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Hepatoprotective Evaluation of *Biophytum umbraculum* Leaf Against Paracetamol and Alcohol Induced Hepatic Damage in Male Wistar Rats

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ABSTRACT: The liver assists in intestinal digestion and performs a diverse variety of functions including bile secretion, bilirubin metabolism, metabolic detoxification, nutrient storage, and metabolism along with other vascular, hematological, and endocrine functions. Liver disease in acute conditions displays hepatitis, steatosis, or both which when left untreated progresses from fibrosis and then to cirrhosis whereas, in chronic conditions, it displays jaundice, encephalopathy, coagulopathy, portal disease, etc. Many synthetic-based drugs have been used to treat liver disorders; however, these medications have negative side effects such as despair, insomnia, vomiting, and constipation. More studies on plants and herbs that might replace chemical-based medications are necessary. The current study focussed on assessing the hepatoprotective activity of *Biophytum umbraculum*. Plant-derived active moieties with their ability to capture free radicals are known to challenge pathophysiological events making them prophylactic as well as curative via phytotherapy. The biochemical constituents reported from *Biophytum umbraculum* include primary components, antioxidants, and antinutrients along with secondary metabolites. This study enumerated the hepatoprotection offered by *Biophytum umbraculum* leaf extract against the paracetamol and alcohol induced hepatocellular damage after examining the acute toxicrange of the extract. Levels of biochemical parameters namely glucose, cholesterol, serum transaminases, alkaline phosphatase, along with total and direct bilirubin revealed adequate protection offered by *Biophytum umbraculum* leaf extract (BULE). Tissue estimates revealed inhibition of lipid peroxidase (LPO) enzyme activity at $p > 0.05$ in both paracetamol and alcohol induced hepatotoxicity, prominent antioxidant potential through reduced glutathione (GSH) with $p \leq 0.001$ in paracetamol, and $p \leq 0.0001$ in alcohol induced hepatotoxic animal models while a non-significant upsurge of superoxide dismutase (SOD) at $p>0.05$ was observed in both paracetamol and alcohol induced hepatotoxicity. All the results accompanied the histopathological evidence in showing dose-dependent protection offered by *Biophytum umbraculum* leaf extract with mild to no signs of necrosis.

Keywords: Liver, phytotherapy, *Biophytum umbraculum*, hepatoprotection, necrosis

INTRODUCTION

The pathological relevance of any disease has a remarkable influence on the homeostatic balance that interferes with the cellular and molecular mechanisms of the body. Each disease entity is presented with its variability and contemplates its abnormality. However, the pathological significance of that particular disease entity is multi-faceted and it influences the vulnerability of the other organ systems. The liver is one such organ that carries a huge disease burden not only for itself but also for other disease-specificities. The liver assists in intestinal digestion and performs a variety of functions including bile secretion, bilirubin metabolism, metabolic detoxification, nutrient storage, and metabolism along with other vascular, hematological, and endocrine functions (Ozougwu, 2017). According to the National Vital Statistics System (NVSS) – Mortality Data (2020), chronic liver disease and cirrhosis account for 15.7 deaths per 0.1 million population (Murray *et al*., 1996). It was documented that sinusoidal endothelial cells (LSECs) in the liver might mediate Coronavirus disease – 19 (COVID-19) induced liver injury via immunocompetent, interleukin (IL-6) which needs intentness for post-covid measures (McConnell *et al*., 2021). Liver disease in acute conditions displays hepatitis, steatosis, or both which when left untreated progresses from fibrosis and then to cirrhosis whereas, in the chronic condition, it displays jaundice, encephalopathy, coagulopathy, portal disease, etc. The diversifying patterns of liver injury in different disease modalities are an emerging concern that needs immediate and adequate attention.

