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ORIGINAL ARTICLE **OPEN ACCESS**

In-Vitro Hepatoprotective Activity of Hydro-Alcoholic Extract of *Dodonaea viscosa* Linn

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ABSTRACT
The liver is a fundamental organ that capacities as a point of convergence of digestion and detoxification. Segregated rodent essential hepatocytes were utilized as an in-vitro model to assess the hepatoprotective movement of hydroalcoholic concentrate of Dodonaea viscosa Linn. Carbon tetrachloride was picked as hepatotoxin and silymarin was the reference hepatoprotective specialist. The hydroalcoholic concentrate of Dodonaea viscosa Linn. was surveyed for the in-vitro hepatoprotective movement on CCl4 prompted hepatotoxic Human Liver cell lines(HEpG-2 cell line) by MTT examine strategy. The confined essential rodent hepatocytes were brooded with normalized hepatotoxic portion of CCl4 (10 mM), and different fixations (10, 50 and 100µg/ml) of the concentrate and silymarin (100µg/ml). Trypan blue prohibition examine was utilized to quantify the cell feasibility. Marker transaminase compounds (GOT, GPT) and Total Protein (TP) in the cell culture not really set in stone. The outcomes uncovered that hydro alcoholic concentrate showed the critical % practicality (56.88%) in a portion subordinate way and contrasted with standard, Silymarin. This outcome proposes that the Dodonaea viscosa Linn. have critical hepatoprotective movement on carbon tetrachloride actuated hepatotoxicity in segregated rodent hepatocytes.
Keywords: *Hepatoprotective, MTT, Dodonaeaviscosa Linn. hepatocytes,cell viability, HEpG-2*

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INTRODUCTION

The liver is a crucial organ that capacities as a point of convergence of digestion of supplements and detoxification of synthetic substances. Any injury to the liver or the debilitation of its capacity might prompt numerous entanglements and influence the human wellbeing. On account of exorbitant openness to risky synthetic substances, the free revolutionaries are delivered that they overwhelm the normal cautious framework prompting hepatic harm, jaundice, cirrhosis and greasy liver [1]. In like manner, a pain relieving and against pyretic medication, Paracetamol and hostile to tubercular medications, isoniazid, rifampicin, pyrazinamide that causes intense liver harm at higher dosages [2-3].

Disregarding that, the openness of manufactured prescriptions for the treatment of liver issue and its related illnesses, still the administration of liver sicknesses is challenge to present day medication. As of now restorative plants and their arrangements have been generally utilized for the treatment of hepatic sicknesses. Natural medications are more broadly utilized than allopathic medications as hepatoprotective on account of its more affordable, better social adequacy, better similarity, with the human body and negligible secondary effects. These home-grown medications have shown the capacity to keep up with the ordinary utilitarian sculptures of the liver with or without less aftereffects [4].

The optional metabolites like Silymarin from *Silybum marianum*, andrographolide from *Andrographis paniculata*, curcumin from *Curcuma longa*, picroside and kutkoside from *Picrorrhiza kurroa*, phyllanthin and hypophyllanthin from *Phyllanthus niruri*, glycyrrhizin from *Glycyrrhiza glabra* are customarily utilized in the treatment of liver infections and address the phytochemical constituents and have been read up for their substance and natural profile and clinical viability [5, 6].

Dodonaea viscosa Linn. (Sapindaceae) regularly known as 'virali' is an evergreen enduring bush broadly disseminated in Western Ghats and Tamilnadu. The fables guarantee uncovers that the leaves have been utilized for the treatment of migraines and spinal pains by the Muthuvan clans of the Kerala locale. High temp water decoction of leaves is utilized to diminish swellings, spinal pains and steam inward breath is utilized to mitigate cold.

Further, in conventional clinical practice *Dodonaea viscosa* Linn. is utilized to ease stomach torment, heaps and ulcer. Past examinations have detailed the mitigating, antimicrobial, neighborhood sedative

and smooth muscle loosening up movement of *D. viscosa* [7, 8]. Thus, the current review was intended to assess the *In Vitro* hepatoprotective action of Hydro alcoholic concentrate of *D. viscosa* against carbon tetrachloride (CCl4) poisonousness by *In vitro* technique on a logical way is without a doubt persuading.

