Invitro, and Invivo Studies on Biomedical Applications of Foliose Lichens Stictaweigelii, Dermatocarponvellereum and Heterodermiaboryi against Clinical Debilities

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Abstract

Preclinical (Anti pyretic & analgesic) studies, Anti Diabetic, Hepatoprotective and and Anti cancer studies (Invivo and Invitro) of three lichen species viz., H.boryi, S.weigelii and D.vellereum were studied in the present work. Antipyretic and analgesic effects were high in H.boryi and D. vellereum respectively. Diabetic kidney treated with D. vellereum and S.weigelii have shown no vascular degenerative changes that possessed more antioxidants which prevented vascular degeneration.There was a remarkable regeneration of hepatocyte was found in H. boryi (200 and 400 mg). Diabetic kidney treated with D.vellereum and S.weigelii shown no vascular degenerative changes. There was a remarkable regeneration of hepatocyte was found in H.boryi (200 & 400mg). The IC_{50} The effect of lichen extracts (acetone, chloroform and diethyl ether) were tested against MCF-7, MG-63 and HeLa cells. The IC_{50}value was very less for the acetone extract of H.boryi (83.29 µg against MCF-7, 70.37 µg against MG-63 cells and 62.51 µg against HeLa cells) against the selected cell lines invitro. It was observed that the chloroform extract of H.boryi have shown 222.7 µg/ml of IC_{50} against MG-63 cells, 208 µg/ml of IC_{50} against HeLa cells . The diethyl ether extract of H.boryi have IC_{50} of about 140.7 µg/ml against MCF-7 cells, 109.2 µg/ml against MG-63 cells and 93.64 µg/ml against HeLa cells.The IC_{50}value was very less for the acetone extract of S.weigelii (151.1 µg against MCF-7 and 83.82 µg against HeLa cells) against the selected cell lines invitro. It was observed that the chloroform extract of S.weigelii have shown 179.7 µg/ml of IC_{50} against MG-63 cells. The diethyl ether extract ofS.weigelii have IC_{50} of about 204.2 µg/ml against MCF-7 cells, 88.91 µg/ml against MG-63 cells. The IC_{50}value was very less for the acetone extract of D.vellereum (101.5 µg against MCF-7, 120.3 µg against MG-63 cells and 120.9 µg against HeLa cells) against the selected cell lines invitro. It was observed that the chloroform extract of D.vellereum have shown 164.2 µg/ml of IC_{50} against MG-63 cells, The diethyl ether extract ofD.vellereum have IC_{50} of about 178.8 µg/ml against MG-63 cells. Cervical cancer induced disease model in animal was evaluated in terms of CEA which is found to be greatly reduced in group treated with (500mg of acetone extract of Sticta weigelii, 500mg of Dermatocarpon vellereum). Hence this work may probably serves as a base line study to explore the impact of depsides of foliose lichens on animal and microbial kingdom.
KEYWORDS – Acute toxicity, MCF-7, HeLa, Histopathology, Cervical cancer

I. Introduction

Lichens have a worldwide distribution, occurring in the highest, hottest, coldest, wettest and driest habitats and they are slow growing organisms, yet they are extremely sensitive to pollution [1]. Lichens are complex organisms involved in the symbiotic relationship between a phycobiont (Cyanobacteria or Green alga, or both) and a mycobiont (a fungus), have attracted considerable attention because of their perceived position on the ladder of evolution to land plants [2]. Lichens are known to

produce various secondary metabolites that are unique with respect to those of higher plants [3]. Many of the lichen substances are used in pharmaceutical sciences. Lichen extracts have been used for various remedies in folk medicine. Screening of lichen extracts has revealed the frequent occurrence of these metabolites with antibiotic, anti-mycobacterial, antiviral, antitumor, analgesic and antipyretic properties. Lichen and lichen products have been used in traditional medicines for centuries and still hold considerable interest as alternative treatments in various parts of the world [4]. Their efficacy is due to the synthesis of unique secondary metabolites that have important biological roles [5].

II.PROPOSED ALGORITHM

III. EXPERIMENT AND RESULT

A. **Sample collection and extraction:** Collected lichen species were washed with clean water and dried under roof shadow for 15 days at 26°C ± 1°C. 20 gram portions of each lichen species were ground and added to 500 ml of different solvents viz acetone, chloroform, diethyl ether. The mixtures were extracted for 48 hours at room temperature in an orbital shaker. The samples were left to stand for a time. Clear solvent content was collected and centrifuged at 2500 rpm. The supernatant was filtered in Whatman no: 1 filter paper, dried and kept at refrigerated condition until analysis.

B. **Preclinical toxicological studies:**Acute toxicological studies were carried in experimental animal after obtaining proper permission from (CPCSEA) Institutional ethical committee (JKKMMRFCP/IAEC/2013/02). Animalsweighing 25 to 30 g bred in M/s. Venkateshwara Animal Breeders Pvt. Ltd, Bangalore, were used. The animals were fed with standard food pellets and water ad libitum. They were maintained in standard laboratory conditions (12 to 12 hour's light and dark cycles with 25 ± 2°C). 54 animals were divided into 9 Groups of 6 animals

each. Healthy animals were fasted overnight and fed with water ad libitum. Basal rectal temperature was measured by inserting lubricated thermostat probe up to 3 cm into the rectal cavity of the animal. The probe was linked to a digital device, which displayed the temperature at the tip of the probe. The values displayed were manually recorded. After measuring the basal temperature, pyrexia was induced by injecting 15% w/v Brewer's yeast suspension (1 ml/100 g) subcutaneously below the nape of the neck. After 18 hours of brewer's yeast injection the rise in rectal temperature was measured. Only rats which were shown an increase in temperature of at least 1°C was used for further experiment. Animals were divided in to 8 groups, each consisting of 6 animals. Control groups were treated with 15% v/v of yeast to increase body temperature. Test groups were treated with 100 and 200 mg/kg body weight of acetone extracts of S.weigelii, H.boryi and D.vellereum. Positive control group was treated with Yeast (15% v/v) and paracetamol 150mg/kg body weight).

C. **Antidiabetic activities of lichens against alloxan induced animal models:**Antidiabetic effect of foliose lichen extracts were carried in experimental animal after obtaining proper permission from (CPCSEA) Institutional ethical committee (JKKMMRFCP/IAEC/2013/02). Animalsweighing 25-30 g bred in M/s. Venkateshwara Animal Breeders Pvt. Ltd, Bangalore, were used. The animals were fed with standard food pellets and water ad libitum. They were maintained in standard laboratory conditions (12 to 12 hour light and dark cycles with 25 ± 2°C). 54 animals were divided into 9 Groups of 6 animals each. Alloxan monohydrate (LOBA Chemie, Mumbai, India) was purchased, preserved at 25°C and used for this study. Glibenclamide is an oral anti-diabetic preparation with an efficient hypoglycemic action. Daonil (Glibenclamide) manufactured by Aventis Pharma Ltd. Goa, India, was used. Diabetes was induced in overnight fasted animals by a single intra peritoneal injection of 130 mg/kg alloxan monohydrate. Alloxan monohydrate was dissolved in normal saline (pH - 4.5). Animals were fed with 5% glucose solution in order to prevent hypoglycemic shock for 18 hours. Hyperglycemia is to be confirmed by elevated blood glucose levels in plasma, determined at 72 hours and then on day 0 after injection. The threshold value of fasting plasma glucose to diagnose diabetes was taken as > 200 mg/dl. Only rats found with permanent diabetes were used for the anti-diabetic study. Diabetic condition was induced by administering alloxan (130mg/kg body weight) and other group was treated with standard drug (Glibenclamide 3mg/kg body weight). The test groups were treated with 10 and 200 mg of acetone extracts of S.weigelii, H.boryi and D.vellereum.

