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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF EXTRACTS OF *BREYNIA VITIS-IDAEA* (BURM.F) C. FISHER LEAVES BY USING CHANG LIVER CELL LINE

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ABSTRACT

The present study was under taken to study the Hepatoprotective activity of ethanol, aqueous and ethyl acetate extracts of *Breyniavitis-idaea* (burm.f) c. fisher. (*Euphorbiaceae*) leaves. Hepatoprotective activities of these extracts were studied using *in-vitro* MTT assay and compared with the toxicants. All the extracts showed significant increase in the % cell viability. Hepatoprotective nature of the extracts may be attributed to the presence of constituents such as alkaloids, glycosides, flavonoids and saponins in plant extracts. This study proven that the potential hepatoprotective nature of the extracts of *Breyniavitis-idaea* leaves.

KEYWORDS

Breyniavitis-idaea, Hepatoprotective activity, Ethanol extract, Aqueous extract and Ethyl acetate extract.

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INTRODUCTION

Herbal medicines are a valuable as well as a precious gift from nature. They were existing even before human beings made their appearance on the earth. Wherever we are born we have around us herbs, shrubs, and plants useful to us¹. It is gratifying to note that in India, the importance and relevance of herbal system (Ayurveda, Unani, Sidda) is increasingly being realised for the last few decades. It is apparent that the sleeping giant of ayurveda is finally waking up².

Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ. Other chemical agents

such as those used in laboratories and industries, natural chemicals and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins.

More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures^{3,4}. India's system of medicine helping the body copes with liver problems, including jaundice, hepatitis, and cirrhosis, these herbs help the liver to eliminate toxins and microbial infections⁵.

In modern medicine corticosteroids and immunosuppressant are commonly used to treat liver disease in allopathic form of medicine. But, these drugs are associated with adverse effects such as immunosuppressant and bone marrow depression⁶. However there are a number of drugs employed in traditional system of medicine for liver affections. About 600 commercial preparations with claimed liver protecting activity are available all over the world. About 100 Indian medicinal plants belonging to 40 families are used for herbal formulation. A few reports on the hepatoprotective activity are cited here, e.g. *Apiumgraveolens* Linn. (*Umbelliferae*), *Euphorbia antisiphilitica* (*Euphorbiaceae*), *Rubbia cordifolia* (*Rubiaceae*), *Solanumlyratum* (*Solanaceae*), *Tylophoraindica* (*asclepiadaceae*)⁷.

The liver is a vital organ present in vertebrates and some other animals and it is the largest glandular organ of the body. It weighs about 3 lb (1.36 kg). It is reddish brown in colour and is divided into four lobes of unequal size and shape. It lies on the Right side of the abdominal cavity beneath the diaphragm. A thick capsule of connective tissue called Glisson's capsule covers the entire surface of the liver. The liver is multi-lobed organ. It has 6 distinct lobes organized into 3 regions, divided into a large right lobe and a smaller left lobe. The falciform ligament divides the two lobes of the liver. Each lobe is further divided into lobules that are approximately 2 mm high and 1 mm in circumference.

Approximately 1500 ml of blood enters the liver each minute, making it one of the most vascular organs in the body. 75% of the blood flowing to the liver comes through the portal vein; the remaining 25% is oxygenated blood that is carried by the hepatic artery. Blood is carried to the liver through two large vessels called the hepatic artery and the portal vein. The hepatic artery carries oxygen rich blood from the aorta. The portal vein carries blood containing digested food from the small intestine. These blood vessels subdivide in the liver repeatedly, terminating in very small capillaries. Liver tissue is composed of thousands of lobules, each lobule is made up of hepatic cells, the basic metabolic cells of the liver⁸.

Functions of the liver⁹

- Secretion and excretion of bile.
- Excretion of bilirubin, cholesterol, hormones and drugs.
- Metabolism of carbohydrates, fats, proteins and various chemicals and drugs.
- Fibrinogens, prothrombin and heparin production.
- Enzyme activation.
- Storage of glycogen, vitamins and minerals (iron and copper).
- Synthesis of plasma proteins, such as albumin, globulin and clotting factors.
- Blood detoxification and purification.

Physiology of liver

It is an understatement to say that the liver is an important organ. Every second the liver cells go through thousands of complex biochemical reactions that influence all the functions of other organs in the body. The liver has reserve functional power and can operate effectively when most of the hepatocytes are not working well. In addition, diseased hepatocytes can actually regenerate and return to normal function. The human body identifies almost all drugs as foreign substances (xenobiotics) and subjects them to various chemical processes i.e. metabolism to make them suitable for elimination. This involves chemical transformations to (a) reduce fat solubility and (b) to change biological activity. Although almost all tissue in the body have some ability to metabolize chemicals, smooth endoplasmic reticulum in liver is the principal "metabolic clearing house" for both endogenous

chemicals (e.g. cholesterol, steroid hormones, fatty acids, proteins), and exogenous substances (e.g. drugs).