MATERIAL AND METHODS

All chemicals used were of analytical grade and procured from Sigma alrich and Fine Chemicals, Mumbai, India. Human hepatoma cell line (HepG2) was obtained from National Center for Cell Science (NCCS), Pune, India.

Plant Materials

The leaves of Dodonaeaviscosa Linn. were gathered in the month August 2018, from Trichy, Tamilnadu, India. The plant material was recognized and verified by the botanist. The plant materials were dried under conceal, cut into little pieces, crushed utilizing a mechanical processor and went through 40 cross section sifter and put away in a sealed shut holder for additional utilization (9).

Preparation of Plant Extracts (10)

The powdered leaves of Dodonaeaviscosa Linn. were separated with hydro liquor at room temperature. Later comprehensive extraction, the dissolvable was gathered and separated. The dissolvable was concentrated under lessen tension at 50-55°C. The concentrated hydro liquor separates were kept in desiccators for additional utilization. The hydroalcoholic concentrate of Dodonaeaviscosa Linn. was evaluated for their in-vitro hepatoprotective movement on CCl4 inebriated Human typical liver cell lines (HEpG-2 cell line) by MTT Assay (11 - 18).

Cell Culture and Sub Culturing of Human Normal Liver Cell Lines

Human typical liver cell lines (HEpG-2 cell line) were refined in DMEM medium Supplemented with 20% hotness inactivated FBS. To forestall the bacterial tainting anti-infection agents, Streptomycin and Penicillin were added. The way of life was cleaned by filtration utilizing 0.2 µm pore size cellulose acetic acid derivation channel. Sub refined is a course of moving few cells into another vessel. FBS was utilized for the appropriate development of Human typical liver cell lines. Trypsinisation is the most common way of utilizing trypsin, a proteolytic chemical what separates proteins to separate follower cells from the Vessels in which they are being refined. The cell lines were washed with PBS and the completely intersecting cells were trypsinised utilizing 500µl of trypsin for 3 minutes at 37oC. Later disaggregation the cell is moved to other carafe and enhanced with media, trypsinized (500µl of 0.025% Trypsin in PBS EDTA Solution) for 2 minutes and moved to T flagons in complete aseptic conditions.

Preparation of Test Solutions

Each weighed hydro alcoholic concentrate was broken up in refined DMSO and the volume was made up with DMEM enhanced with 2% inactivated FBS to get a stock arrangement of 1000 µg/ml fixation and cleaned by filtration. From this stock arrangement, three diverse lower weakening (250, 125 and 62.5 µg/ml) were ready (10).

Induction of CCl4 Hepatotoxicity

Carbon tetra chloride was used to induce hepatotoxicity on human normal liver cell lines. Under asceptic conditions, the cells were treated with 0.1% CCl4.

Procedure

The monolayer cell culture was trypsinized and the cell count was acclimated to 1.0 x 105 cells/ml utilizing DMEM containing 10% FBS. 0.1 ml of the weakened cell suspension (roughly 10,000 cells) was added to each well of the 96 well microtitre plates. Later 24 h, when a fractional monolayer was framed, the supernatant was flicked off and washed the monolayer once with medium. To this, 100 microlitre of various test convergences of hydroalcoholic concentrate of D. viscosa was added separately on to the halfway monolayer in microtitre plates. The plates were then brooded at 370C for 3 days in 5% CO2 air, and minuscule assessment was completed and perceptions were noticed each 24 h span. Later 72 h, the test substance in the wells was disposed of and 50 microlitre of MTT in PBS was added to each well. The plates were delicately shaken and brooded for 3 h at 370C in 5% CO2 environment. The supernatant was taken out and 100 microlitre of propanol was added. Every one of the plates were delicately shaken to solubilize the shaped formazan. Then, at that point, the absorbance was estimated utilizing a microplate peruse at a frequency of 540 nm utilizing UV-apparent spectrophotometer. The CTC50 still up in the air from the portion reaction curve (7).

