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Hepatoprotective activity of *Ficus semicordata* Buch.-Ham. ex Sm. leaves aqueous extract on D-galactosamine induced toxicity in HepG2 cell line

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The leaves of *Ficus semicordata* Buch-Ham.ex Sm., locally in North India is referred to as *Bhumi udumbara* have been reported for their traditional use to cure jaundice and various liver ailments. Despite its extensive usage in northeastern states of India, there is a lack of scientific substantiation on the safety and pharmacotherapeutic efficacy. The present study was carried out to evaluate the hepatoprotective activity of *F. semicordata* (FS) leaves aqueous extract on HepG2 cell line to validate its ethnic claim in the management of liver disorders. D-galactosamine induced HepG2 cell toxicity model was used to evaluate its hepatoprotective activity. The cells were treated with different concentrations of aqueous extracts and dexamethasone as a standard. MTT assay was performed to determine the % inhibition of hepatotoxicity. The result indicates that the toxicity induced by D-galactosamine is reduced by FS aqueous extract group which is better when compared with the standard drug. This study revealed the hepatoprotective potential of *F. semicordata* on HepG2 cell line.

Keywords: D-galactosamine, Dexamethasone, *Ficus semicordata* Buch. Ham. ex Sm, Hepatoprotective, HepG2.

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Introduction

Ficus semicordata Buch.-Ham. ex Sm., commonly known as drooping fig is a medium-sized evergreen tree, having oblong leaves with scabrid petioles. The tree typically lacks aerial roots. This plant with grey bark and pendulous branches bears pear-shaped figs. These figs often mature underground and hence the term *Bhumi udumbara*[1,2]. This plant is commonly found in India, Nepal, China, Bhutan and South-east Asian countries. It is distributed in forests ranging from Chenab to Myanmar[3]. The various parts of *F. semicordata* reported for medicinal uses are leaves, roots, bark, figs and latex and these are advocated in the management of liver ailments, jaundice, stomach disorders, wound, scabies, leprosy, indigestion and skin diseases[4,5]. The whole plant has been reported to possess steroids, terpenoids, flavonoids and glycosides[5]. Its leaves, in particular, possess catechins, quercetin, quercitrin and flavonoids[6]. It is also reported for anti-oxidant[7], anti-bacterial activity[8] and anti-diabetic activity[9]. Inspite of its extensive use in liver disorders and jaundice, the validated data for the claim is deficient. Hence, this study was planned to evaluate the hepatoprotective activity of *F. semicordata* leaf to validate its ethnic claim in the management of liver disorders.

Material and Methods

Plant collection and identification

The plant *F. semicordata*, growing naturally in Gandhamardana hills, Paikmal, Odisha, was identified by local Vaidya and taxonomist. The botanical name, *F. semicordata* was confirmed by studying the morphological characters in various floras. Sample specimen was authenticated by Dr Harisha C R, the head of pharmacognosy laboratory of Gujarat Ayurved University, Jamnagar. Sample herbarium was deposited to institute's pharmacognosy museum (6249/17-18) in November 2017 and also to Botanical Survey of India (CNH/Tech.II/2018/11)[10].

Sample preparation of plant extracts

Aqueous extract of *F. semicordata* (FS) was prepared as per protocol mentioned in Ayurvedic Pharmacopoeia of India[11]. The leaves of FS were shade dried at room temperature. Dried leaves were pulverized to obtain a coarse powder. Five grams of coarse powder was subjected to extraction with

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100 mL of distilled water (six hours of shaking and 18 hours stand still method) to maximally yield soluble compounds. Later, this was filtered through Whatman filter paper, and the filtrate obtained was lyophilized using Lyophilizer (Labconco) at -50 °C and 0.020 mBarr pressure for 4-6 h to yield dried extract powder. The dried extract obtained was 0.7 g after lyophilization (14% yield) which was stored in an airtight container till further use.

Animal tissue culture

In this study, HepG2 cell line was procured from NCCS (National Centre of Cell Science), Pune. These cells were cultured and maintained in Minimum Essential Medium (MEM, Gibco-Invitrogen) media supplemented with 10% fetal bovine serum (FBS), Penicillin (100 U/mL) and Streptomycin (100 µg/mL) and incubated at 37 ^{0}C in a humidified incubator with 5% CO_2, 95% air. Cultures were regularly examined using an inverted microscope at 20X magnification and media changes were given every alternate day. Cells were grown till 70% confluency was achieved i.e.~1x10^6/mL in T-25 tissue culture flask and then were sub-culturedas per the ATCC guidelines.

Cytotoxicity assay

Cytotoxicity assay was performed using MTT- [3-(4, 5 – dimethylthiazol–2-yl)-2,5-diphenyl tetrazolium bromide salt]. The cells were treated with different concentrations (50, 100, 150 and 200 µg/mL) of the plant extract and the absorbance was recorded using ELISA plate reader (EPOCH) at 540 nm[12].

