernatant was aspirated off and the loosely packed pellet of cells is gently re-suspended in calcium free HEPES buffer. This washing procedure was repeated three times. Cell viability was determined by the Trypan blue dye exclusion method<sup>[4]</sup>. These isolated hepatocytes are cultured in Ham's F12 medium, supplemented with 10% new-born calf serum, antibiotics, 10<sup>-6</sup>M dexamethasone and 10<sup>-8</sup> bovine insulin. The cell suspension was incubated at 37°C for 30 min in a humidified incubator under 5% CO<sub>2</sub>.

**Table 1:** Experimental protocol for *in vitro* study of D-GalN induced hepatotoxicity in rat hepatocytes<sup>[3]</sup>  
About 1ml of hepatocyte suspension is incubated for 24 h following the protocol given below:

Control	: 0.1 ml of hepatocyte suspension + 0.1 ml vehicle (distilled water) + 0.1 ml PBS (phosphate buffer saline, pH 7.4.)
Toxicant	: 0.1 ml of hepatocyte suspension + 0.1ml of toxicant ( <i>D-Galactosamine</i> 40mM) + 0.8 ml PBS.
Standard	: 0.1 ml of hepatocyte suspension + 0.1 ml of toxicant ( <i>D-galactosamine</i> 40mM) + 0.1 ml of silymarin (100 µg/ml, suspended in distilled water) + 0.7 ml PBS.

Test substance	0.1 ml of hepatocyte suspension + 0.1 ml of toxicant ( <i>D-galactosamine</i> 40mM) + 0.1 ml of IB-4 (1000, 100, 10 µg/ml, suspended in distilled water) + 0.7 ml PBS.
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Each set contained three samples

## 2.6 Assessment of anti-hepatotoxic Activity

### 2.6.1 Cell Viability Assay

Cell viability was evaluated by trypan blue dye exclusion test [4]. Aliquot of cell suspension (2 ml) is combined with 0.08% trypan blue (2 ml) for 3 min. Then, 500 µl of the mixture are counted for cells using a hemocytometer. Cell viability was defined by the following formula:

$$\text{Cell viability} = \frac{\text{Cells excluding trypan blue}}{\text{Total cells}} \times 100$$

### 2.6.2 Determination of AST, ALT, ALP, γ GTP & total protein levels

The hepatocyte suspension after drug treatment was centrifuged at 5000 rpm for 10 in and the supernatant

obtained is used for estimation of AST, ALT, ALP, γ GTP levels [5, 6, 7].

### 2.6.3 Determination of Lipid Peroxidase (LPO) and Glutathione (GSH) Levels

The hepatocyte suspension after drug treatment was centrifuged at 5000 rpm for 10 in and the supernatant obtained is used for estimation of LPO & GSH levels [8, 9].

### 2.6.4 Statistical Analysis

The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Newmann Keul's multiple range tests. The values are represented as Mean±SEM. Probability value of  $P < 0.01$  was determined to be statistically significant.

## 3. Results

### 3.1 Characterization of isolated compounds

**Table 2:** Results of the TLC profile of isolated compound ethanolic extract of *Indigofera barberi*

Solvent system	Solvent Ratio (V/v)	R <sub>f</sub> value
Toluene: ethyl acetate : methanol	5:3:2	0.49
Toluene: ethyl acetate : formic acid : methanol	5:2:1:1	0.48

The IB-4 compound was isolated from column chromatography as mentioned in the experimental section. Compound IB-4 as colorless semi solid, m.p.314 °C was found to be homogeneous on TLC profile.

### 3.2 Results of the isolated compound IB-4 from *Indigofera barberi* in freshly isolated rat hepatocytes

The effects of the IB-4 on freshly isolated rat hepatocytes intoxicated with D-GalN were recorded in Table 3.

The hepatoprotective activities of IB-4 were assessed by measuring their effects on the release of AST, ALT, ALP, γGT and cell viability from primary cultures of rat hepatocytes injured with D-GalN, are studied lipid peroxidase (LPO) and Glutathione (GSH) levels.