Drug metabolism is usually divided into two phases: Phase 1 reaction is thought to prepare a drug for Phase 2. However many compounds can be metabolized by Phase 2 directly. Phase 1 reaction involves oxidation, reduction, hydrolysis, hydration and many other rare chemical reactions. These processes tend to increase water solubility of the drug and can generate metabolites, which are more chemically active and potentially toxic. Most of Phase 2 reactions take place in cytosol and involve conjugation with endogenous compounds via transferase enzymes. Chemically active Phase 1 products are rendered relatively inert and suitable for elimination by this step.

A group of enzymes located in the endoplasmic reticulum, known as Cytochrome P-450, is the most important family of metabolizing enzymes in the liver. Cytochrome P-450 is the terminal oxidase component of an electron transport chain. It is not a single enzyme, rather consists of a family of closely related 50 forms, six of them metabolize 90% of drugs. There is a tremendous diversity of individual P-450 gene products and this heterogeneity allows the liver to perform oxidation on a vast array of chemicals in Phase 1. Three important characteristics of the P-450 system have roles in drug induced toxicity¹⁰.

Genetic diversity

Each of the P-450 proteins is unique and accounts to some extent for the variation in drug metabolism between individuals. Genetic variations (polymorphism) in CYP-450 metabolism should be considered when patients exhibit unusual sensitivity or resistance to drug effects at normal doses. Such polymorphism is also responsible for variable drug response among patients of differing ethnic backgrounds.

Change in enzyme activity

Many substances can influence P-450 enzyme mechanism like drugs interact with the enzyme family in several ways. Drugs that modify Cytochrome P-450 enzyme are referred to as either inhibitors or inducers. Enzyme inhibitors block the metabolic activity of one or several P-450 enzymes; this effect usually occurs

immediately and on the other hand inducers increase P-450 activity by increasing its synthesis. Depending on inducing drug's half-life, there is usually a delay before enzyme activity increases.

Competitive inhibition

Some drugs may share the same P-450 specificity and thus competitively block their biotransformation. This may lead to accumulation of drugs metabolized by the enzyme. This type of drug interaction may also reduce the rate of generation of toxic substrate.

Liver Injury

The primary liver diseases include viral hepatitis, hepatocellular carcinoma, alcoholism, extra hepatic infection disseminated cancer, cirrhosis, hepatic failure etc.

Toxins

There are literally thousands of chemicals that could be toxic to the liver and a few examples of these chemicals (Table No.1) that are commonly used in the treatment include:

- Rimadyl (arthritis treatment) in Labradors
- Thiacetarsamide (heartworm treatment)
- Ketoconazole (fungal treatment)
- Tylenol (acetaminophen)
- Glucocorticoids (cortisone)
- Anthelmintics (deworming medication)
- Parasiticides

Breyniavitis-idaea (Burm.F.) C. fisher.

(Euphorbiaceae) is an evergreen, glabrous tree or large shrub. Found in the genetic plain, western peninsula, China, India, Malay Peninsula and Sri Lanka. Bark is yellowish gray, leaves are alternate dark brown when dry, and flowers are small, greenish yellow and dull red, purple or white berries. Root, leaves and bark are medicinal. Root decoction is used as mouthwash. A new sulphur-containing spiroketal glycoside, breynin I and a new terpenic glycoside, breyniaionoside E together with 10 known compounds, were isolated from the plant¹³.

EXPERIMENTAL SECTION

Plant material

The leaves of *Breyniavitis-idaea* were collected from surrounding area of Maddur, Mandya District in Karnataka, India. The plant was identified and

authenticated by Botanist Prof. Nagendra. T. (Specimen no: 594) Bharathi College, Bharathinagar, Mandya District, Karnataka, India. The leaves were dried under shade then pulverized into coarse powder by a mechanical grinder and used for extraction.

Extraction Process

About 200 grams of dry powder of leaf of *Breyniavitis-idaea* was extracted first with ethanol for 72 hrs. The powdered drug was dried and packed well in Soxhlet apparatus and extracted. The extract was concentrated and dried using Rotary vacuum evaporator. It was kept in desiccators until used. The marc left after the extract was dried and subsequently extracted with ethyl acetate. The extraction was continued up to 72 hrs. The extract was concentrated and dried using Rotary vacuum evaporator. It was kept in desiccators until used. The marc left after the extract was dried and subsequently extracted with water. The extraction was continued up to 72 hrs. The extract was concentrated and dried using Rotary vacuum evaporator. It was kept in desiccators until used^{14,15}.