Cytoprotective Effect

The essential rodent hepatocytes hence acquired were utilized for concentrating on the cytoprotective movement of hydroalcoholic separate. Fixed number of hepatocytes (1×106 cells/ml) was hatched with various fixations (10, 50 and 100 µg/ml) of JG concentrates and hepatotoxin 10 mM poison (CCl4) in sterile test tubes. The above cell suspension was hatched for 3 hour and later 3hrs of brooding the cell reasonability was dictated by the trypan blue prohibition examine. Later wards cell suspension was

centrifuged at low speed (2000 rpm) for 15 min. The supernatant arrangement was utilized for the assessment of marker catalysts like Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT) and Total Protein (TP). All judgments were done in Semi Auto analyzer (Microlab 300) utilizing Mark symptomatic packs. In the CCl4 control tubes media was added rather than extricate while in typical cylinders contains no CCl4. Silymarin (100µg/ml) was utilized as standard reference hepatoprotective.

Statistical Analysis

All the data were presented as mean ± SEM. Statistical analyses were performed by one way ANOVA followed by Newman-Keuls Multiple Comparison Test. Differences were considered statistically significant at the value of probability less than 5% ($P < 0.05$).

RESULTS

Dodonaea viscosa Linn, inebriated human cell lines treated with hydroalcoholic extricate displayed the greatest % practicality (56.88%) on CCl4 incited hepatotoxic Human Liver cell lines. The rate practicality of control, CCl4, standard and hydro alcoholic of *Dodonaea viscosa* Linn. bunches were recorded in table 1. Every one of the outcomes got were contrasted and the standard medication, silymarin.

Table: 1. Hepatoprotective effect of hydroalcoholic extract of *Dodonaea viscosa* Linn. on CCl4 induced heptotoxic HepG-2 cell line

S.No.	Extract tested	Test Conc. (µg/ml)	Absorbance	% Viability
1	Control		1.182	-
2	Carbon tetrachloride	0.1	0.198	-
3	Standard (Silymarin)	200 400	1.196 1.152	93.42±1.26** 94.51±4.24**
4	hydroalcoholic extract	250 125 62.5	0.668 0.584 0.322	56.88±6.14*** 43.50±1.82** 12.60±3.32**

Significant at*P <0.001 compared to control group; *P <0.05, **P <0.01; ***P <0.001 compared to CCl4 group.

The cytoprotective impact of hydroalcoholic concentrate of Dodonaeaviscosa Linn is portrayed in table 2. Detached rodent hepatocytes brooded with 10mM CCl4 brought about enlistment of critical ($P<0.05$) sub maximal poisonousness, a height around 141.45% and 216.62% of GOT and GPT level are noticed individually upon inebriated with CCl4. Disengaged rodent hepatocytes pretreated with hydroalcoholic concentrate of Dodonaeaviscosa Linn in the centralization of 10-100 µg/ml showed a moderate defensive impact by reestablishing the suitability of hepatocytes (10.81-22.82%), TP content (23.76-28.73%), GOT (14.04-32.84%) and GPT (19.75-30.52%). The most extreme huge ($P<0.05$) hepatoprotective movement was seen at 100 µg/ml focus while no/least action was delivered at 10 µg/ml fixation. The reference standard medication silymarin showed great hepatoprotective impact by reestablishing practicality (81.92%), GOT (92.52%), GPT (77.21%) and TP (78.48%).

Table: 2. Effect of hydroalcoholic extract of *Dodonaea viscosa* Linnon CCl4 induced hepatotoxicity in isolated rat hepatocytes

Treatment	Viable cell (%)	GOT (IU/L)	GPT IU/L	TP g/dl
Normal	95.66±0.88	64.33±0.33	94.33±0.33	0.91±0.025
CCl4 Control	29.57±0.50	155.33±0.88	298.67±1.20	0.43±0.015
Silymarin 100 µg/ml	85.05±0.58** (81.92)	72.00±1.53* (92.52)	143.33±1.45* (77.21)	0.81±0.019** (78.48)
Hydroalcoholic extract 10 µg/ml	25.33±1.20 (-6.38)	144.33±1.20* (14.04)	260.33±1.33 (19.75)	0.39±0.015 (-8.27)
Hydroalcoholic extract 50 µg/ml	37.33±0.67** (10.81)	134.67±1.45* (22.87)	245.66±1.45* (26.94)	0.54±0.014** (23.76)
Hydroalcoholic extract 100 µg/ml	44.33±0.88** (22.82)	126.33±0.67* (32.84)	238.33±1.35* (30.52)	0.56±0.018** (28.73)