**Table 3:** Results of the effect of different concentrations of IB-4 on % viability and biochemical parameters of rat hepatocytes intoxicated with D-GalN

Treatment	Concentration(µg/ml)	% Viability	AST (U/L)	ALT (U/L)	ALP (U/L)	γGTP (U/L)
Normal control	-	95.06 ± 1.62	115.26 ± 2.10	54.26 ± 1.18	195.12 ± 2.20	123.7 ± 2.45
D- Galactosamine (Toxic control)	40mM	18.75 ± 0.97*	296.5 ± 3.05*	178.5 ± 1.23*	410.38 ± 3.25*	209.6 ± 2.17*
Silymarin (Positive control)	100	85.47 ± 1.63**	149.7 ± 2.43**	83.6 ± 1.10**	265 ± 2.16**	132.5 ± 1.86**
IB-4 (Isolated compound)	10	34.10 ± 1.21*	225.8 ± 1.24*	152.6 ± 2.32*	312.46 ± 2.52*	179.8 ± 1.92*
	100	56.24 ± 1.83**	176.3 ± 1.42**	116.3 ± 1.15**	276.58 ± 1.47**	162.4 ± 1.23**
	1000	79.23 ± 1.47**	160.4 ± 1.35**	92.7 ± 1.28**	219.14 ± 2.36**	145.7 ± 1.10**

Values (% viability) are mean ±S.E.M of three readings in each group

\* – values are significantly different from Normal control at  $P < 0.01$

\*\* – values are significantly different from Toxic control at  $p < 0.01$ .

### 3.3 Cell viability

D-GalN treatment for 24 h caused a loss of about 80% of the cell viability. When hepatocytes were treated with the IB-4 just before the addition of D-GalN, a significant increase in cell viability was observed in a dose-dependent manner. About 80% cell viability is restored at an IB-4 concentration of 1000 µg/ml for 24 h incubation. When silymarin was used instead of the IB-4, the cell viability was restored almost complete.

### 3.4 Cellular leakage

The AST, ALT, ALP and γGT leakage from hepatocytes treated with D-GalN and the isolated compound IB-4. AST, ALT, ALP and γGT leakage was elevated with respect to normal hepatocytes upon D-GalN treatment indicating severe cellular damage. IB-4 treatment before D-GalN intoxication significantly ( $P < 0.01$ ) inhibited the damages as evident from less AST, ALT, ALP and γGT leakage. Cellular leakages were decreased linearly with increase in IB-4 concentration and at the concentration of 1000 µg/ml; the level was reduced to near normal. Silymarin administration also showed similar result.

**Table 4:** Results of the effect of different concentrations of IB-4 on Lipid peroxidase and glutathione of rat hepatocytes intoxicated with D-GalN

Treatment	Concentration ( $\mu\text{g/ml}$ )	LPO ( $\mu$ mole of MDA / min/mg protein)	GSH ( $\mu\text{g/mg protein}$ )
Control	-	$7.2 \pm 0.15$	$5.6 \pm 0.04$
D-Galactosamine	40mM	$18.5 \pm 0.21^*$	$1.8 \pm 0.03^*$
Silymarin	100	$8.6 \pm 0.13^{**}$	$4.5 \pm 0.07^{**}$
IB-4	10	$15.9 \pm 0.17^*$	$2.2 \pm 0.06^*$
	100	$12.6 \pm 0.14^{**}$	$3.8 \pm 0.06^{**}$
	1000	$9.7 \pm 0.10^{**}$	$2.9 \pm 0.09^{**}$

Values are mean  $\pm$  S.E.M of three readings in each group

\* – values are significantly different from Normal control at  $P < 0.01$

\*\* – values are significantly different from Toxic control at  $p < 0.01$ .

LPO -  $\mu$  mole of MDA/ min /mg protein)

GSH -  $\mu\text{g/mg protein}$

### 3.5 Results of the effect of isolated compound IB-4 on the D-GalN -induced lipid peroxidation

MDA level indicates the extent of lipid peroxidation. The D-GalN induced lipid peroxidation, an indicator of membrane damage. D-GalN administration increased the lipid peroxidation to 18.5 with respect to the normal cells. IB-4 treatment prior to D-GalN administration caused inhibition in the lipid peroxidation in a linear fashion. Significant membrane damage recovery has been observed at an IB-4 concentration of 100  $\mu\text{g/ml}$  and at a concentration 1000  $\mu\text{g/ml}$ , the damage was recovered almost to its normal level.