METHOD OF HEPATOPROTECTIVE ACTIVITY^{16,17}

Maintenance of cell lines

Cell lines were grown in 25 cm² tissue culture flasks containing Dulbecco's modified eagles medium supplemented with 10% Foetal bovine serum, 1% L-glutamine and 50µg/ml gentamycin sulphate at 37°C in CO₂ incubator in an atmosphere of humidified 5% CO₂ and 95% air. The cells were maintained by routine sub culturing in 25cm² tissue culture flasks.

Sub culturing process of cell lines

The culture media from the flasks containing monolayer culture was aspirated and washed with sterile phosphate buffered saline. To the flasks 3 ml of 0.2% trypsin-EDTA solution was added, after few seconds it was aspirated and flask was kept in incubator 2-3 min for detachment. The flasks were removed from the incubator and gently tapped to detach all the adhering cells. The cell detachment was confirmed by observing under an inverted microscope. Once the cells were completely detached from the flasks, 2-3 ml of Dulbecco's modified eagles medium media containing 10% Foetal bovine serum was added and mixed well. Cell viability was checked with a

small sample of the suspension by trypan blue dye exclusion test. From the stock cell suspension, 1 x 10⁴ viable cells/ml suspended in media were seeded in 25cm² tissue culture flask containing about 4ml of fresh media and incubated until the flasks attained 60-70% confluence.

Trypsinization

To obtain a single cell suspension from a monolayer culture, cells were dislodged from the culture flasks by trypsinization. From a 60-70% confluent flask, the culture media was aspirated out using a micropipette. Cells were washed with 3 ml of Phosphate buffer saline to remove trace amount of media. To each culture flask 3 ml of trypsin-EDTA was added and after few seconds it was aspirated and the flask was kept in the incubator for 3-4 min for cell detachment. Culture flasks were observed under an inverted microscope to ensure that cells were completely dislodged. Trypsin activity was stopped by adding 2-3ml media containing 10% Foetal bovine serum.

Cryopreservation of the cell lines

Tumour cells from the first and second passage of transplantation were stored in liquid nitrogen in cry vials containing Dulbecco's modified eagle's medium supplemented with 10% Foetal bovine serum and 10% Dimethyl sulphoxide as preservative at a concentration of 1x 10⁶ cells/ml. This constituted the tumour bank. After every 10 passages, that tumour cell line was discarded and new passage was started using the original tumour cells from the tumour bank.

MTT assay principle

This is a colorimetric assay that measures the reduction of yellow 3-(4, 5-dimethylthiazol-2-yl) - 2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent (e.g. isopropanol) and the released solubilized formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

Working Procedure

Trypsinize one T-25 flask and add 5 ml of complete media to trypsinized cells. Count and record cells per ml. Remember to remove the cells aseptically when counting. The monolayer cell culture of Chang liver cells was trypsinized and the cell count was adjusted to 1.0×10^6 cells/mL using medium containing 10% new born calf serum. To each well of the 96 well micro titre plates, 0.1 mL of the diluted cell suspension (approximately 10,000 cells) was added. Treat cells on day two (after 24 hrs) with standard or drug. If removing media, do very carefully. This is where most variation in data may occur. Final volume should be 100 μ l per well. Incubate it for 24hrs. After 24hrs i.e., on 3rd day, Add 20 μ l of 5 mg/ml MTT to each well. Include one set of wells with MTT but no cells (control). All should be done aseptically. Incubate for 3.5 hrs at 37 °C in culture hood. Carefully remove media. Do not disturb cells and do not rinse with Phosphate buffer saline. Add 80 μ l Dimethyl sulphoxide. Cover with tinfoil and agitate cells on orbital shaker for 15 min. Read absorbance at 540 nm by using Micro plate reader. Storage: The MTT reagent must be kept at 4°C in the dark.

Calculation

Percentage cytotoxicity calculated from the formula: $[(AC - AB) - (AT - AB)] / (AC - AB) * 100$.

Where AC, AT and AB are absorbance of control, test and blank respectively. IC₅₀ values can be determined from the dose response plots, by linear regression.

In Vitro Hepatoprotection

In vitro hepatoprotective activity against alcohol and CCl₄ induced toxicity

The *in vitro* hepatoprotection study is based on the principle of MTT assay of cytotoxicity. The cells get pre-treated with the compound for 24 hrs then challenged by toxicant for next 24hrs. Finally cells get exposed to tetrazolium salt 3-(4, 5 dimethyl thiazole-2 yl) - 2, 5-diphenyl tetrazolium bromide (MTT) which gets cleaved into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used.