Medicinal plants and herbs on the other hand provide promising benefits in managing diseases and their exclusive secondary constituents elaborate their hepatoprotective potential (Flora *et al*., 1996) and herbal medicines in particular, pose a feasible option for several hepatic conditions (Izzo *et al*., 2016). Dependency on herbal medicines is increasing in demand which proposes scientific evaluation and utilization of medicinal plants for the betterment of health care with effective therapeutic strategies. A major concern that outlays the utilization of medicinal plants and herbs lies in their undetermined chemical compositions (Ling *et al*., 2009). Plant-derived active moieties with their ability to capture free radicals are known to challenge pathophysiological events making them prophylactic as well as curative via phytotherapy (Munteanu and Apetrei 2021). Plants acquire their significant aroma and pigments based on the phytochemicals generated in the process of environmental interactions and defenses against environmental hazards (Koche*et al*., 2016) which are considered important for disease prevention. Several plants and herbs have been thoroughly investigated for their phytochemical hepatoprotective potential (Ali *et al*., 2018). The aqueous extract of *Artemisia absinthium* L. (Amat *et al*., 2010) showed hepatoprotective activity in mice against chemically and immunologically induced liver injuries. Likewise, ethanolic extract of flowers of *Vitex trifolia* exhibited potency against CCl_4-induced liver damage in rats (Anandan *et al*., 2009) and cinnamon showed a protective effect on hepatocytes in rats induced with liver injury (Alshathly *et al*., 2013). *In vitro* regenerated *Boerhaavia* plants in CCl_4 model (Gudipati *et al*., 2015), *Ficus carcia* leaf extract in rifampicin-induced liver damage (Gond and Khadabadi 2008), Silymarin (extract from *Silybum marianum*) (Surai, 2015), *Nelumbo nucifera* leaf extract in CCl_4 model (Theplantlist, 2013), *Solanum nigrum* L extract (Hsieh *et al*., 2008); *Tecomella undulata* (Sm.) Seemstem bark (Khatri *et al*., 2009) in thioacetamide-induced models and *Thymus vulgaris* leaf extract in sodium nitrite-induced model (Soliman *et al*., 2021) has shown hepatoprotective activity. The plant under current investigation *Biophytum umbraculum* (Lourteig, 1981), is a known medicinal herb of the family Oxalidaceae and is popularized as *Biophytum petersianum* with ethnopharmacological importance (Malterud, 2017) in India along with two other species *B. sensitivum* DC or *Oxalis sensitivum* and *B. reinwardtii* Edgew (Pawar and Vyawahare 2014). The biochemical constituents reported from *Biophytum umbraculum* include primary components, antioxidants and, antinutrients along with secondary metabolites (Mohammad *et al*., 2018). The plant extract is used against cerebral malaria with a 56% fidelity level by inhibiting the complement system and reducing inflammation (Austarheim *et al*., 2016). Semi-ethanolic extract reported a dose dependent increase in adrenal secretion and mediate hypotensive and hypoglycemic activities in contrast to the conventional antihypertensive medication (Kodjo *et al*., 2006) while methanolic extract showed the cytokine profile and thereby inhibited the expression of cyclooxygenase -2 (COX-2) (Guruvayoorappan and Kuttan 2008). Hydroalcoholic extract of the plant also revealed calcium antagonism towards aorta rings in Wistar rats by blocking receptor and voltage regulated calcium channels (Titrikou *et al*., 2007). Pectin polysaccharides derived from the extract prepared in hot water with the aerial parts of the plant indicated immunomodulatory effects through activation of macrophage as well as dendritic cells (Inngjerdingen *et al*., 2008) whereas the pectic polysaccharide fragments reported immunomodulatory action against macrophages and cells from the Peyer's patch in the intestine (Grønhaug *et al*., 2011). This plant is also used as an antitoxin for snake and scorpion bites (Burkill, 1994). Leaves are reported for their wound healing (Diallo *et al*., 2002). Saponins derived from the plant are investigated for improved ruminal fermentation which might help in the mitigation of environmental pollution (Santoso *et al*., 2007). The crude methanolic extract of the plant is enriched with flavones namely cassiaoccidentalin A, C-glycosyl isovitexin, and β- D glycosyl isoorientin (Pham *et al*., 2013). This isoorientin revealed strong α, α-diphenyl-β-picrylhydrazyl scavenging, 15-Lipoxygenase inhibitory, and moderate xanthine oxidase inhibitory activities. A significant anticonvulsant potential of hydroalcoholic extract was reported in pentylenetetrazole (PTZ), and maximal electroshock seizure (MES) models (Fisseha, 2022). Methanolic and dichloromethane extracts exhibited cytotoxic effects against cancer cells in humans (Darwati *et al*., 2019). All the evidence directed the present research to emphasize the hepatoprotective ability of the extract of *Biophytum umbraculum* leaf.

METHODS AND MATERIALS

A. Test system

The test systems in this evaluation were subjected to acclimatization for a minimum period of 7 days and were observed regularly for clinical representations. The housing of animals followed standard laboratory conditions and the facility was air-conditioned with proper fresh air supply (air changing at 12-15/hr), room temperature 20.2 to 22.7°C, relative humidity 49-63 %, with 12/24 dark-light cycle. Each animal was individually housed in cages made of stainless-steel mesh top grills that can hold pellet feed and also drinking water. They were given a standard laboratory rodent diet. The drinking water was provided freely.

B. Materials

Paracetamol, alcohol, silymarin, dimethyl sulfoxide (DMSO), formaldehyde; normal saline from BD, USA; liquid paraffin, paraffin wax, xylene from Oxford Labs, India; hematoxylin (H) and eosin (E) from Santa Cruz Biotechnology, Inc., USA. All the biochemical diagnostic kits used for the study were purchased from Tulip Diagnostics (P) Ltd. and assayed as per the standard methods. Colorimetric assay kits for Lipid peroxide/LPO (E-BC-K176-M), reduced glutathione/GSH (E-BC-K030-M), and superoxide

dismutase/SOD (E-BC-K020) were purchased from Elabsciences.