### 3.6 Results of effect of the IB-4 on the D-GalN -induced oxidative stress

D-GalN treatment decreased GSH level to 1.8. The IB-4 treatment prior to D-GalN administration increased the GSH level in a dose-dependent manner. GSH level was significantly decreased at a concentration at a concentration 100  $\mu\text{g/ml}$  of isolated compound IB-4. At the concentration of 1000  $\mu\text{g/ml}$ , the level was decreased to near normal. Silymarin administration also showed similar result.

## 4. Discussion

D-GalN is known to selectively block the transcription and indirectly hepatic protein synthesis and as a consequence of endotoxin toxicity, it causes fulminant hepatitis [10, 11]. The toxicity of D-GalN results from inhibition of RNA and protein synthesis in the liver. The metabolism of D-GalN may deplete several uracil nucleotides including UDP-glucose, UDP-galactose and UTP, which trapped in the formation of uridine- diphospho galactosamine. Accumulation of UDP-sugar nucleotide may contribute to the change in the rough endoplasmic reticulum and to the disturbance of protein metabolism. Intense D-GalN of the membrane structures was thought to be responsible for loss in the activity of ionic pumps. The impairment in the calcium pumps, with consequent increase in the intracellular calcium is considered to be responsible for cell death. D-GalN intoxication is known to cause marked elevation in liver enzyme levels. Silymarin is used as standard hepatoprotective compound since it is reported to have a protective effect on the plasma membrane of hepatocytes [12].

Phytochemical screening of ethanolic extracts of *indigofera barberi* were shows the presence of flavonoids. Since the isolated compound IB-4, further it was confirmed by simple qualitative analysis for the existence of flavonoids [13]. Studies on the anti-oxidative potency of various flavonoids have confirmed the importance of the distribution and quantity of the hydroxyl groups. In general, the anti-oxidative properties of polyphenols depend on hydroxylation of ring B. Nevertheless, the meta-hydroxyl groups in ring A at positions 5 and 7 play a minor role in the anti-oxidative activity of flavonoids such as quercetin [14]. Recent studies of the

metabolism of the dietary flavonoids by human intestinal and hepatic cell lines as well as rat hepatocytes demonstrated glucuronidation and sulfation to be the rate limiting metabolic reactions [15].

At a certain concentration of D-GalN cell viability was minimal and did not change further with the increased concentration of D-GalN. Treatment with the IB-4 prior to D-GalN treatment not only increased the cell viability gradually to about 78% with respect to the control but also significantly reduced the enzyme leakage.

D-GalN caused severe damages to cells by reacting with cellular macromolecules and causing increased production of ROS in hepatocytes as evidenced from the decreased cell viability, enhanced AST, ALT, ALP and  $\gamma$ GT, decreased levels of GSH content and enhanced levels of lipid peroxidation. With increase in D-GalN concentration cell viability is decreased; on the other hand, AST, ALT, ALP and  $\gamma$ GT levels were increased in the extracellular part (Table 3). It has been shown that the leakage of enzymes correlates with cellular viability and hence a useful indicator of membrane damages. Treatment with isolated compound IB-4 exhibited significant restoration of the altered biochemical parameters towards normal in D-GalN intoxicated rat hepatocytes. The protective activity of IB-4 as comparable with that of standard silymarin use in both the test concentration. The maximum protective effect is observed in concentrations of 1000  $\mu\text{g/ml}$  and 100  $\mu\text{g/ml}$  against D-GalN induced toxicities.

D-GalN has been found to stimulate lipid peroxidation by generation of ROS. D-GalN also decreased the GSH content, altered these levels of enzymes and increased the susceptibility of hepatocytes to *in vitro* lipid peroxidation. Similar results have also been observed in our studies. Isolated compound IB-4 treatment prior to D-GalN administration showed practically no enhancement of MDA content in hepatocytes suggesting that the D-GalN might have a protective effect against the ROS induced membrane damages. D-GalN also produced oxidative stress by depleting the GSH level suggesting the presence of free radicals generated by D-GalN. When the hepatocytes were incubated with the IB-4 prior to D-GalN administration, the consumption of GSH by the hepatocytes was reduced. Result suggests that the IB-4 increased the amount of hepatic GSH and maintained its normal level in presence of D-GalN. It could be said that D-GalN caused the cellular damage by inhibiting the activity of the antioxidant enzymes and that could be prevented by the IB-4 treatment. The protective activity of the IB-4 against D-GalN induced injury in hepatocytes may be due to its radical scavenging and anti-oxidative properties. The isolated compound IB-4 seems to protect hepatocytes from D-GalN induced injury by maintaining the level of GSH and by inhibiting the production of MDA. The effect of isolated compound IB-4 on the D-