***In-vitro* hepatoprotective activity**

Hepatotoxicity was induced in the cells using 15 mM of D-galactosamine as per the method described by Brindha *et al.,* (2015)[12]. The concentration of D-galactosamine was fixed after standardization. The test drug stock solution (2 mg/mL) was prepared by reconstituting the lyophilized FS in Milli-Q water during every experiment. The hepatotoxicity induced cells were treated with four different concentrations of the FS extract (FS1-50 µg/mL, FS2-100 µg/mL, FS3-150 µg/mL and FS4-200 µg/mL). Dexamethasone at 1.4 µM concentration was used as a standard drug against the D-galactosamine induced cells[13].

Data analysis

Data obtained has been represented as mean±SD from triplicate per treatment group. The statistical analysis was done using 'GraphPad Prism'statistical software. One-way analysis of variance (ANOVA) was used.

Results

Cytotoxicity assay

The cytotoxicity assay results are represented in Fig. 1 wherein HepG2 cells treated with various concentrations of test drug showed similar cell viability as in the cell blank. Thus, it is revealed that the test drug does not exhibit any significant toxicity up to 200 µg/mL. The evaluation of cytotoxicity was necessary for ruling out any hepatotoxicity of test drug itself.

Hepatoprotective activity

The results obtained are given in Table 1-3 and Fig 2. D-galactosamine control cells exhibited a significant reduction in cell number as compared to cell control, which can be reversed by the treatment with hepatoprotective agents. The inhibition of D-galactosamine induced cytotoxicity with dexamethasone treatment is suggestive of its ability to reverse the damage and protective role. When the D-galactosamine induced toxic cells were treated with various concentrations (50-200 µg/mL), it showed highly significant protection against toxicity as compared to D-galactosamine control. This protective effect was comparable to the control (cell blank) indicative of its ability to normalize the damaged cells. As seen in the graph the cell number has gradually increased as the concentration of the test

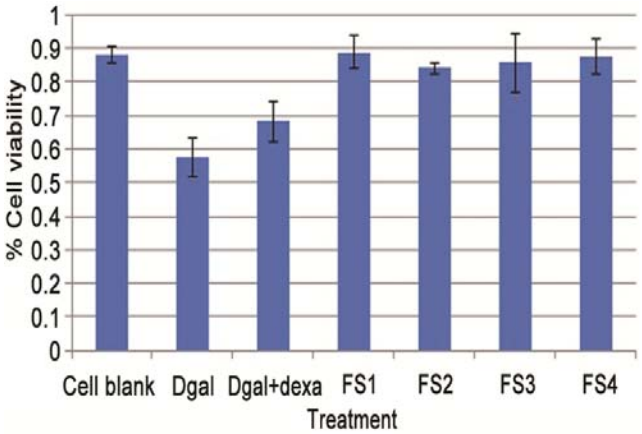

Fig. 1 — HepG2 cells (Control) treated without D-galactosamine and test drugs (FS1-FS4)

Table 1 — Application of ANOVA showing statistical significance at p <0.05

ANOVA Table	SS	Df	MS
Treatment (between columns)	0.4922	6	0.08204
Residual (within columns)	0.1312	21	0.006247
Total	0.6234	27	

Table 2 — Application of Dunnett's multiple comparison test showing statistical significance at p <0.05 in control vs test drug groups (FS1-FS4)

Dunnett's multiple comparison test	Mean Difference	q	Significant? P < 0.05?	Summary	95% CI of diff
Control vs Dgal	0.4080	7.300	Yes	***	0.2520 to 0.5640
Control vs Dgal+dexa	0.2000	3.578	Yes	**	0.04400 to 0.3560
Control vs FS1+Dgal	0.1925	3.444	Yes	*	0.03650 to 0.3485
Control vs FS2+Dgal	0.08250	1.476	No	ns	-0.07350 to 0.2385
Control vs FS3+Dgal	0.05875	1.051	No	ns	-0.09725 to 0.2148
Control vs FS4+Dgal	0.01575	0.2818	No	ns	-0.1403 to 0.1718

***- highly significant; **-significant

Table 3 — Application of Dunnett's multiple comparison test showing statistical significance at p <0.05 in D-galactosamine versus test drug groups (FS1-FS4)

Dunnett's multiple comparison test	Mean difference	q	Significant? P <0.05?	Summary	95% CI of diff
Dgal vs Control	-0.4080	7.300	Yes	***	-0.5640 to -0.2520
Dgal vs Dgal+dexa	-0.2080	3.722	Yes	**	-0.3640 to -0.05200
Dgal vs FS1+Dgal	-0.2155	3.856	Yes	**	-0.3715 to -0.05950
Dgal vs FS2+Dgal	-0.3255	5.824	Yes	***	-0.4815 to -0.1695
Dgal vs FS3+Dgal	-0.3493	6.249	Yes	***	-0.5053 to -0.1932
Dgal vs FS4+Dgal	-0.3923	7.018	Yes	***	-0.5483 to -0.2362