C. Instruments

The instruments used in the present investigation include a Remi -8 °C centrifuge; Biorad micropipettes (2-10µl, 10-100µl, and 100-1000µl); Tarsons microcentrifuge tubes, Hitachi Semi-Autoanalyser; Inverted binocular biological microscope from Biolinkz, India; multichannel pipettes and pipettor from Benchtop; USA; 37 °C incubator from Heal Force, China; 96-well plate cell from Corning, USA; 96 well Plate reader (ELX-800, BioTek, CA, USA).

D. Experimental Design and Schedule

Experimental test system I for paracetamol-induced hepatotoxicity: The hepatoprotection of BULE was determined using a paracetamol-induced hepatotoxic animal model in Wistar rats. Animals under examination were randomized into 6 groups with 6 animals each, and administered orally with the following: (i) Group I/ GI as normal/ vehicle control received demineralized (DM) water (ii) Group II/ GII as induction control received paracetamol 2gm/Kg. (iii) Group III/ GIII received 200 mg/kg silymarin along with paracetamol at 2 gm/Kg; (iv) Group IV/ GIV received 100 mg/kg BULE along with paracetamol at 2 gm/Kg; (v) Group V/ GV received 200 mg/Kg BULE along with paracetamol at 2 gm/Kg; (vi) Group VI/ GVI received 400 mg/Kg BULE along with paracetamol at 2gm/Kg p.o.

Experimental test system II for alcohol-induced hepatotoxicity: The hepatoprotection of BULE was also determined using alcohol induced hepatotoxic animal model in Wistar rats. Animals under study were randomized into 6 groups with 6 animals each, and administered orally with the following: (i) Group I/ GI as normal/ vehicle control received demineralized (DM) water (ii) Group II/ GII as induction control received alcohol 5 g per Kg of 25% w/v; (iii) Group III/ GIII received 200 mg/kg silymarin along with alcohol 5 g per Kg of 25% w/v; (iv) Group IV/ GIV received 100 mg/kg BULE along with alcohol 5 g per Kg of 25% w/v; (v) Group V/ GV received 200 mg/Kg BULE along with alcohol 5 g per Kg of 25% w/v; (vi) Group VI/ GVI received 400 mg/Kg BULE along with alcohol 5 g per Kg of 25% w/v.

E. Methods

(i) Acute toxicity studies. The minimal lethal dose (MLD) of the plant extract under study was >2000mg per kg body weight, according to the findings of acute oral toxicity research (OE CDTG 423) conducted on rats. According to this guideline, the test compound can be classified under Globally Harmonized System or GHS category 5 based on the observed MLD value which proposed the selection of 1/5th, 1/10^{th,} and 1/20th doses for the efficacy study.

(ii) Induction of Hepatotoxicity. Paracetamol is generally used against pyrexia at higher doses in liver deplete reduced glutathione and causes accumulation of a toxic compound by the name N-acetyl-1,4-benzoquinone imine (Akther *et al*., 2013) which strongly binds with sulfhydryl functional groups; forms protein adducts. After overnight fasting for about 12-16 hours, animals in group II to group VI in system I were administered paracetamol regularly at 2 gm/Kg dose orally for 7 days. Likewise, alcohol (Balakrishna and Lakshmi 2017) being toxic in itself releases still more toxic metabolites which when present in excess amounts lead to steatosis, inflammation, hepatocyte necrosis, and pericentral sclerosis (Keegan *et al*., 1995) due to inflammation, oxidative stress, endotoxemia, and other cellular mechanisms. Similar to System I, animals in Group II to Group VI in System II were administered 5 gm/Kg of 25% w/v alcohol at a regular daily dose orally for 21 days. At study termination, rats were given anesthesia for withdrawing the blood required for biochemical analysis. Livers were gathered, measured for weight, and a part of the liver was used for the antioxidant capacity of the extract while the rest was dipped in a 10% formalin solution and subjected to histopathology examination.

(iii) Serum Biochemical Estimates. The collected blood sample was subjected to centrifuge for 15 min at 4000 rpm to separate the serum. Post-serum collection, the selected biochemical parameters namely glucose, cholesterol, liver enzymes, and total and direct bilirubin were measured from the separated serum by using respective diagnostic kits.

(iv) Tissue Estimates. Liver tissues were weighed after collection and washed in normal saline. The tissue samples were minced into smaller pieces and 1gm of fresh tissue was washed with homogenization (0.01M phosphate-buffered saline (PBS) or 20 mM tris(hydroxymethyl) aminomethane (TRIS-HCl) medium maintained at 2-8°C at 9:1 ratio and it was subjected to tissue homogenizer (60 Hz, the 90s) in the ice bath. Then, the tissue homogenate was subjected to centrifugation at 4°C and 2000 rpm for about 10 minutes, and the supernatant collected was used for the following antioxidant marker evaluation.