Preparation of Test solutions

1 mg of the drug samples were weighed accurately and separately dissolved in 10 μ L of Dimethyl sulphoxide and made up the volume to 1 ml with maintenance medium. These solutions were serially diluted with maintenance medium to obtain the lower dilutions.

Procedure

The monolayer cell culture of Chang liver cells was trypsinized and the cell count was adjusted to 1.0×10^6 cells/mL using medium containing 10% new born calf serum. To each well of the 96 well micro titre plates, 0.1 mL of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hrs, when a partial monolayer was formed, 100 μ L of different drug concentrations was added to the cells in micro titre plates. Plates were then incubated at 37 °C for next 24 hrs in 5% CO₂ atmosphere, and microscopic examination was carried out and observations recorded every 24 hrs. After 48 hrs, 100 μ L of a fixed concentration of hepatotoxin (either alcohol or CCl₄) was added to the cells in micro titre plates. The plates were then incubated at 37 °C for next 24 hrs in 5% CO₂ atmosphere After 72 hrs, the solutions from the wells were discarded and 50 μ L of MTT was added to each well. The plates were gently shaken and incubated for 3 hrs at 37 °C in 5% CO₂ atmosphere. The supernatant was removed and 100 μ L of Dimethyl sulphoxide was added and the plates were gently shaken to solubilise the formed formazan. The absorbance was measured using a micro plate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the formula below:

% Growth Inhibition = $100 - \frac{\text{Mean OD of individual Test group}}{\text{Mean OD of Control group}} \times 100$

Statistical analysis

All results of experiment were expressed as mean \pm sem. Statistical analysis were carried out with Prism version-5.0 using ANOVA followed by Dennett's test (P<0.05).

RESULTS AND DISCUSSION

In the present study, *B.vitis-idaea* leaf parts were extracted with ethanol, ethyl acetate and water were analysed for presence of phytochemicals and subjected to *in-vitro* hepatoprotective activity. Phytochemical

analysis showed that the presence of alkaloids, flavonoides, glycosides, and saponins in both ethanol and aqueous extracts. Ethyl acetate extracts shows presence of glycosides and flavonoides. This showed in Table No.2 and 3.

Above three extracts of plant were tested at different dose levels (62.5-500µg/ml). All the extracts showed cell viability of more than 80% at 500µg/ml concentrations. Indicating the IC₅₀ values more than 500 µg/ml. This result suggest that extracts are nontoxic to chang liver cells up to the dose of 500µg/ml. Based upon the study we selected doses (25-200µg/ml) were selected for hepatoprotective against liver toxicant (alcohol or CCl₄) Table No.4.

Evaluation the Hepatoprotective effect of extracts on alcohol induced and CCl₄ induced liver toxicity in chang liver cell line

Hepatotoxicity was induced by CCl₄ and Alcohol in chang liver cells. Alcohol produced about 65% death of the chang liver cells indicating toxic effect on hepatocytes. Pre-treatment with plant extracts at

different concentrations could able to protect alcohol induced chang liver cell damage. CCl₄ treatment resulted in 77% cell death; pre-treatment with plant extracts at different dose levels protect CCl₄ induced hepatic cell damage.

In the assessment of liver damage by hepatotoxins like alcohol and CCl₄ the determination of cell damage by morphological characters and followed by MTT assay. Hepatoprotective activity of aqueous, ethanol, and ethyl acetate extracts were evaluated using standard procedure in human chang liver cell cultures.

In MTT assay different four concentrations were used. All the extracts exhibited a percentage viability of 85 to 95% on chang liver cells. And finally it has shown treatment with plant extracts at different dose levels possesses a protection against alcohol and CCl₄ induced hepatic cell damage of chang liver cell line. The results are depicted as in the following given Table No.6.

Table No.1: Classification of hepatotoxins and also mechanism of action of each group of Hepatotoxins^{11,12}

Category of agents	Mechanism of action	Histological lesion	Examples
Intrinsic toxicity			
Direct	Direct physiochemical destruction by per oxidation of hepatocytes	Necrosis And / or Steatosis	Carbon tetrachloride, Phosphorous
Indirect cytotoxic	Interference with Hepatocellular metabolic Pathways	Steatosis or necrosis	Ethionine, ethyl alcohol, tetracycline
Host idiosyncrasy			
Hypersensitivity	Drug allergy	Necrosis or cholestasis	Chlorpromazine, phenytoin sulphonamides
Metabolic	Production of hepatotoxic metabolites	Necrosis or cholestasis	Isoniazide, valproic acid.