***- highly significant; **-significant

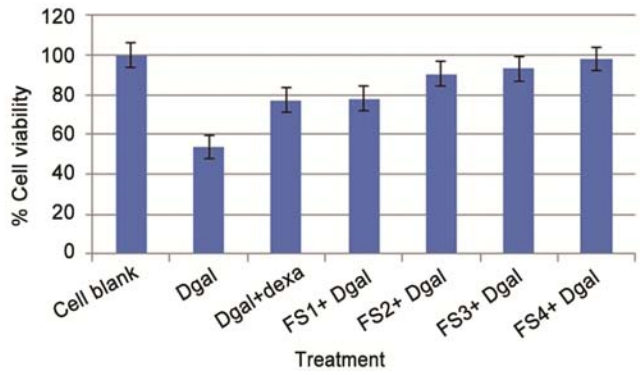

Fig. 2 — HepG2 cells treated with D-galactosamine and test drugs (FS1-FS4).

drug increases which is suggestive of dose-dependent response. When the hepatotoxic cells were treated with 50 µg/mL, the cells showed inhibition similar to the standard drug Dexamethasone, and the test drug at 200 µg/mL concentration showed viable cell number which is closer to the control, thus we can say that the toxicity of D-galactosamine is inhibited at a greater extent. The maximum drug concentration was restricted to 200 µg/mL because it will not be feasible to prescribe the drug in human for achieving concentration above 200 µg/mL for bioavailability in the blood. This depicts the hepatoprotective role of FS against D-galactosamine induced toxicity in HepG2 cell line.

Discussion

This study was planned to get an insight into the mode of action at the cellular level using *in vitro* cell-based model. Further, in the case of the hepatoprotective assay, D-galactosamine was chosen for inducing hepatotoxicity as this is a well-established hepatotoxin which generates damage analogous to viral hepatitis in humans regarding morphologic and functional characteristics of liver. A single dose can cause hepatocellular necrosis and fatty liver[14,15]. The toxicity of D-galactosamine is mainly linked to the reduction of uridine pools that are associated with limited ribonucleic acid (RNA) and protein synthesis, thus disturbing hepatocellular function[16]. Cholestasis caused by galactosamine is due to its damaging effect on bile ducts thereby reducing bile flow of bile salts, cholic acid and deoxycholic acid[17]. It has been reported that Dexamethasone counteracts hepatic inflammation and oxidative stress in cholestatic rats[18]. Thus, it was used as the standard in the experiment which inhibits the D-galactosamine induced hepatotoxicity.

The test drug showed evidence of the hepato-protective activity in a dose-dependent manner ranging from 50-200 µg/mL after which the effect may remain at pace with the untreated cells irrespective of dose. The leaves of FS have been reported to be rich in tannins, catechins and flavonoids namely quercetin[2]. The hepatoprotective

effect of FS may be due to its phytoconstituent quercetin a flavonoid and catechin which have antioxidant effect thereby preventing ROS formation and damage to hepatocyte as observed in the study[19,20]. This hepatoprotective activity of FS leaves substantiates the ethnomedicinal claim in management of liver disorders and leaf decoction in combination with other plant extract is taken orally to get relief from jaundice[21]. The jaundice is described in Ayurveda under *Kamala* which is mainly due to *Pittaja* vitiation. The liver is a *mulasthana* (primary site) of *Raktajavyadhi* (blood disorders). In a preliminary study in healthy volunteers, the *Rasa* of dried powder of leaves of FS is found to be *Kashaya* (astringent taste), *madhura* (sweet taste)[22]. *Kashaya* rasa is reported to be Kledamedavishoshan (useful in dyslipidemia having scraping action) and hence could have a possible role in reducing cholestasis. Also, it is tvakprasadana (~enhancing dermal health by removing toxins which here can be correlated to epithelium of liver) which can be possibly correlated to the repair of liver tissue and can be attributed in management of drug-induced liver injury[23].

Conclusion

The present study substantiates the hepato-protective potential of aqueous extract of *F. semicordata* leaves by alleviating the *in vitro* D-galactosamine induced hepatotoxicity in HepG2 cells. *In vitro* cell-based assay results observed in the present study must be correlated with suitable *in vivo* model and could give better insights into the role of *F. semicordata* in evaluating hepatoprotective activity clinically. There is a need for chemical evaluation to identify the active constituent, which is in process.

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Conflict of interest

The authors declare no conflict of interest.

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