Estimation of glycated Heamoglobin: Fasting blood glucose levels of all rats was determined prior to the start of the experiment.Blood samples from all groups were collected at weekly intervals from tail vein puncture till the end of study. In the course of study (21days), blood glucose levels for all animals were determined by one touch glucometer at 0^{th},7^{th} and 14^{th} day. On 14^{th} day, samples were collected by cardiac puncture under mild anesthesia from overnight fasted rats and used for the estimation of both serum glucose and glycated haemoglobin. Blood samples from each group were collected in K3EDTA pre coated tubes. Glycosylated haemoglobin was estimated by HbA1c test kit by IFCC method after sacrificing the animals from each group. 5 µl whole blood was added to the test tube pre-filled with R1reagent (0.2ml) and mixed well. The tube was left at room temperature for minimum 2 minutes and maximum 3 minutes. Equilibrate the R1/Reagent to room temperature (20 - 25°C) before use. Remix to obtain a homogenous suspension. 25 µl of the reaction mixture

was added to TD/Test Device by holding the pipette approx. 0.5 cm above the test well. The reaction mixture was allowed to soak completely into the membrane for 10 seconds). 25 µl of R2 washing solution was added to the TD/Test Device and it was allowed to soak completely into the membrane. After 10 seconds, reading was taken in NycoCard READER II (Axis - Shield Diagnostics Ltd, UK).

- **Histopathological studies of kidney and pancreatic tissues from treated animals:** The animals were sacrificed by cervical dislocation. Kidneys and pancreas were excised from each group, washed with phosphate saline (pH -7.0) and blotted with tissue paper. Organs were transferred to formasaline solution for histopathological studies. The organ tissues were processed with paraffin for embedding and sections of 5 µm thickness were taken by microtome sectioning. Sections were subjected to H & E staining and were examined under microscope for histopathological changes [6].

D. Hepatoprotective effect of lichen extracts against paracetamol induced liver damage in exmeprimental animal models: Hepatoprotective effect of foliose lichen extracts were carried in experimental animal after obtaining proper permission from (CPCSEA) Institutional ethical committee (KMCRET/Ph.D/01/2014-15). Animalsweighing 25-30 g bred in M/s. Venkateshwara Animal Breeders Pvt. Ltd, Bangalore, were used.The animals were fed with standard food pellets and water ad libitum. They were maintained in standard laboratory conditions (12 to 12 hour's light and dark cycles with 25 ± 2°C). 54 animals were divided into 9 Groups of 6 animals each. Liver damage in animals was induced with single dose of paracetamol (750 mg/kg body weight) on the first day. Diseased animals were treated with silimarin (100 mg/kg body weight) before and after administration of paracetamol for 5 days and 4 days respectively. In same manner all the three lichens extracts (each extract for 200 mg/kg and 400 mg/kg body weight for each group) were administered for the test groups, before (for 5 days) and after (for 4 days) administration of paracetamol.

- **Determination of SGOT:** The reaction systems used for this study included blank, standard, test (for each serum sample) and control (for each serum sample). 0.5 ml of buffered substrate was added into all the test tubes. Then 0.2 ml of serum was added to the test group tubes and 0.05 ml - 0.20 ml of working pyruvate standard into the standard tubes. After proper mixing, all the tubes were kept for incubation at 37°C for 60 min, after which 0.5 ml of 2, 4 - DNPH reagent was added into all the tubes. Then, 0.05 ml of distilled water and 0.1 ml of each serum sample was added to the blank and the serum control tubes respectively. The mixture was allowed to stand at room temperature for 20 min. After incubation, 5.0 ml of sodium hydroxide was added to all test tubes, further incubated for 10 minutes. Mixed properly and optical density was measured in a spectrophotometer at 510 nm within 15 min [7].
- **Determination of SGPT:**The reaction systems used for this study included blank, standard, test (for each serum sample) and control (for each serum sample). 0.5 ml of buffered substrate was added into all the test tubes. Then 0.2 ml of serum was added to the test group tubes and 0.05 ml - 0.20 ml of working pyruvate standard into the standard tubes. After proper mixing, all the tubes were kept for incubation at 37°C for 60 min, after which 0.5 ml of 2, 4 - DNPH reagent was added into all the tubes. Then, 0.05 ml of distilled water and 0.1 ml of each serum sample was

added to the blank and the serum control tubes respectively. The mixture was allowed to stand at room temperature for 20 min. After incubation, 5.0 ml of sodium hydroxide was added to all test tubes, further incubated for 10 minutes. Mixed properly and optical density was measured in a spectrophotometer at 510 nm within 15 min [8].

- **Determination of ALP:** The working solution was prepared by reconstituting one vial of buffered substrate with 2.2 ml of water. 0.5 ml of working buffered substrate and 1.5 ml of purified water was dispensed to blank, standard, control and test. Mixed well and incubated at 37°C for 3 min. 0.05 ml each of serum and phenol standard were added to test and standard test tubes respectively. Mixed well and incubated for 15 min at 37°C. Thereafter, 1 ml of chromogenic reagent was added to all the test tubes. Then, added 0.05 ml of serum to control [9]. Mixed well after addition of each reagent and the O.D of blank, standard, control and test were read against purified water at 510 nm.
- **Determination of total Bilirubin:** 500 µl of working diazo reagent was added to 200 µl of rat serum and make up the volume to 2.0 ml of distilled water and then 0.5 ml of diazo reagent blank and 2.5 ml of methanol were added. The tubes were incubated in dark for 30 minutes at 37°C. Absorbance was measured at 540 nm against the standard [10].
- **Histopathological studies of liver tissues from treated animals:** The animals were sacrificed by cervical dislocation & liver was excised, washed with phosphate saline (pH - 7.0) and blotted with tissue paper. A part of the lobe of liver was transferred to forma saline (40% formaldehyde) solution for histopathological studies. The liver tissues were processed with paraffin for embedding and sections of 5 µm thickness were taken by microtome sectioning. Sections were subjected to H & A staining and were examined under microscope for histopathological changes [6].

E. Invitro studies of anti cancerous effect of selected lichen species on cancerous cell lines: Human mammary carcinoma (MCF-7), human osteosarcoma (MG-63) and human cervical adenocarcinoma (HeLa) cells were obtained from NCCS, Pune and grown in EMEM supplemented with 10% v/v FBS, 2 mM L - glutamine, 100U/ml penicillin and 100 µg/ml streptomycin. Cells were routinely grown in 75 cm2 culture flasksmaintained at 37°C, 5% CO_2, 95% air and 100% relative humidity. Cells were maintained by passaging weekly and changing culture medium twice a week. The monolayer cells were trypsinized from the culture flasks with trypsin - EDTA when they reached approximately 80% confluence. To make single cell suspensions and viable cells were counted by tryphan blue exclusion assay using hemocytometer. The cell suspension was diluted with medium containing 5% FBS to give final density of $1x10^5$cells/ml. 100µl of cell suspension of each cell line were seeded into 96-well plates, each well with initial density of 1×10^4 cells and incubated at 37°C, 5% CO_2, 95% air and 100% relative humidity. After 24 hours, the medium was replaced with 100 µl of fresh medium supplemented with 5% FBS containing 0.25 µm filter sterilized lichen extracts (dissolved in sterile DMSO) at the required concentrations (serial dilutions between 18.75, 37.5, 75, 150 and 300 µg/ml). 100 µl of each dilution were added to appropriate wells containing 100 µl of medium, resulting with required final concentrations. Following the addition of lichen extracts, the plates were incubated for an additional 48 hours at 37°C, 95% air and 100% relative

humidity. Each concentration was tested in triplicate and control wells were exposed to medium containing 5% FBS alone.

- **MTT assay:** The MTT assay is based on the protocol first described [11]. After 48 hours incubation, 15 µl of MTT in PBS (5 mg/ml) was diluted to 1: 10 ratio in FBS - free medium without phenol red. Plates were then further incubated at 37°C for 4 hours. The medium with MTT was then flicked off and so formed formazan crystals were solubilized in 100 µl of DMSO. Plates were then placed in a micro titre plate shaker for 10 min at room temperature and the absorbance of the resulting solution was measured at 570 nm using a Bio-Rad 550 micro titre plate reader. The effect of each treatment was calculated as the percentage of tetrazolium salt reduction by viable cells against the untreated cell control (cells with medium only).