Table No.2: Phytochemical analysis of the extracts of *breyaniavitis-idaea*

S.No	Extracts	Alkaloids	Flavonoides	Glycosides	Saponins
1	Aqueous extract	+	+	+	+
2	Ethanol extract	+	+	+	+
3	Ethyl acetate extract	-	+	+	-

Table No.3: Absorbance of Dimethyl sulphoxide (control) at 540nm

S.No	Control	Trial-1	Trial-2	Trial-3	Average
1	DMSO (0.5%)	0.398	0.408	0.352	0.386

Table No.4: Determination of CTC₅₀ by MTT Assay in Chang Liver cell line by using various extracts of plant CTC₅₀ – Cytotoxicity concentration 50%

S.No	Compound Name	Conc (µg/ml)	Read at 540nm			% Cell death			Mean% cell death	% Cell Viability			Mean % viability	SEM	IC ₅₀ (µg/ml)
1	Aqueous extract	500	0.32	0.33	0.32	17.1	12.9	17.1	15.7	82.9	87.0	82.9	84.3	1.4	>500
		250.0	0.33	0.33	0.33	12.1	13.9	12.1	12.7	87.8	86.0	87.8	87.2	0.6	
		125.0	0.36	0.32	0.32	6.74	15.5	15.5	12.6	93.3	84.5	84.5	87.4	2.9	
		62.5	0.36	0.37	0.38	4.92	2.85	0.52	2.76	95.1	97.2	99.5	97.2	1.3	
2	Ethanol extract	500	0.34	0.37	0.37	11.9	2.85	3.11	5.96	88.1	97.2	96.9	94.0	3.0	>500
		250.0	0.34	0.35	0.37	9.84	8.03	1.81	6.56	90.2	92.0	98.2	93.4	2.4	
		125.0	0.30	0.33	0.38	20.2	12.1	0.00	10.7	79.8	87.8	100	89.2	5.9	
		62.5	0.34	0.36	0.39	11.1	5.44	-1.0	5.18	88.9	94.6	101	94.8	3.5	
3	Ethyl acetate extract	500	0.25	0.30	0.30	33.1	22.0	20.2	25.1	66.8	78.0	79.8	74.9	4.0	>500
		250.0	0.32	0.30	0.26	16.8	21.7	30.8	23.1	83.2	78.2	69.2	76.9	4.1	
		125.0	0.33	0.30	0.28	12.4	22.0	25.1	19.8	87.6	78.0	74.9	80.1	3.8	
		62.5	0.36	0.28	0.38	6.48	26.9	0.26	11.2	93.5	73.1	99.7	88.8	8.1	

Values represent in Mean % of cell viability and SEM in different extracts of plant. * represents P<0.05 when compared with Control. **represents P<0.01 when compared with Control.*** represents P<0.001 when compared with Control.

Table No.5: Raw data of cell viability with plant extracts of *Breyniavitis-idaea* for control, alcohol and CCl₄

S.No	Trial	Raw data			Mean	SEM	% cell death	% cell viability
1	Medium control	0.636	0.709	0.627	0.657	0.026	0.00	100.0
2	Alcohol	0.255	0.305	0.322	0.294	0.02	65.3	34.7
3	CCl ₄	0.125	0.158	0.167	0.15	0.013	77.2	22.8

Table No.6: Protective effect of extracts of *Breyniavitis-idaea* on alcohol induced and CCl₄ induced liver toxicity in chang liver cell line

Group No.	Compound Name	Con'c (µg/ml)	Read at 540nm			% Cell death			mean %cell death	% Cell Viability			mean % cell Viability	SEM
1	Aqueous extract + Alcohol	25	0.24	0.23	0.26	13	14.2	10.2	12.5	86.9	85.7	89.7	87**	1.2
		50	0.25	0.28	0.25	11.6	7.1	10.9	9.88	88.3	92.9	89.1	90.1*	1.4
		100	0.24	0.26	0.27	13	9.69	10.6	11.1	86.9	90.3	89.3	88.8*	1.3
		200	0.21	0.24	0.26	10.9	2.73	9.23	7.65	89.0	97.2	90.7	93**	2.2
2	Aqueous extract + CCl ₄	25	0.07	0.07	0.07	8.74	9.2	8.59	8.84	91.2	90.8	91.4	91.1*	0.2
		50	0.15	0.12	0.16	7.18	11.5	9.29	9.35	92.8	88.4	90.7	90.6*	2.1
		100	0.15	0.13	0.15	6.88	9.01	6.42	7.43	93.1	90.9	93.5	92.6*	0.8
		200	0.17	0.18	0.20	13.6	12.6	9.12	11.8	86.3	87.3	90.8	88**	1.4
3	Ethanol extract + alcohol	25	0.26	0.30	0.27	9.08	3.45	8.62	7.05	90.9	96.5	91.8	92.95	1.8
		50	0.26	0.28	0.28	9.84	7.1	7.1	8.01	90.1	92.9	92.9	91.9*	0.9
		100	0.16	0.18	0.22	4.29	1.55	6.38	4.07	95.7	98.5	93.6	96**	2.3
		200	0.15	0.15	0.15	7.33	7.33	6.88	7.18	92.6	92.6	93.6	93**	0.2