GalN induced lipid peroxidation showed that IB-4 treatment prior to D-GalN administration caused inhibition in the lipid peroxidation in a linear fashion. Significant membrane damage recovery has been observed at an IB-4 concentration of 100µg/ml and at a concentration 1000µg/ml, the damage is recovered almost to its normal level. The D-GalN induced oxidative stress, isolated compound IB-4 treatment prior to D-GalN administration increased the GSH level in a dose-dependent manner. GSH level is significantly decreased at a concentration 100 µg/ml of isolated compound. At the concentration of 1000 µg/ml, the level was decreased to near normal and was comparable with silymarin.

## 5. Conclusion

In conclusion, the present study findings clearly show the significant evidence that *Indigofera barberi* Gamble is one of the promising herbal drug for improving defense mechanisms in the physiological systems against oxidative stress caused by the D-GalN. The probable action could be due to stabilizing the hepatocellular membrane, inhibiting neutrophils infiltration into the liver cells, preventing the process of lipid peroxidation, preventing inactivation of antioxidant enzymes. It might be used in the treatment of viral hepatitis.

## 6. References

1. Harborne JB. Phytochemical methods 11 Edition. In Chapman &, Hall. New York, 1984, 4-5.
2. Seglen PO. Isolation of Hepatocytes. In Cell Biology: A Laboratory Handbook, Academic Press. Inc. 1994; 1:96-102.
3. Kucera O, Lotková H, Kand'ár R, Hézová R, Muzáková V, Cervinková Z. The model of D-galactosamine-induced injury of rat hepatocytes in primary culture. Acta medica (Hradec Králové). 2006; 49(1):59-65.
4. Kristine Louis S, Andre Siegel C. Cell Viability Analysis Using Trypan Blue: Manual and Automated Methods, Protocol, Mammalian Cell Viability. The series Methods in Molecular Biology. 2011; 740:7-12.
5. Reitman S, Frankel S. *In vitro* determination of transaminase activity in serum. American Journal of Clinical Pathology. 1957; 28(1):56-63.
6. Kind PRN, King EJ. Estimation of Plasma Phosphatase by Determination of Hydrolysed Phenol with Amino-antipyrine. Journal of Clinical Pathology. 1954; 7(4):322-326.
7. Szasz G. A kinetic photometric method for serum gamma-glutamyl transpeptidase. Clinical Chemistry. 1969; 15(2):124-126.
8. Devasagayam TP, Tarachand U. Decreased lipid peroxidation in the rat kidney during gestation. Biochemical and Biophysical Research Communications. 1987; 145(1):134-138.
9. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hateman DG, Hoekstra WG. Selenium; Biochemical roles as a component of glutathione peroxidase. Science. 1973; 179(4073):588-590.
10. Nakagiri R, Hashizume E, Kayahashi S, Sakai Y, Kamiya T. Suppression by Hydrangeae *Dulcis folium* of D-galactosamine-induced liver injury *in vitro* and *in vivo*. Bioscience Biotechnology and Biochemistry. 2003; 67(12):2641-2643.
11. Xin-Hui Tang, Ling Gao, Jing Gao, Yi-Mei Fan, Li-Zhi Xu, Xiao-Ning Zhao *et al.* Mechanisms of hepatoprotection of *Terminalia catappa* L. extract on D-

Galactosamine-Induced Liver Damage. The American Journal of Chinese Medicine. 2004; 32(4):509-519.

12. Ramellini G, Meldolesi J. Liver protection by Silymarin: *in vitro* effect on dissociated rat hepatocytes. Arzneimittelforschung-Drug Research. 1976; 26(1):69-73.
13. Srinivasan N, Sathyanarayana D. Pharmacognostical investigation of *Indigofera barberi* Gamble (Fabaceae) – A Threatened medicinal herb. Hygeia: journal for drugs and medicines. 2014; 6(2):45-56.
14. Torel J, Cillar J, Cillard P. Antioxidant activity of flavonoids and reactivity with peroxy radical. Phytochemistry. 1986; 25(2):383-385.
15. Galijatovic A, Otake Y, Walle UK, Walle T. Extensive metabolism of the flavonoid chrysin by human Caco-2 and Hep G2 cells. Xenobiotica. 1999; 29(12):1241-1256.