F. Antiprolieferative activity of lichen extracts on benzapyrene induced cervical cancer in experimental animal model: Female Sprague Dawley rats (25-30 g) were purchased from M/s. Venkateshwara Animal Breeders Pvt. Ltd, Bangalore, with age of 21 days and were used for the study. All rats were kept at room temperature of 22°C under 12 hours light/12 hours dark cycle in animal house. Animals were fed with commercial pellet diet and water ad libitum freely throughout the study. All animal procedures were performed after approval (KMCRET/Ph.D/01/2014-15) from the ethics committee and in accordance with the recommendations for the proper care and use of laboratory animals. The Benzo(a)pyrene was purchased from Sigma chemicals, Mumbai, India, Benzo(a) pyrene was prepared by the company when the order is placed and is shipped on dry ice. Upon arrival, Benzo(a)pyrene was stored at -20°C to prevent its decomposition. Benzo(a)pyrene was dissolved immediately prior to its use in Corn oil (1 ml). The solution was used within 20 min after its preparation.The cervical cancer was induced in the animals by intraperitonial administration of Benzo(a)pyrene at a dose of 10mg/kg body weight, weekly twice for first two months. 5-flurouracil (Standard) was dissolved in normal saline as vehicle [12]. 54 animals were divided into 9 Groups of 6 animals each. During the course of treatment, the body weight of animals in each group was measured once in four weeks. All the animals, after treatment were sacrificed by cervical dislocation, and the cervical tissues were studied for tumor burden and tumor weight.

- **Estimation of Carcino embryonic antigen (CEA):** The CEA was estimated in MODULAR ANALYTICS E170, cobas e 602 immunoassay analyzer. The blood samples from the animals by retro orbital bleeding were collected in K3-EDTA tubes. Serum was separated by centrifuging at 1500 rpm. The collected serum samples were stored at 4°C and immediately analyzed. 10 µl of serum sample was added with biotinylated monoclonal CEA - specific antibody labeled with ruthenium complex. After addition of streptavidin coated microparticles, the complex bound to the solid surface on the well. After 10 minutes of incubation at room temperature, the reaction mixture was aspirated into the measuring cell. The unbound substances are then removed with ProCell/Procell M. The analyzer, automatically calculates the CEA concentration of each serum sample in ng/ml. 1ng/ml CEA corresponds to 16.9 U/ml of serum. Results were determined via a calibration curve, which is specifically generated by a 2 - point calibration and a master curve provided.

- **Histopathological studies of cervical tissues from treated animals:** The animals were sacrificed by cervical dislocation & cervical tissues were excised, washed with phosphate saline (pH - 7.0) and blotted with tissue paper. A part of the cervical tissue was transferred to forma saline (40% formaldehyde) solution for histopathological studies. The liver tissues were processed with paraffin for embedding and sections of 5 µm thickness were taken by microtome sectioning. Sections were subjected to H & A staining and were examined under microscope for histopathological changes [6].

RESULT

A. Preclinical toxicological studies:

Studies on the antipyretic effect of collected lichens species was carried out and the results were presented in table: 1.0. At the 0^{th} hour,temperature in control group was 31.8°C and was reduced significantly (P<0.001) in standard, S. weigelli 100 mg and 200 mg, where the temperature was 28.6°C, 28.5°C and 27.8°C respectively. D.vellereum showed nonsignificant results (P> 0.05) when compared to the control, where the temperature was 29.9°C and 30.9°C in 100 and 200 mg respectively. Whereas 100 mg and 200 mg of acetone extract of H. boryi have shown moderate (29.1°C) and high (27.8°C) significant reduction in temperature when compared to control group. After 1^{st} hour of treatment, standard drug and all the lichen extracts, shown high significant reduction (P<0.001) in temperature, when compared to control. The temperature of standard, 100 and 200 mg concentrations of S. weigelii, D. vellereum and H. boryi were 26.1°C, 27.9°C, 26.4°C, 29.2°C, 30.1°C, 27.4°C and 25.6°C respectively, whereas the temperature in control was 31.3°C. During 2^{nd} hour, S. weigelii (100 mg and 200 mg) H. boryi (100 mg and 200 mg) and standard shown highly significant reduction (P<0.001) in temperature (27.2°C, 26.2°C, 27.8°C, 24.4°C and 25.3°C respectively), whereas, D. vellereum (100 mg) shown a nonsignificant reduction (30.4°C). 200 mg of D. vellereum extract shown less significant temperature reduction (31.3°C) when compared to control (30.2°C). At 3^{rd} hour of treatment, control group shown the temperature of 30.2°C. Standard, S.weigelii (200 mg) and H.boryi (100 mg and 200 mg) shown a high significant reduction (P<0.001) in temperature (29.5°C,25.6°C, 26.8°C and 26.8°C respectively), whereas S. weigelii (100mg) D.vellereum (100mg and 200mg) shown a nonsignificant reduction in temperature (30.1°C, 29.3°C and 29.2 respectively) when compared to control. At 4^{th} hour, control animals shown 27.6°C temperature. The standard drug shown a high significant reduction in temperature (26.5°C), whereas, S.weigelii (100 mg), H.boryi (100mg and 20mg) shown a nonsignificant reduction in temperature (27.1°C,28.5°C and 28.7°C respectively). S.weigelii (200mg) and D.vellereum (100mg and 200mg) have a significant increase in temperature (29.6°C, 29.2°C and 28.3°C respectively). At 5^{th} hour after treatment, there was a significant increase in temperature in all the extracts (26.5°C in standard, 28.9°C in 200mg S.weigelii, 28.8°C in 100mg D.vellereum, 29.4°C and 28.5°C in 100 mg and 200mg of H.boryi respectively). From the results it was revealed that, H.boryi (100 and 200 mg) and 200 mg of S.weigelii extracts and standard have shown significant reduction in temperature up to 3^{rd} hour when compared to control, in

D.vellereum (100 and 200mg) treated groups, the temperature reduction was noticed only upto 1st hour. Among all the treatments given, H.boryi have shown a good antipyretic activity, when compared to the standard drug (Paracetamol) followed by S.weigelii. D.vellereum has shown less antipyretic activity, when compared to standard and other two lichens extracts.

Treatments	Temperature(°C)					
	0 hour	1st hour	2nd hour	3nd hour	4th hour	5th hour
Pyretic induced	31.8±0.64	31.3±0.35	30.2±0.23	30.2±0.27	27.6±0.21	23.4±0.17
Pyretic treated	28.6±0.00	26.1±0.28	25.3±0.26	29.5±0.18	26.5±0.16	26.5±0.18
S. weigelii(100 mg)	28.5±0.69***	27.9±0.28***	27.2±0.21***	30.1±0.26 NS	27.1±0.23 NS	23.4±0.32 NS
S. weigelii(200 mg)	27.8±0.37***	26.4±0.11***	26.2±0.21***	25.6±0.14***	29.6±0.42***	28.9±0.30***
D.vellereum (100 mg)	29.9±0.33 NS	29.2±0.32***	30.4±0.21 NS	29.3±0.21 NS	29.2±0.28***	28.8±0.14***
D.vellereum (200 mg)	30.9±0.40 NS	30.1±0.33***	31.3±0.15*	29.2±0.27 NS	28.3±0.17***	22.6±0.15 NS
H.boryi (100 mg)	29.1±0.26**	27.4±0.18***	27.8±0.26***	26.8±0.18***	28.5±0.31 NS	29.4±0.12***
H.boryi (200 mg)	27.8±0.26***	25.6±0.17***	24.4±0.17***	26.8±0.18***	28.7±0.24 NS	28.5±0.15***

Values are expressed as mean ± SEM (n=6). *** - P< 0.001, ** - P< 0.01,* - P< 0.05, NS - non significant(P> 0.05)

Table: 1.0 - Antipyretic activity of ichen extracts

The analgesic activity of acetone extracts of lichens was done by hot plate method and the results were presented in table: 2.0. The results obtained from the Hot plate method showed that, at 0 minutes of treatment, there was not much difference was observed between control and treated animals, except with 200 mg of H.boryi, in which the retention time as significantly increased (7.5 sec). After 15 minutes of treatment, retention time was increased significantly in all the extracts and standard (P<0.001). The highest retention time was noticed in D.vellereum, 100mg and 200mg with 13.6 and 12.48 sec respectively, when compared to control (5.4 sec), followed by H. boryi and S. weigelii. After 30 minutes, there was a high significant increase in retention time was noticed in standard and all other extracts when compared to control (3.66 sec). Among all the extracts, high retention time was noticed in standard (16.91 sec), followed by 200 mg of D. vellereum (12.43 sec). After 45 minutes also, high significance (P<0.001) retention time was noticed in standard and all other extracts (between 6.66 to 10.46 sec), when compared to control (4.83 sec). Among all treatments, high retention time was noticed in 100 mg H. boryi (10.46 sec). After 45 minutes till 90 minutes, H. boryi (200 mg) and D. vellereum (100 mg) have shown non-significant retention time change (P>0.05) when compared to control. After 60 minutes time interval, all the extracts have shown significant increase in retention time except D.vellereum (100 mg) and H. boryi (200 mg). After 75 and 90 minutes intervels, standard and all other extracts have shown significant increase (P>0.001) except D. vellereum (200mg) and H. boryi (200mg).