4	Ethanol extract + CCl ₄	25	0.14	0.15	0.16	8.4	5.96	5.66	6.67	91.6	94.0	94.3	93.33	0.9
		50	0.15	0.15	0.17	6.12	5.96	2.77	5.91	93.8	94.0	97.2	94.09	1.1
		100	0.19	0.19	0.21	9.88	9.88	8.05	9.27	90.1	90.1	91.9	90.73*	0.6
		200	0.22	0.23	0.25	6.53	14.8	11.2	10.8	93.4	85.1	88.8	89.3**	1.6
5	Ethyl acetate extract + Alcohol	25	0.26	0.28	0.28	9.99	6.49	6.95	7.81	90	93.5	93.0	92.19	1.1
		50	0.25	0.31	0.30	11.3	12.3	13.9	12.5	88.6	87.6	86.1	87.46	2.8
		100	0.16	0.16	0.19	15.0	15.8	10.3	13.7	84.9	84.1	89.6	86.26	1.7
		200	0.11	0.12	0.12	13.2	11.1	10.9	11.7	86.7	88.8	89.0	88.2*	0.7
6	Ethyl acetate extract + CCl ₄	25	0.14	0.15	0.17	8.25	6.88	13.5	9.55	91.7	93.1	86.4	90.45	1.4
		50	0.15	0.15	0.16	16.5	6.88	15.3	12.9	83.4	93.1	84.6	87.07	0.5
		100	0.15	0.14	0.17	6.72	8.55	4.29	6.52	93.2	91.4	95.7	93.48	1.2
		200	0.19	0.18	0.19	10.7	11.4	9.88	10.6	89.2	88.6	90.1	89.3*	0.4

Values represent in Mean % of cell viability and SEM in different extracts of plant.* represents P<0.05 when compared with Control.**represents P<0.01 when compared with Control.*** represents P<0.001 when compared with Control.