Data represents the Mean±SEM of three values, JG: Justiciagendarussa extract.
*Significant reduction compared to hepatotoxin (CCl4) ($P < 0.05$).
** Significant increase compared to hepatotoxin (CCl4) ($P < 0.05$)

DISCUSSION AND CONCLUSION

The liver is one of the most crucial organs in the body and principally capable in the guideline of assorted cycles. Liver cells are harmed because of the consistent openness of poisons and medications, ongoing liquor utilization and organisms which influence the human wellbeing. In excess of 700 mono or poly-home grown arrangements as decoction, color, tablets and case are accessible as hepatoprotective with or without market norms.

The current study was performed to survey the hepatoprotective movement in segregated rodent hepatocytes against carbon tetrachloride actuated intense hepatic injury. CCl4 initiated hepatic injury is normally utilized as a trial technique for the investigation of hepatoprotective impact of restorative plants. The hepatotoxicity instigated by CCl4 is because of its metabolite CCl3*, a free extreme that ties to lipoprotein and prompts peroxidation of lipids of the endoplasmic reticulum (19).

The capacity of a hepatoprotective medication to lessen the harmful impacts, or to save the typical hepatic physiological instruments which have been upset by a hepatotoxin, is a record of its defensive impacts. In spite of the fact that transaminase chemicals levels are not an immediate proportion of hepatic injury, they show the situation with the liver. The bringing down of marker liver compounds is an unequivocal sign of hepatoprotective activity of the medication. The transaminase compounds GOT and GPT levels are solid markers of liver capacity (20).

The previous finding gives solid proof and started the examination work with the theory that Dodonaeaviscosa Linn additionally may display hepato defensive movement. The outcomes uncovered the very that cell which are pretreated with extricates showed an increment in rate reasonability when contrasted with non-treated CCl4 inebriated cells in a portion subordinate way. Inebriated human cell lines treated with hydroalcoholic separate displayed the greatest % suitability (55.57%) at a convergence of 250 µg/ml on CCl4 incited hepatotoxic Human Liver cell lines. No brief % suitability was seen in the inebriated human cell lines treated with ethyl acetic acid derivation and pet ether extricates at the different focus utilized. Every one of the outcomes acquired were contrasted and the standard medication, silymarin.

Confined hepatocytes have become important devices to assess the conceivable defensive impact of medications in the new past. The methods for high return disengagement of rodent hepatocytes are made it as helpful model (21). Hepatotoxin like CCl4, paracetamol, thioacetamide and so forth have been displayed to bring about the decrease of cell feasibility just as rise in marker proteins, comparative changes in the current review affirms these progressions and shows palatable normalization of our detachment and culture strategy. In the current review, the hepatotoxin utilized lessens cell feasibility conceivably because of injury to plasma layers of hepatocytes bringing about the spillage of cell chemicals. Brooding of segregated hepatocytes with hydroalcoholic concentrate of *Dodonaea viscosa* Linn tolerably reestablished their feasibility just as adjusted biochemical boundaries actuated by hepatotoxin.

In this study, we have noticed huge poisonousness later 3hr brooding with hepatotoxin. The hydroalcoholic concentrate of Dodonaeaviscosa Linn at the portion of 100 µg/ml has delivered huge ($P<0.05$) assurance against CCl4 initiated hepatotoxicity, but the hydroalcoholic concentrate of *Dodonaea viscosa* Linn has shown no defensive impact at 10 µg/ml focus. It very well might be conjectured that, the flavonoids, which are available in JG remove, could be considered liable for the hepatoprotective action.

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CONFLICT OF INTEREST

There is no conflict of interest.

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