Treatments	Reaction time (seconds)						
	0 minutes	15 minutes	30 minutes	45 minutes	60 minutes	75 minutes	90 minutes
Control	4.8±0.30	5.4±0.13	3.6±0.10	4.8±0.30	5.8±0.20	3.6±0.32	1.7±0.10
Pentazocine treated	3.4±0.13***	7.8±0.30***	16.9±0.41***	8.8±0.30***	9.8±0.30***	7.0±0.17***	4.8±0.30***
S. weigelii (100 mg)	4.6±0.33 NS	3.8±0.30***	5.6±0.12***	7.6±0.33***	9.3±0.16***	7.0±0.17***	4.7±0.16***
S. weigelii (200 mg)	3.4±0.13***	7.0±0.10***	5.6±0.12***	7.5±0.12***	10.2±0.13***	8.4±0.15***	5.6±0.14***
D. vellereum (100 mg)	2.7±0.22***	13.6±0.14***	9.6±0.10***	8.2±0.25***	5.6±0.14 NS	3.2±0.13 NS	1.6±0.13 NS
D. vellereum (200 mg)	3.1±0.09***	12.4±0.17***	12.4±0.16***	9.8±0.30***	7.4±0.15***	3.2±0.13 NS	2.6±0.13*
H. boryi (100 mg)	3.1±0.11***	6.6±0.13**	10.1±0.16***	10.4±0.16***	9.4±0.14***	7.5±0.14***	5.3±0.24***
H. boryi (200 mg)	7.5±0.13***	8.6±0.13***	6.6±0.11***	6.6±0.12***	5.2±0.27 NS	3.2±0.13 NS	1.5±0.10 NS

Values are expressed as mean ± SEM (n=6). ***- $P< 0.001$, ** - $P< 0.01$, * - $P< 0.05$, NS - non significant($P> 0.05$)

Table:2.0 – Analgesic activity activity of ichen extracts (Hot plate method)

The analgesic activity of acetone extracts of lichens was done by tail flick method and the results were presented in table: 2.1. At 0 minutes between control and treatments, a non-significant retention time (P>0.05) was observe, whereas, at 15 minutes, there was a significant increase (P<0.001) was noticed in all extracts (between 2 to 4.33 sec) and standard, when compared to control (1.66 sec). At 30 minutes, all the extracts have shown significant decrease in retention time (2 to 3.33 sec), when compared to control (2.66 sec), whereas, the standard drug have shown significant increase (P<0.001) in retention time (12 sec). At 45 minutes, retention time of control was 3 sec. the retention time significantly increase (P<0.001) in standard, S. weigelii (100 and 200 mg) treated animals (6, 5.66 and 5 sec respectively), whereas all other extracts had shown significant decrease of retention time (P<0.01) with retention time of 2 minutes. After 75 and 90 minutes the retention time was non-significant (P>0.05) in standard and all the extracts (between 1 to 1.33 sec), when compared to control (1 and 1.33 sec in 75 and 90 minutes respectively).

Treatments	Reaction time (seconds)						
	0 minutes	15 minutes	30 minutes	45 minutes	60 minutes	75 minutes	90 minutes
Control	2±0.63	1.66±0.21	2.66±0.21	3±0.63	2.66±0.21	1.33±0.21	1±0.00
Pentazocine treated	2±0.00	4.33±0.76	12±1.09	6±1.46	2±0.36	1.33±0.21	1±0.00
S. weigelii (100 mg)	2±0.36 NS	2±0.36***	2.33±0.21***	5.66±0.21 NS	2±0.00 NS	1±0.00 NS	1±0.00 NS
S. weigelii (200 mg)	1.66±0.42 NS	2±0.36***	3.33±0.55***	5±0.63 NS	3±0.36 NS	1.33±0.21 NS	1±0.00 NS
H. boryi (100 mg)	1.33±0.21 NS	2.33±0.21**	2.33±0.21***	2±0.00**	1.33±0.21 NS	1±0.00 NS	1±0.00 NS
H. boryi (200 mg)	1.33±0.21 NS	2±0.00***	2±0.00***	2±0.36**	1±0.00 NS	1±0.00 NS	1±0.00 NS
D. vellereum (100 mg)	1±0.00 NS	2.33±0.21**	2.33±0.21***	2±0.00**	1.33±0.21 NS	1±0.00 NS	1±0.00 NS
D. vellereum (200 mg)	1.33±0.21 NS	2±0.00***	2±0.00***	2±0.00**	1.33±0.21 NS	1±0.00 NS	1±0.00 NS

Values are expressed as mean ± SEM (n=6). *** - $P< 0.001$, ** - $P< 0.01$, NS - non significant($P> 0.05$)

Table:2.1 – Analgesic activity activity of ichen extracts (Tail flick method)

B. ANTIDIABETIC EFFECT OF LICHENS EXTRACTS

- **Levels of serum glucose on the course of treatment:** The level of serum glucose in standard drug (paracetamol) treated and lichens extracts treated animals were studied and the results were presented in table: 3.0. At 0^{th} day day, the serum glucose level of paracetamol and lichens extracts given animal was significantly high ($P< 0.001$) when compared to control animals. At 7^{th} day when compared with control (524.83 g/dl) all other treatments showed high significant decrease ($P<0.001$) of serum glucose levels (402, 407, 364, 494, 263, 476 and 310 mg/dl in glibenclamide, S. weigelii, H. boryi and D. vellereum [100 mg and 200 mg] respectively). At 14^{th} day, compared to control (526.6 mg/dl) all the treatments have shown significant decrease ($P< 0.001$) of glucose level. The glucose level of standard drug, S. weigelii, D. vellereum and H. boryi (100 and 200 mg) were 146, 287, 193, 342 101, 364 and 170 mg/dl respectively. In both 7^{th} day and 14^{th} day, the level of serum glucose was decreased in groups treated with 200 mg of all three lichen extracts was noticed. Among all the treatments, H. boryi (200 mg) was found that blood glucose level was effectively reduced when compared to all other extracts and standard. The levels of serum glucose very less in animals treated with 200 mg acetone extract of H. boryi (262.5 mg/dl on 7^{th} day treatment; $P<0.001$ and 100.6 mg/dl on 14^{th} day treatment; $P<0.001$). Most of the lichen extracts have shown a remarkable decrease on serum glucose at 0^{th} day. A moderate decrease in glucose was observed after 7^{th} day treatment. Only H.boryi had shown a significant decrease in blood glucose after 14^{th} day treatment. The other extracts have no significant effect at the end of 14^{th} day treatment.

Treatments	0th day	7th day	14th day
Control	93.4±0.27	97.73±0.30	92.5±0.50
Alloxan treated	404.6±1.14	524.83±1.16	526.6±0.80
Glibenclamide treated	594±3.07	402.46±0.54	146±0.51
S. weigelii (100 mg)	465±1.52***	406.63±0.46***	287±0.51***
S. weigelii (200 mg)	525±1.17***	363.73±1.10***	192.66±0.71***
H. boryi (100 mg)	575±1.12***	494.16±1.19***	341.6±0.61***
H. boryi (200 mg)	513.16±1.16***	262.5±0.92***	100.6±0.84***
D. vellereum (100 mg)	585.66±0.98***	476.16±0.47***	364.16±1.04***
D. vellereum (200 mg)	371±1.21***	310±0.89***	170.16±0.90***

Values are expressed as mean ± SEM (n=6). *** - $P< 0.001$

Table: 3.0 – Levls of serum glucose (mg/dl blood)

- **Levels of glycated Haemoglobin:** The level of glycated haemoglobin and the percentage of glycated haemoglobin were studied with standard glibenclamide and lichen extracts. The results were presented in table 3.1. The percentage of glycosylation and the amount of

glycosylated haemoglobin was found to significantly decreased (P< 0.001), when compared to control, where the percentage of glycosylation and haemoglobin bound glucose level was 7.3% and 162 mg/dl respectively. Whereas, the percentage of glycosylation in Glibenclamide treated, S.weigelii, H. boryi and D. vellereum (100 mg and 200 mg) treated animals was found to be 7.1%, 6.3%, 8.1%, 4.4%, 6.8%, 5.2% and 5.6% respectively. The haemoglobin bound glucose in glibenclamide treated, S. weigelii, H.boryi and D.vellereum (100 mg and 200 mg) treated animals was found to be 157, 134, 185, 78, 148, 102, 114 mg/dl respectively. When compared to all the extracts H. boryi (100 mg) have shown a high significant decrease in percentage of glycosylation and haemoglobin bound glucose level, followed by D. vellereum (100 mg and 200 mg).