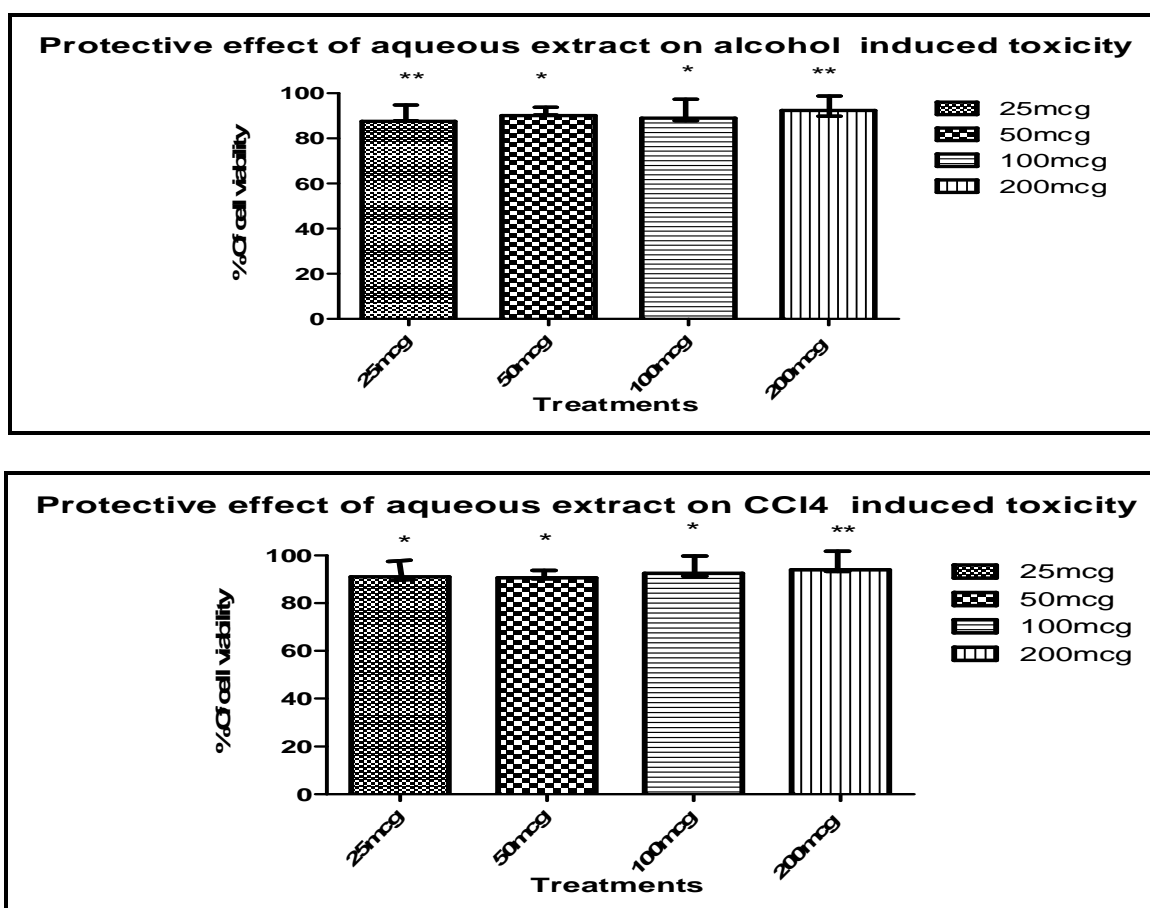


Figure No.1: Protective effect of aqueous extract on alcohol and CCl₄ induced toxicity

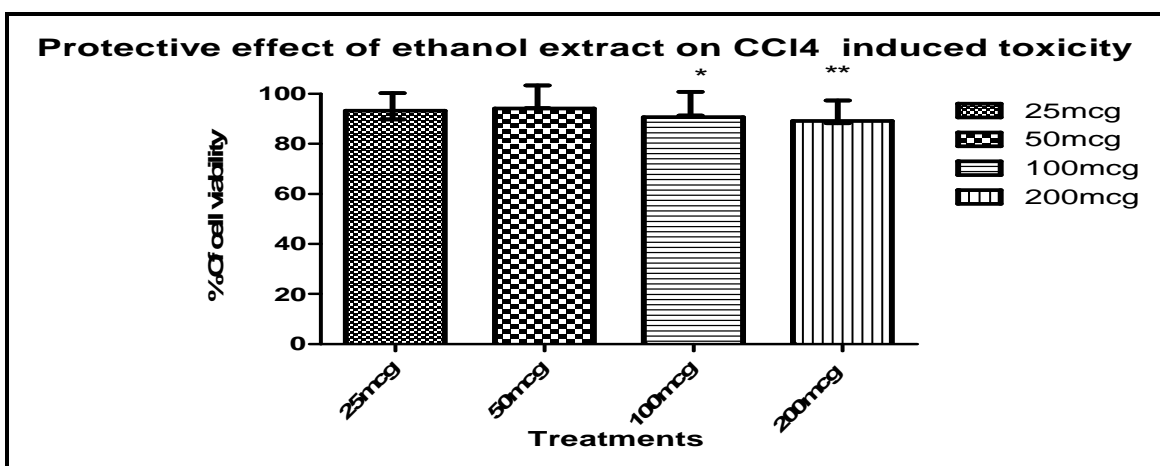
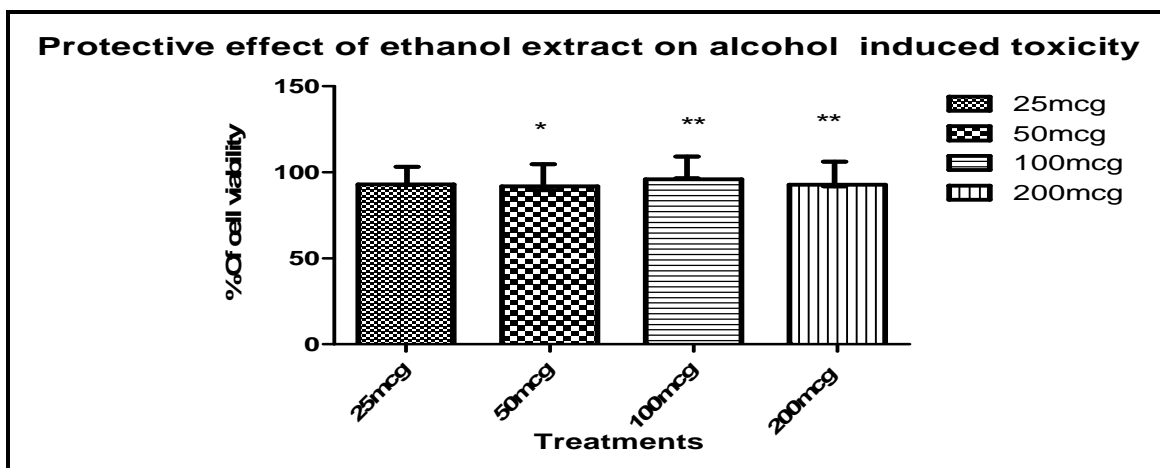
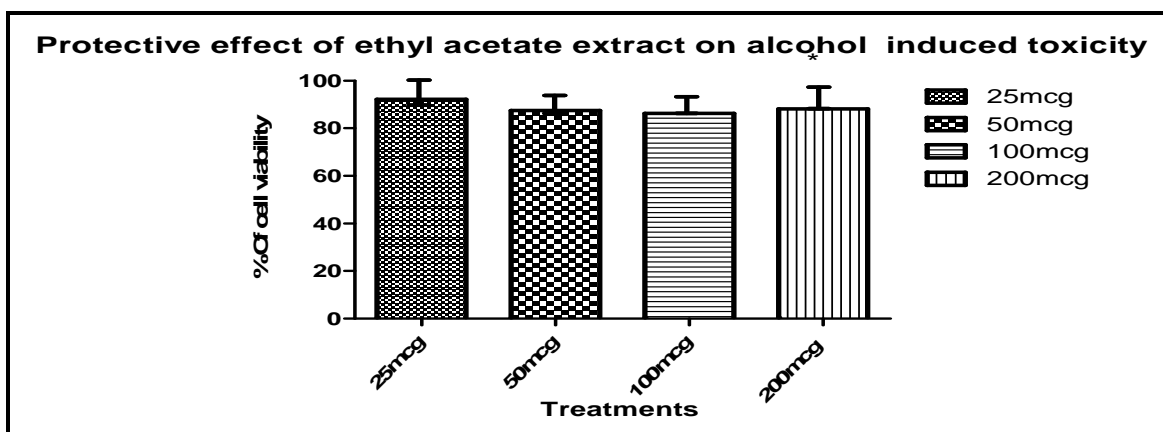