Lipoxygenase (LPO) Assay. Lipid peroxidation serves as a marker of the cellular level and tissue level damage through oxidative stress and yields carbonyl end products namely lipoxygenase, malondialdehyde, 4-hydroxynonenal, etc. According to Ohkawa's (1979) method, the levels of MDA in the homogenate (Ohkawa *et al*., 1979) help to determine the quantity of LPO. 0.2 mL of tissue homogenate was added to 0.2 mL sodium dodecyl sulfate (SDS) (8.1%), 1.5 mL of acetic acid (20%), and 1.5 mL of thiobarbituric acid (8%) and the prepared volume was then adjusted to 4 mL by using distilled water. It was then heated in a water bath at 95°C for an hour in a condensed atmosphere. The tubes were then cooled to room temperature and adjusted to a final volume of 5 mL. 5 ml of a 15:1 ratio mixture of butanol: pyridine (Placer *et al*., 1966) was added and vortexed for 2 min. The mixture was allowed to centrifuge at 3000 rpm for about 10 minutes, and the obtained supernatant with an organic layer was collected to verify the optical density at a wavelength of 532 nm against a blank. The lipid peroxides formed were expressed in the form of a

number of moles of the reactive substances of thiobarbituric acid for amilligram of the protein.

Reduced Glutathione/ GSH Assay. Glutathione is a general reducer in different biochemical pathways (Lushchak, 2012) associated with catalytic reactions, metabolism, and transport mechanisms. Dysregulated glutathione system results in excess ROS generation. Elman's method (1959) was in general used to determine the GSH potential (Ellman, 1959). The homogenate used in the reaction was prepared in 0.1 M phosphate buffer with 7.4pH. This buffered homogenate was added in an equal ratio of 20% of trichloroacetic acid with 1 mM ethylene diamine tetra acetic acid to encourage protein precipitation. Then the mixture is subjected to centrifuge (10 min at 2000 rpm) after 5-minute incubation. 200 µL of the supernatant was collected into tubes and mixed with 1.8 mL of 0.1 mM Ellman's reagent (a 5,50 -dithiobis-2- nitrobenzoic acid prepared in 0.3 molar phosphate buffer, 1% of sodium citrate and volume adjusted to 2 mL). The solutions were tested at a wavelength of 412 nm against the blank and the absorbance values were compared with the standard absorption curve of GSH.

Superoxide dismutase (SOD) Assay. Superoxide anion chemically is a weak oxidant that produces hydroxyl radicals along with singlet oxygen that contributes to oxidative stress (Hayyan *et al*., 2016). According to Beauchamp and Fridovich's method of Nitro blue tetrazolium (NBT) assay, xanthine-xanthine oxidase was utilized for the generation of superoxide flux (Beauchamp and Fridovich, 1971). NBT-mediated reduction by superoxide radicals to produce formazan (blue) was further analyzed at a wavelength of 560 nm and results were compared with the reference values. The amount inhibited was defined as the percent of the NBT reduction reference rate devoid of SOD activity. This data was plotted as % inhibition on Y (%) vs concentration of protein on the X-axis respectively. The unit activity was termed as the amount of protein required to bring the reference rate down to 50 percent of the max. inhibition. Low-speed centrifugation of the sample and reference cuvettes were performed, if the turbidity was too great. The results were represented in units of SOD activity for mg of protein.

(iv) Histopathological studies. The liver tissue of the rats was excised and washed in normal saline to fix them in formalin 10%. Each formalin-fixed liver tissue was washed under water, dehydrated in ethanol, and cleared in xylene which is finally embedded in wax. Each section 4-5 µm thick was cut from the paraffin-embedded tissue block and subjected to staining with hematoxylin and eosin to study the histopathological changes.

(v) Statistical Analysis. The tests were performed in triplication and the results are shown with mean ± SEM values. Inhibitory concentration 50 (IC_{50}) values were recognized using regression analysis and Pearson's correlation coefficient (r) was estimated using Excel 2007 while the statistical significance was determined with a two-way analysis of variance/ ANOVA test using GraphPad Prism 9 for Windows among various treatments. A statistical significance with "$p < 0.05$" is expressed as significant.

RESULTS AND DISCUSSION

Liver being the second large organ that performs remarkable duties in the body. It is a vulnerable target for a wide variety of toxicants (Mayer and Kulkarni 2001). Several hepatotoxins are used in preclinical investigations to induce potential hepatic damage namely carbon tetrachloride (CCl_4), galactosamine, D-galactosamine/lipopolysaccharide (GalN/LPS), thioacetamide (TAA), paracetamol, arsenic, alcohol, isoniazid, rifampicin, antibiotics, aflatoxin, antitubercular drugs etc.

Each hepatotoxin induces hepatotoxicity by different mechanisms. Drug-induced liver toxicity was found to be responsible for 50% of liver failure cases in theacute state out of which 39% were caused by paracetamol (Mohi-Ud-Din *et al*., 2019). Paracetamol induces hepatotoxicity by forming protein adducts (McGill *et al*., 2013) through an oxidative metabolite named 'NAPQI' (Nelson, 1990). NAPQI mainly targets mitochondrial proteins (Du *et al*., 2016) and interferes with electron transport causing electron leakage and which thereby forms superoxide radicals (Yan *et al*., 2010) that interact with nitric oxide in the mitochondria and initiate the formation of peroxynitrite ($ONOO^-$) which compromises the cellular functions (Ramachandran *et al*., 2011). The hepatic damage further leads to altered mitochondrial permeability and consequent alteration in adenosine triphosphate (ATP) production, which eventually triggers the regulated necrosis or necroptosis (Schwabe and Luedde 2018) by recruiting necrosome complex (Vandenabeele *et al*., 2010) that finally induces the hepatotoxic damage (McGill *et al*., 2013).