Treatments	Percentage of glycosylation (%)	Haemoglobin bound glucose (mg/ml)
Control	5.5	110.45 ±1.44
Alloxan treated	7.3	162.05 ± 1.15
Glibenclamide treated	7.1	157.4 ± 0.72
S. weigelii (100 mg)	6.3	134.03 ± 0.69***
S. weigelii (200 mg)	8.1	185.45 ± 0.97***
H. boryi (100 mg)	4.4	78.83 ± 0.50***
H. boryi (200 mg)	6.8	148.46 ± 0.39***
D. vellereum (100 mg)	5.2	102.4 ± 0.29***
D. vellereum (200 mg)	5.6	114.55 ± 0.45***

Values are expressed as mean ± SEM (n=6). *** - $P< 0.001$

Table: 3.1 – Levls of glycated Hemoglobin (mg/dl blood)

- **AlkalinePhosphatase:** The level of ALP was significantly decreased (P<0.01) in the all the lichens extracts treated groups except for S. weigelii (400mg) when compared to control (441.01 EU/l). S. weigelii at 200 mg concentration had shown significant reduction in the level of ALP 404.79 EU/l. and 400mg have shown slight increase in ALP (467.11 EU/l), whereas H.boryi has shown significant reduction (P<0.01) of ALP (432.7 and 185.98 EU/l) at concentrations of 200 mg and 400 mg respectively. D. vellereum at 200 and 400 mg have shown 287.9 and 299.38 EU/l respectively (table: 4.0).
- **Serum Glutamate Oxaloacetate Transferase:** 200mg of S.weigelii shown significant increase (108.9 EU/l; P<0.01) in SGOT and 400mg shown and show significant decrease (P<0.01) when compared to control (101.01 EU/l), whereas, H.boryi (200mg and 400mg) shows significantly decreased (P<0.01) SGOT as 57.43 and 58.81 EU/l respectively. D.vellereum (200mg) shown significantly increased (P<0.01; 107.5 EU/l) and a non-significant decreased (P>0.05; 39.2 EU/l) for D.vellereum at 400mg when compared to control group (table: 4.0).

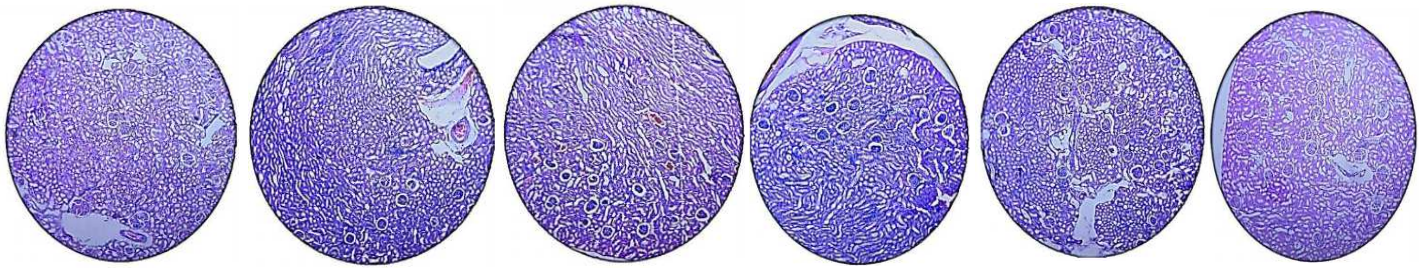
- **Serum Glutamate Pyruvate Transaminase:** The level of SGPT was estimated in all the treated groups and standard. These results have shown that in all the lichen extracts, SGPT activity was found to be less, when compared to control (171.65 IU/l), except, S. weigelii (200 mg), where the SGPT activity was significantly increased (P< 0.01; 215.8 IU/l). H. boryi and D. vellereum (200 mg and 500 mg) extracts have shown 83.25, 73.78, 77.45, 156.18 and 36.16 IU/l respectively (table: 4.0).
- **Total Bilirubin:** Lichen extracts have shown non-significant (P>0.05) variation in the level of total bilirubin except S.weigelii with 200mg and 400mg (0.83mg/dl) have shown significant increase (P<0.01) in bilirubin content. The other two lichen extracts (H.boryi and D.vellereum) at 200 and 400 mg have shown non-significant variation (P>0.05) and found to be at the normal range of bilirubin (0.46, 0.53, 0.58 and 0.56 mg/dl respectively) (table: 4.0).

Treatments	Alkaline phosphatase (EU/L)	SGOT (IU/L)	SGPT (IU/L)	Total bilirubin (mg/dl)
Control	246.5±0.83	37.56±0.71	44.86±0.69	0.55±0.07
Paracetamol treated	336.05±0.90	62.34±0.66	73.56±0.83	3.55±0.11
Silymarin treated	441.01±0.95	101.01±1.03	171.65±0.93	3.86±0.01
S. weigelii (200 mg)	404.79±0.83**	108.94±0.95**	215.8±0.85**	0.83±0.01**
S. weigelii (400 mg)	467.11±1.33**	81.13±0.93**	83.25±0.98**	0.83±0.01**
H. boryi (200 mg)	432.73±1.09**	57.43±0.86**	73.78±0.93**	0.46±0.01^{NS}
H. boryi (400 mg)	185.98±0.89**	58.81±0.68**	77.45±0.96**	0.53±0.01^{NS}
D. vellereum (200 mg)	287.99±1.53**	107.51±0.87**	156.18±0.98**	0.58±0.01^{NS}
D. vellereum (400 mg)	299.38±1.02**	36.21±0.99^{NS}	36.16±0.98**	0.56±0.08^{NS}

Values are expressed as mean ± SEM (n=6), ** - P< 0.01, NS - non significant(P> 0.05)

Table: 4.0 – Hepatoprotective effects of lichen extracts

- **Histopathological studies of kidney tissues from treated animals:** Histopathological changes in kidney and pancreas of diabetes induced animals treated with lichen extracts showed that kidney treated with D.vellereum and S.weigelii have shown no vascular degenerative changes. But it was observed in H. boryi treated groups, pancreatic tissue had shown interstitial oedema which might be due to the usage of crude extract, hence if it is purified, it may reduce the risk of excretory problems and reduce the damage of kidney and pancreas (fig:1 & 2)



NORMAL
Normal architecture.Absence of degenerative changes.Absence of oedema and mesangial hyperplasia

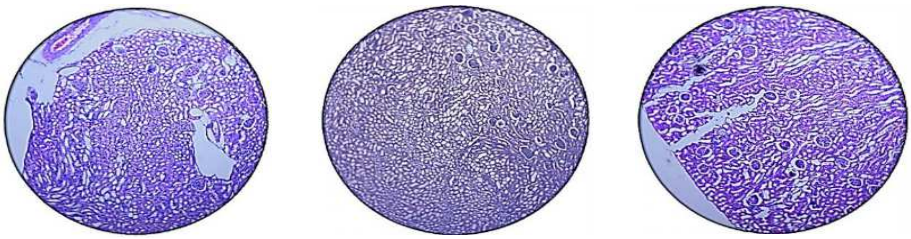
INDUCED
Lymphocytic infilteration.Degenerative changes in glomerular lobes. Distortion of tubular epithelial cells with interstitial oedema and hvaline casts

TREATED
Capillary basement thickening. Absence of degenerative changes. Absence of oedema and mesangial hyperplasia with hyaline casts

***S.weigelii* (100mg)**
Mild ischemic changes. Basement membrane thickening. Tubules with mild dilation and intraluminal casts

***S.weigelii* (200mg)**
Mesangial sclerosis. Focal and cresent shaped mesangial vessels. Mild distortion of tubules with interstitial oedema