Figure No.2: Protective effect of ethanol extract on alcohol and CCl₄ induced toxicity



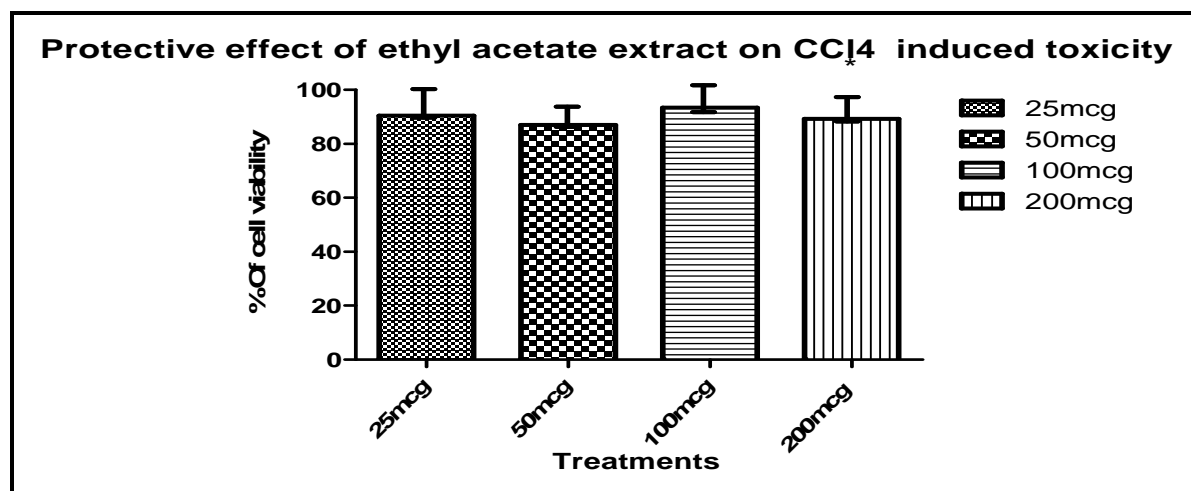


Figure No.3: Protective effect of ethyl acetate extract on alcohol and CCl₄ induced toxicity

Values represent in Mean % of cell viability and SEM in different extracts of plant. * represents P<0.05 when compared with Control. **represents P<0.01 when compared with Control.*** represents P<0.001 when compared with Control.

CONCLUSION

The activity performed was targeted the toxicity protection and the outcome of the results are highly encouraging and matching. Based on the result we concluded that aqueous, ethanol and ethyl acetate extracts of plant proves the hepatoprotective action in change liver cell lines against alcohol and carbon tetra chloride induced toxicity in cell line. The extracts were found to be nontoxic. By doing further research in *Breynia vitis-idaea* plant even we can develop phytotherapeutic for cancer. Also we can use plant for wound healing.

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CONFLICT OF INTEREST

None declared.

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