Alcohol on the other hand is involved in the hepatotoxic mechanisms that are characterized based on acute exposure or chronic exposure. Acute exposure shows effects like redox changes, fatty liver, and exacerbated porphyrias whereas chronic exposure shows effects like redox changes, fatty liver, necrosis, cirrhosis, etc (Badawy, 1980). Three different systems namely the alcohol dehydrogenase system, catalase system, and microsomal ethanol-oxidizing system also called Meos are involved in alcohol metabolism (Teschke *et al*., 2021). All these oxidative pathways release free radicals and initiate lipid peroxidation via ethoxy radicals leading to tissue damage and other impaired metabolic processes (Zakhari, 2006). The oxidative metabolism of alcohol creates a highly reduced environment in the hepatocytes and makes them vulnerable to tissue damage while forming acetaldehyde along with free radicals (Comporti *et al*., 2010). Acetaldehyde undergoes yet another rapid metabolism in the mitochondria to release acetate and reduced nicotinamide adenine dinucleotide. Excessive acetaldehyde may deplete glutathione reserves and promotes fibrosis that may further lead to chronic liver tissue damage associated with cirrhosis, steatosis, etc. In the liver, the structural organization of the sinusoidal

spaces facilitates rapid and efficient transport of drugs from the portal blood while the higher biotransformation capacity of the hepatocytes addresses the generation of reactive metabolites which are known to cause hepatic damage (Yuan and Kaplowitz 2013).

Hepatotoxicity with its huge disease burden encounters serious adverse effects and frequent drug product recalls (Onakpoya *et al*., 2016). Thus, emerging a need to identify, characterize and optimize alternative therapeutic strategies where medicinal plants and herbs are considered natural repositories for potential drugs while their plant-specific structural components challenge the therapeutic benefit. Interestingly, polyphenols by acting as hydrogen donors withhold the antioxidant potential and help in alleviating the pathological mechanisms of disease. Compounds present in the plant extracts show their efficacy based on their structural properties, temperature, concentration, physical state, and substrate susceptibility whereas the antioxidant efficiency is specifically dependent on structure-derived intrinsic activity, interface distribution (Shahidi and Zhong 2011), and their rate kinetics (Antolovich *et al*., 2002). The scavenging capability of the plant compounds on the free radicals and reactive oxygen groups was mainly due to hydroxyl groups in the phytochemicals of *Biophytum umbraculum* (Collin, 2019).

The *Biophytum umbraculum* leaf extract (BULE) in this study was investigated for the serum levels of glucose, cholesterol, liver enzymes, and total and direct bilirubin, and the extract was also investigated for the tissue estimates of LPO, GSH, and SOD in both of the induction models as shown in the figures 1 to 4 and tables 1-2. The glucose and cholesterol levels at 400 mg per Kg are relative to the Silymarin (standard) at 200 mg per Kg in both paracetamol and alcohol inductions models. The reduced glucose levels due to BULE might be due to two mechanisms namely enhanced insulin release from the existing β cells of the pancreas in similarity with the action showed by sulfonylureas and increased glycolysis due to hexokinase activity (Ananda *et al*., 2012). The reduced cholesterol level (Puri, 2003) might be attributed to the ability of the phytochemicals in BULE to inhibit the mevalonate pathway (Laka *et al*., 2022). The elevated liver enzymes like SGOT, SGPT, and ALP in paracetamol and alcohol inductions might be because of the altered transport function of the hepatocytes leading to the disruption of the membrane (Rajesh and Latha, 2004) and further promotion of the enzyme leakage into the blood. The study investigated transaminases like SGOT, SGPT, and ALP levels where the administration of BULE at different doses showed a considerable decrease in the respective enzyme levels. Treatment proceeded with 200, 400 mg/Kg doses of BULE in paracetamol-induced hepatotoxicity showed protection at $p \leq 0.05$ in the SGOT levels and SGPT levels whereas the same level of protection at $p \leq 0.05$ was offered at 400 mg/Kg in alcohol induced hepatotoxicity. Interestingly, the protection offered to the ALP levels at all the doses of BULE was found to be significant at $p \leq 0.0001$ in both paracetamol and alcohol induced hepatic damage indicating the efficiency of the phytochemicals present in the BULE On the other hand, bilirubin acts as an indicator of bile tract function (Baiocchi *et al*., 2019) whose raised levels confirm the improper functioning of the liver in paracetamol and alcohol induction models but a considerable decrease in total and direct bilirubin levels at $p > 0.05$ was observed at all the selected doses of BULE.