***H.boryi* (100mg)**
No interstitial oedema. Absence of degenerative changes. Mild vacuolar degenerative changes



***H.boryi* (200mg)**
Mild mesangial and arteriolar sclerosis. Mild tubular distortive changes. Presence of interstitial oedema and tubular hyaline casts

***D.vellereum* (100mg)**
Eosinophilic deposits. Dilation of proximal and distal convoluted tubules. Presence of interstitial oedema

***D.vellereum* (200mg)**
Dilation of Bowman's capsule. Cellular infiltration in glomerulus. Increased thickness of renal arteries

Fig: 1 – Histopathological findings of diabetic induced and treated kidneys of Wistar rats

NORMAL
Normal architecture. Mild fibrosis in interstitial area. Thick walled and congested blood vessels

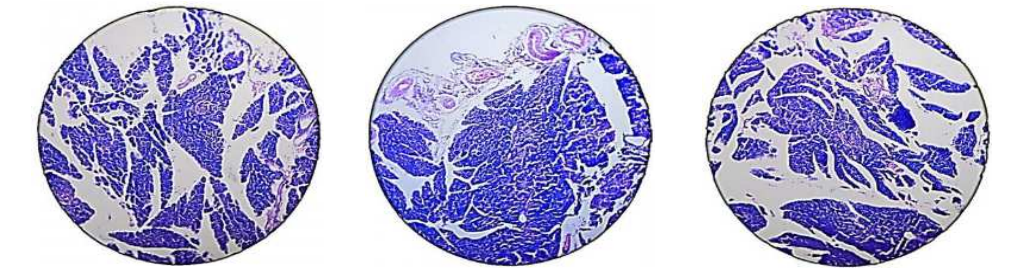
INDUCED
Hyalinization and focal degenerative changes in islets of langerhan's cells. Oedema with fibrosis. Focal lymphocytic infiltration

TREATED
Tissues with normal architecture. Regular islets of langerhan's cells and acini

***S.weigelii* (100mg)**
Mild degenerative changes. Interstitial oedema. Dilated thick walled and congested blood vessels. Focal lymphocytic infiltrate

***S.weigelii* (200mg)**
Mild hyalinization of islets. Interstitial areas with mild fibrosis. Focal lymphocytic infiltration with congested blood vessels

***H.boryi* (100mg)**
Mild degenerative changes. Interstitial oedema and lymphocytic infiltrate. Congested blood vessels. Acini shows focal degenerative changes



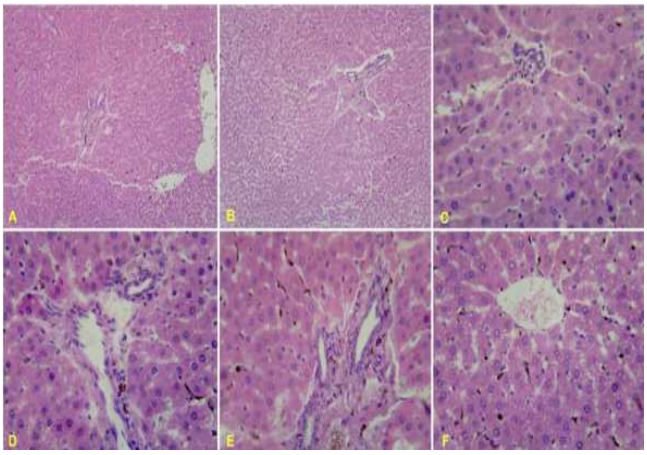
***H.boryi* (200mg)**
Mild focal degenerative changes. Mild fibrosis in interstitial area. Focal lymphocytic infiltrations

***D.vellereum* (100mg)**
Mild degenerative changes in islets of langerhan's cells. Interstitial oedema. Acini with focal vacuolar degenerative changes

***D.vellereum* (200mg)**
Mild hyalinization of langerhan's cells. Flattened acini cells. Mild fibrosis with focal lymphocytic

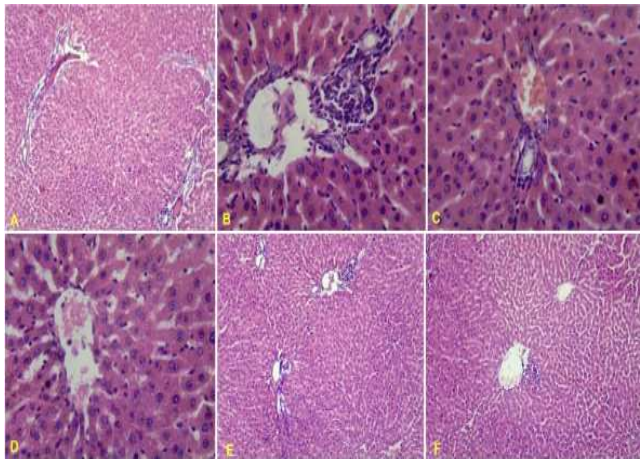
Fig: 2 – Histopathological findings of diabetic induced and treated pancreas of Wistar rats

- **Histopathological studies of liver tissues from treated animals:** Histopathological changes observed in lichens extracts treated animal groups have shown remarkable regeneration of hepatocyte was found in H.boryi (200 and 400 mg), whereas, D.vellereum and S.weigelii have shown good regeneration only in high concentration (400 mg). Hence, when compared to S.weigelii and D.vellereum, H.boryi at low concentration will have a good hepatoprotective activity. This might be due to the antiinflammatory activity of atranorin present in acetone extract of H.boryi, whereas other two extracts does not have atranorin and any other antiinflammatory substances in significant level (fig:3).



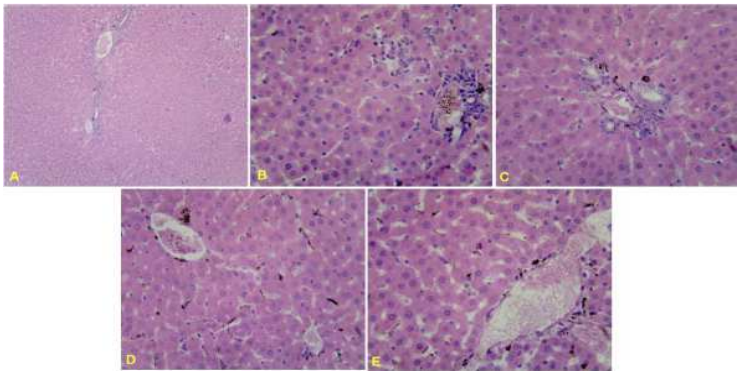
LIVER TREATED WITH SILYMARIN

- Normal lobular architecture (A-C). Portal tract with mild periportal inflammation composed of lymphocytes
- Normal hepatocytes. Congested central vein with no hemorrhage and necrosis (D-F)



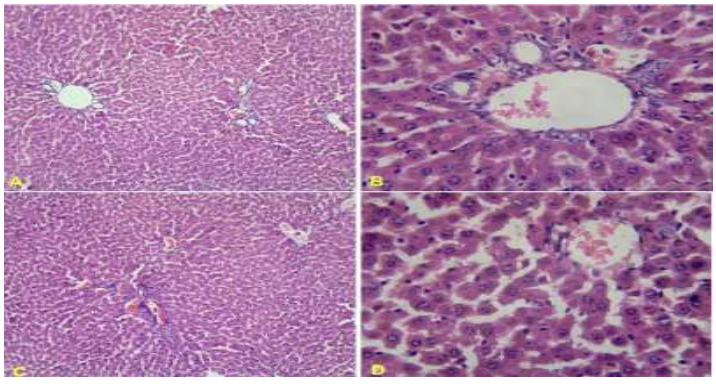
LIVER TREATED WITH *S.weigelii* (200mg)

- Preserved lobular architecture (A)
- Portal tract shows mild lobular inflammation (B)
- Central veins with mild congestion with normal sinusoids (E and F)