Table 1: Effects of *Biophytum umbraculum* leaf extract (BULE) on serum estimates in Paracetamol-induced hepatic damage in male Wistar rats.

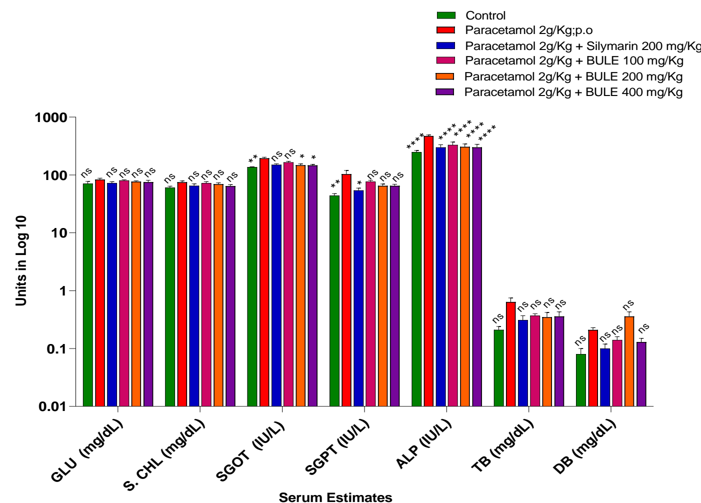
Groups in System I	GLU (mg/dL)	S. CHL (mg/dL)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TB (mg/dL)	DB (mg/dL)
G1: Control	70.97 ± 6.26	60.50 ± 3.39	136.6 ± 2.33	43.97 ± 3.54	247.77 ± 17.19	0.21 ± 0.03	0.08 ± 0.02
G2: Positive control (Paracetamol 2g/Kg; p.o.)	83.63 ± 4.51	75.17 ± 3.80	194.32 ± 8.64	103.62 ± 16.21	472.25 ± 18.56	0.64 ± 0.11	0.21 ± 0.02
G3: Paracetamol 2g/Kg; p.o. + Silymarin 100 mg/Kg	72.53 ± 4.15	64.83 ± 5.29	149.96 ± 5.91	54.02 ± 5.30 *	299.15 ± 34.85****	0.31 ± 0.06	0.10 ± 0.02
G4: Paracetamol 2g/Kg; p.o. + BULE 100 mg/Kg	80.13 ± 2.60	72.00 ± 4.50	165.44 ± 6.94	76.62 ± 4.56	329.65 ± 41.97****	0.37 ± 0.03	0.14 ± 0.02
G5: Paracetamol 2g/Kg; p.o. + BULE 200 mg/Kg	76.83 ± 2.48	69.33 ± 3.85	147.32 ± 7.68*	65.13 ± 4.78	307.06 ± 36.99****	0.35 ± 0.07	0.13 ± 0.02
G6: Paracetamol 2g/Kg; p.o. + BULE 400 mg/Kg	75.50 ± 5.60	64.33 ± 3.85	146.45 ± 5.55*	64.95 ± 4.18	301.49 ± 36.58****	0.36 ± 0.07	0.13 ± 0.02

GLU= Glucose; S. CHL= Serum Cholesterol; liver enzymes: SGOT, SGPT, and ALP= Serum glutamic-oxaloacetic transaminase, Serum glutamic pyruvic transaminase, and Alkaline phosphatase respectively; TB= Total Bilirubin; DB= Direct Bilirubin; BULE= *Biophytum umbraculum* leaf extract. All the values were expressed in Mean ± S.E.M, where N = 5; **** p< 0.0001; *** p< 0.001; ** p<0.01; *p<0.05; ns - non-significant. All the treated groups were compared against the induction control.

Table 2: Effects of *Biophytum umbraculum* leaf extract (BULE) on serum estimates in Alcohol induced hepatic damage in male Wistar rats.