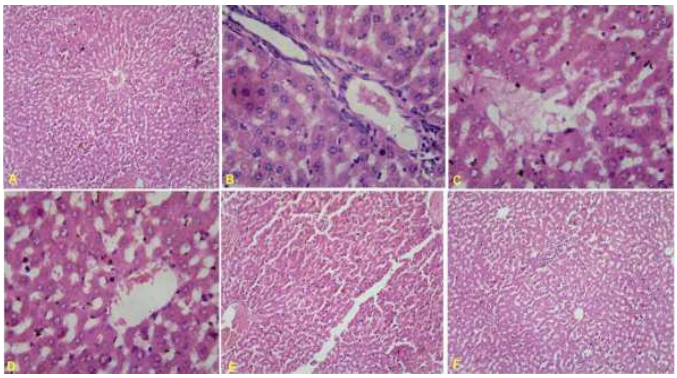
LIVER TREATED WITH *S.weigelii* (400mg)

- Normal lobular architecture with inflammatory areas and lymphocyte deposits on portal tract (A and B)
- Normal central vein with sinusoids (C and D)
- No evidence of necrosis and fibrosis (E)



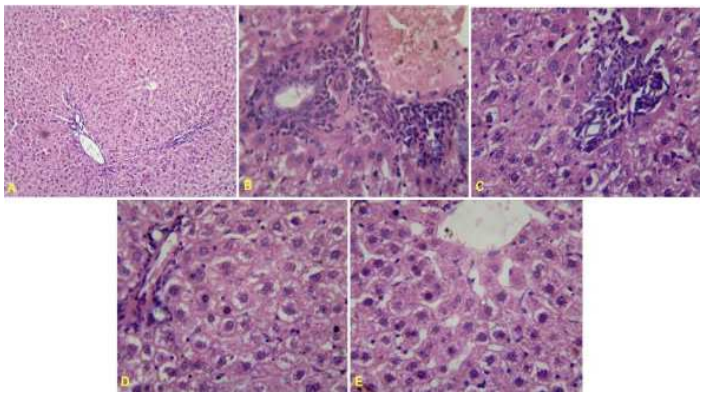
LIVER TREATED WITH *H.boryi* (200mg)

- Normal lobular architecture with normal portal tracts (A)
- Mild congested central veins and sinusoids (B and C)
- No evidence of inflammation or fibrosis (D)



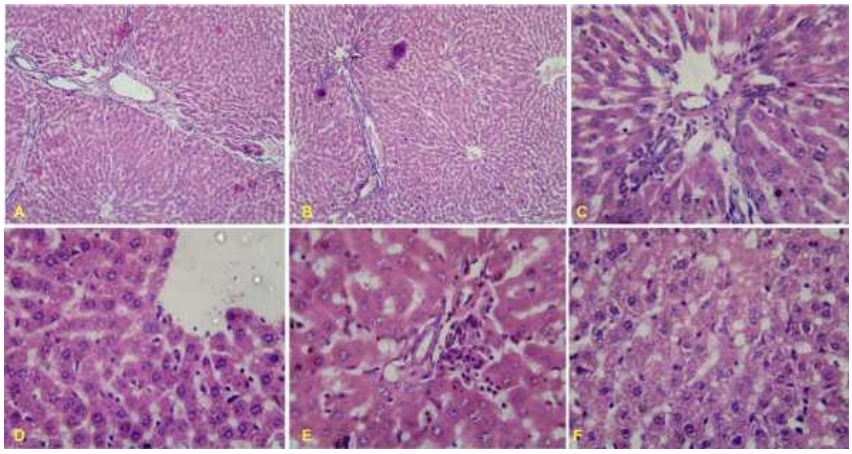
LIVER TREATED WITH *H.boryi* (400mg)

- Normal lobular architecture with normal portal tracts (A)
- Normal hepatocytes with congested veins and mild dilated sinusoids (B-D)
- No evidence of inflammation or fibrosis (E and F)



LIVER TREATED WITH *D.vellereum* (200mg)

- Normal lobular architecture (A)
- Portal tracts with dense inflammatory areas with lympho plasmacytes (B)
- Hepatocytes with cytoplasmic vacuolation (C and D)
- Central veins having congested regions with normal sinusoids (E)



LIVER TREATED WITH *D.vellereum* (400mg)

- Normal lobular architecture with inflammatory portal tracts (A and B)
- Central veins with mild congestion and dilated sinusoids (C-E)
- No evidence of fibrosis (F)

Fig: 3 – Histopathological findings of hepatocytes in liver damage induced and treated Wistar rats

C. **Invitrostudies of anti-cancerous effect of selected lichen species on cancerous cell lines:** The effect of lichen extracts were tested against MCF-7 cells (table: 5.0). IC50value was very less for the acetone extract of H. boryi (83.29 µg/ml) followed by acetone extract of D. vellereum (101.5 µg/ml). The IC50value was 140.2 µg/ml, in chloroform extract of H. boryi and 151.1 µg/ml for acetone extract of S. weigelii. The chloroform extract of all the three lichens and diethyl ether extract of H. boryi were not effective against MCF-7 cells (IC50 value > 300 µg/ml). The cytotoxic effect of lichen extracts were tested against MG-63 cells and presented in table 4.2.2. The IC50value of acetone extract of H. boryi and diethyl ether extract S. weigelii were found to be 70.37 and 88.91 µg/ml respectively. This was followed by diethyl ether extract of H. boryi, acetone and chloroform extracts of D.vellereum (109.2 µg/ml, 120.3 µg/ml and 164.2 µg/ml respectively). The IC50 value was found to be 178.8 µg/ml and 179.7 µg/ml for diethyl ether extract of D. vellereum and chloroform of S. weigelii. It was observed that the chloroform extract of

H. boryi have shown 222.7 µg/ml of IC50 value against MCF-7 cells. The acetone extracts of H. boryi and S. weigelii have shown IC50values of 62.51 µg/ml and 83.82 µg/ml respectively against HeLa cells. Whereas, the acetone extract of D. vellereum have shown IC50value 120.9 µg/ml. Diethyl ether and chloroform extracts of H. boryi have 208 µg/ml and 93.64 µg/ml IC50value against HeLa cells.The other lichen extracts doesn't have effective cytotoxicity (>300µg/ml) that includes chloroform and diethyl ether extracts of S.weigelii and D.vellereum.

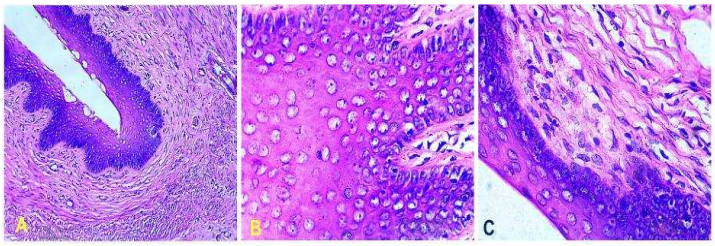
Cell line	Extract	IC50 values of Lichen extracts (mg/ml)		
		H.boryi	S.weigelii	D.vellereum
MCF-7	Acetone	83.29	151.1	101.5
	Chloroform	>300	>300	>300
	Diethyl ether	140.7	204.2	>300
MG-63	Acetone	70.37	>300	120.3
	Chloroform	222.7	179.7	164.2
	Diethyl ether	109.2	88.91	178.8
HeLa	Acetone	62.51	83.82	120.9
	Chloroform	208.0	>300	>300
	Diethyl ether	93.64	>300	>300

Table: 5 - IC50 value of lichen extracts

D. **Antiprolieferative activity of Lichen extracts on benzapyrene induced cervical cancer in experimental animal model:**

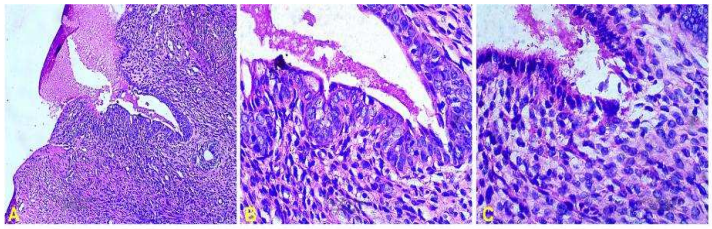
- **Levels of Carcino Embryonic Antigen (CEA):**The levels of Carcino embryonic antigen was estimated in the serum samples of cancer induced rats with various treatment and the results were presented in table: 5.0. The levels of CEA was found to be 5.1 ng/ml in cancer induced control animals. It was about 2.3 ng/ml in standard (5 fluoro uracil) treated group and significantly reduced in lichen extracts treated animals. The CEA concentration in S.weigelii, D.vellereum and H.boryi (250 and 500 mg) treated groups were found to be 3.1, 2.4, 1.5, 2.0, 3.4 and 3.2 ng/ml respectively.