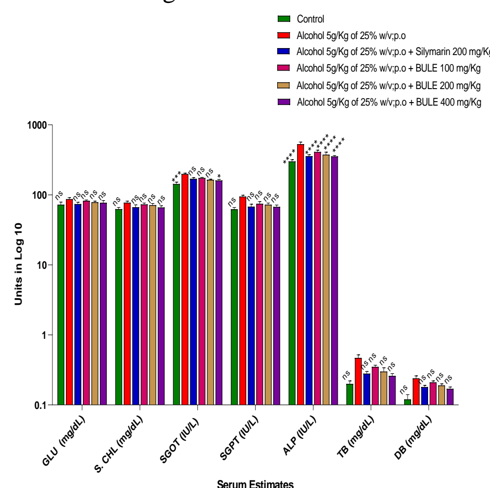
Groups in System II	GLU (mg/dL)	S. CHL (mg/dL)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TB (mg/dL)	DB (mg/dL)
G1: Control	72.57 ± 6.23	62.67 ± 3.50	142.93 ± 9.04	62.43 ± 3.73	299.82 ± 18.53	0.20 ± 0.02	0.12 ± 0.02
G2: Positive control (Alcohol 5g/Kg of 25% w/v; p.o.)	87.00 ± 4.56	77.50 ± 4.05	198.52 ± 5.83	94.72 ± 4.13	530.55 ± 37.59	0.47 ± 0.05	0.24 ± 0.02
G3: Alcohol 5g/Kg of 25% w/v; p.o. + Silymarin 100 mg/Kg	74.00 ± 4.45	66.50 ± 5.42	169.83 ± 6.20	67.78 ± 5.77	357.20 ± 16.94****	0.28 ± 0.02	0.18 ± 0.01
G4: Alcohol 5g/Kg of 25% w/v; p.o. + BULE 100 mg/Kg	82.22 ± 2.55	73.17 ± 3.44	173.90 ± 3.63	74.90 ± 5.18	410.58 ± 25.29****	0.35 ± 0.02	0.21 ± 0.01
G5: Alcohol 5g/Kg of 25% w/v; p.o. + BULE 200 mg/Kg	78.78 ± 2.60	71.50 ± 3.91	164.10 ± 3.25*	72.40 ± 4.06	376.23 ± 31.86****	0.30 ± 0.04	0.19 ± 0.01
G6: Alcohol 5g/Kg of 25% w/v; p.o. + BULE 400 mg/Kg	77.03 ± 6.01	66.17 ± 3.70	160.72 ± 5.67*	67.47 ± 4.27	354.52 ± 11.67****	0.26 ± 0.02	0.17 ± 0.01

GLU= Glucose; S. CHL= Serum Cholesterol; liver enzymes: SGOT, SGPT, and ALP = Serum glutamic-oxaloacetic transaminase, Serum glutamic pyruvic transaminase, and Alkaline phosphatase respectively; TB= Total Bilirubin; DB= Direct Bilirubin. BULE= *Biophytum umbraculum* leaf extract. All the values were expressed in Mean ± S.E.M, where N = 5; **** p< 0.0001; *** p< 0.001; ** p<0.01; *p<0.05; ns - non-significant. All the treatment groups were compared against the induction control.



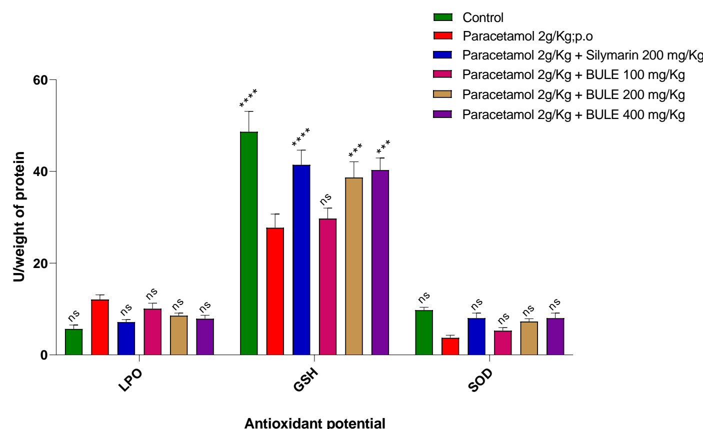
GLU= Glucose; S. CHL= Serum Cholesterol; liver enzymes: SGOT, SGPT, and ALP = Serum glutamic-oxaloacetic transaminase, Serum glutamic pyruvic transaminase, and Alkaline phosphatase respectively; TB= Total Bilirubin; DB= Direct Bilirubin. All the groups were compared with disease control and all the values are expressed in Mean ± S.E.M, where N = 5; **** p< 0.0001, *** p< 0.001, ** p<0.01, *p<0.05, ns - non-significant. Results were analyzed by Two-way ANOVA ensued by Dunnett's post-hoc test. All the treated groups were compared with Paracetamol 2g/Kg B. Wt., p.o.

Fig. 1. Effects of *Biophytum umbraculum* leaf extract (BULE) on serum estimated in paracetamol-induced hepatic damage in male Wistar rats.