❖ Histopathological studies of cervical tissues from treated animals:



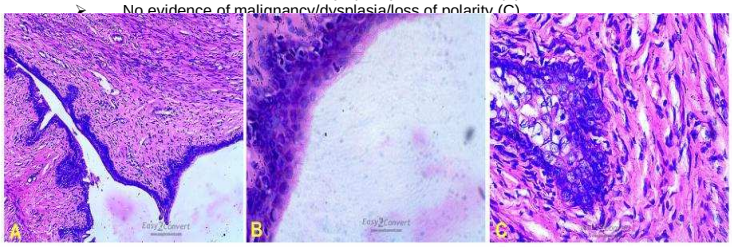
NORMAL CERVIX

- Normal mucosal architecture (A)
- Stromal cells scattered with lymhoplasmacytic infiltrates (B)
- No evidence of malignancy/dysplasia (C)



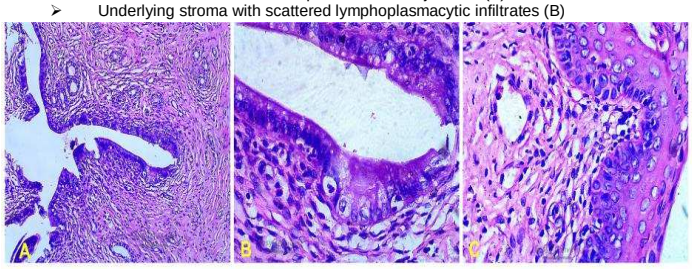
CERVICAL CANCER TREATED WITH 5 FLUOROURACIL

- Cervical tissue showing fragments of endocervix and ectocervix (A)
- Stromal areas with scattered lymphocytic infiltrations (B)
- No evidence of malignancy/dysplasia/loss of polarity (C)



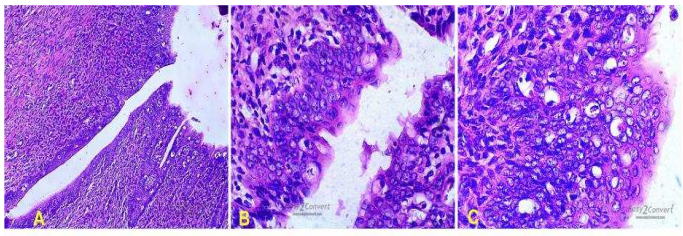
CERVICAL CANCER TREATED WITH *S.weigelii* (500mg)

- Cervical tissue with normla squamo columnar junction (A)
- Underlying stroma with scattered lymphoplasmacytic infiltrates (B)



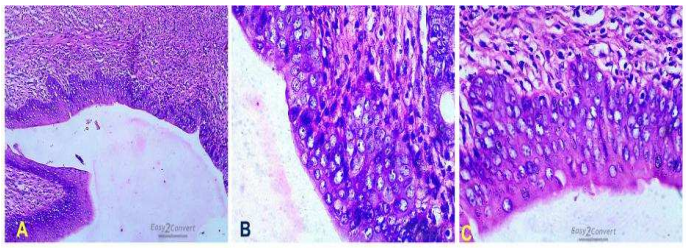
CERVICAL CANCER TREATED WITH *D.vellereum* (500mg)

- Cervical tissues with normal mucosa (A)
- Stromal cells with scattered lymphoplasmacytic infiltrates (B)
- No evidence of malignancy/dysplasia/loss of polarity (C)



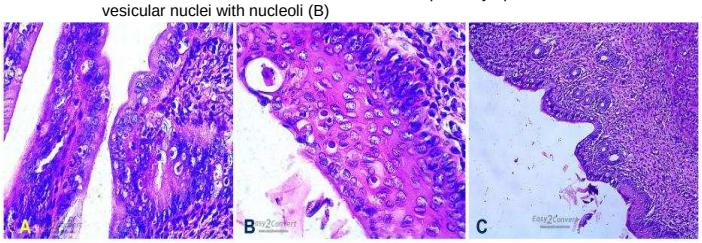
CERVICAL CANCER TREATED WITH *H.boryi* (500mg)

- Cervical tissues showing mild dysplasia and loss of polarity in lower 2/3rd of mucosa (A)
- Individual cells were round with moderate eosinophilic and round to oval vesicular nuclei and showing nucleoli (B)
- No evidence of invasion having 1-2 mitotic index (C)



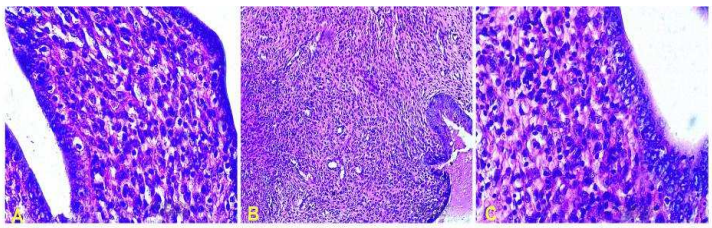
BENZO(A) PYRENE INDUCED CERVICAL CANCER

- Mild dysplasia and loss of polarity in the lower2/3rd mucosa (A)
- Round individual cells with moderate eosinophilic cytoplasm and round to oval vesicular nuclei with nucleoli (B)



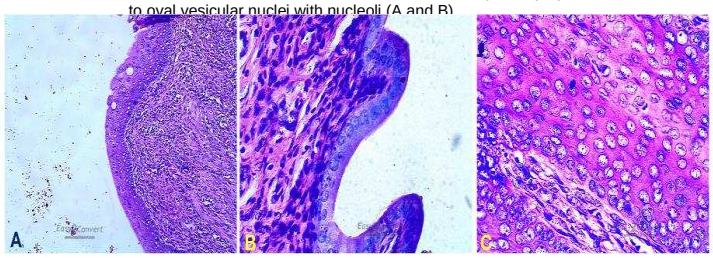
CERVICAL CANCER TREATED WITH *S.weigelii* (250mg)

- Cervical tissue with mild dysplasia and loss of polarity in lower 1/3rd of mucosa (A)
- Round to oval individual cells with moderate eosinophilic cytoplasm and vesicular nuclei and nucleoli (B)
- No evidence of invasion (C)



CERVICAL CANCER TREATED WITH *D.vellereum* (250mg)

- Cervical tissues showing mild dysplasia and loss of polarity in lower 2/3rd of the mucosa (A)
- Individual are round to oval with moderate eosinophilic cytoplasm and round to oval vesicular nuclei with nucleoli (A and B)



CERVICAL CANCER TREATED WITH *H.boryi* (250mg)

- Cervical tissues showing fragments of endocervix and ectocervix with normal mucosa (A)
- Stromal region shows scattered lymphoplasmacytic infiltrates (B)

IV. CONCLUSION

Lichens depsides play a key role in the pharmacological importance that contributes in managing clinical disorders. This will provide further clues whether the exogenous depsides regulates cell growth, revealing new communication avenues between organisms of different kingdoms. In the present research, the study on foliose lichens (Sticta weigelii, Dermatocarpon vellereum and Heterodermia boryi), H.boryi have shown interesting results by exerting its anticancer, analgesic, antipyretic, antidiabetic and hepatoprotective property. Subsequently, S.weigelii has proved to be a good candidate of possessing antioxidant property. D.vellereum has shown considerable antioxidant and hepatoprotective properties. Hence, if we formulate the drug in combination with these three lichens it may have good pharmacological importance without any side effects. The symbiotic relationship between lichenized fungi and algae may be studied on the basis of influencing depsides production. Growth parameter like media designing may be optimized for culturing lichen species under in vitro conditions. The selected compound (atranorin) may be purified and checked for its individual activity (pharmacokinetics and pharmacodynamics) and further commercialized for drug designing. The extracts of lichen species that gave significant results in the effectiveness against plant pathogenic fungi may be further formulated for the development of biopesticides. Molecular mechanisms of cytotoxicity by atranorin may be investigated in detail by apoptotic studies (caspase activities). All the above studies can be further carried out with purified compound to confirm their pharmacological properties. At this juncture, it needs to be stated that such a cross-kingdom communication so far, has not been previously considered for the lichen species of this study. Hence this work may probably serves as a base line study to explore the impact of depsides of foliose lichens on animal and microbial kingdom.

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