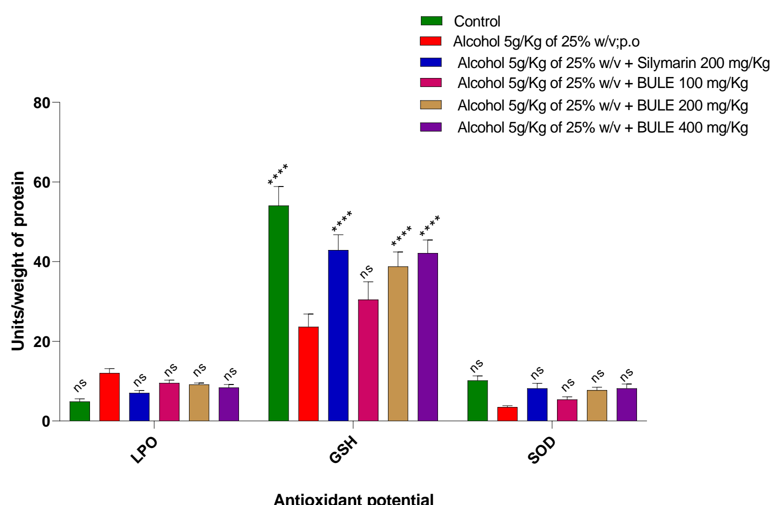
GLU= Glucose; S. CHL= Serum Cholesterol; liver enzymes: SGOT, SGPT, and ALP = Serum glutamic-oxaloacetic transaminase, Serum glutamic pyruvic transaminase, and Alkaline phosphatase respectively; DB= Direct Bilirubin. All the values were expressed in Mean ± S.E.M, where N = 5; **** p< 0.0001; *** p< 0.001; ** p<0.01; * p<0.05; ns - non-significant. Results were analyzed by using Two-way ANOVA followed by Dunnett's post-hoc test. All the treated groups were compared with Alcohol 5g/Kg of 25% w/v., p.o.

Fig. 2. Effects of *Biophytum umbraculum* leaf extract (BULE) on serum estimated in alcohol-induced hepatic damage in male Wistar rats.



LPO= Lipoxygenase; GSH= Reduced Glutathione; SOD= Superoxide dismutase. All the values are expressed in Mean ± S.E.M, where N = 5; **** p<0.0001; *** p< 0.001; ** p<0.01; * p<0.05; ns - non-significant. Results were analyzed by Two-way ANOVA ensued by Dunnett's post-hoc test. All the treated groups were compared with Paracetamol 2g/Kg weight., p.o.

Fig. 3. Effects of *Biophytum umbraculum* leaf extract (BULE) for antioxidant potential in paracetamol-induced hepatic damage in male Wistar rats.



LPO= Lipoxygenase; GSH= Reduced Glutathione; SOD= Superoxide dismutase. All the values are represented in mean ± S.E.M, where N = 5; **** p< 0.0001; *** p< 0.001; ** p<0.01; * p<0.05; ns - non-significant. Results were analyzed by Two-way Analysis of Variance ensued by Dunnett's post-hoc test. All the treated groups were compared with Alcohol 5g/Kg of 25% w/v., p.o.

Fig. 4. Effects of *Biophytum umbraculum* leaf extract (BULE) for antioxidant potential in alcohol-induced hepatic damage in male Wistar rats.

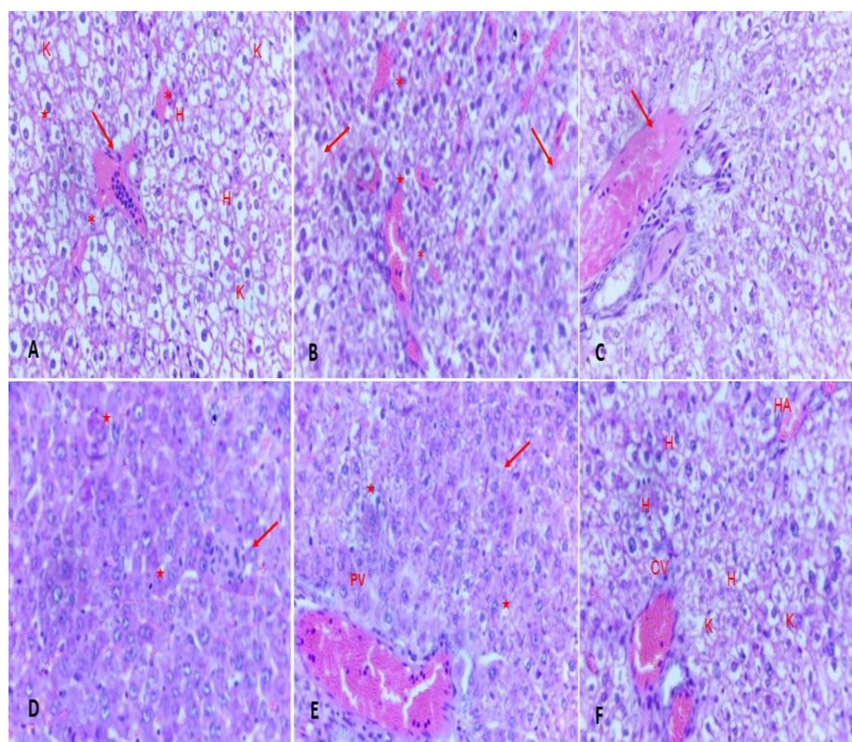
It is interesting to note that research is evident in discussing that the phytochemicals behave as potential antioxidants and they prohibit oxidative damage to the cellular contents in disease interventions (Lee *et al*., 2017). Therefore, the present study investigated for LPO, GSH, and SOD levels in liver tissue homogenates, and the BULE at different doses offered protection by inhibition in lipoxygenase activity and anupsurge in reduced glutathione as well as superoxide dismutase activity as shown in Table 3. Lipoxygenase assay revealed a non-significant protection at all the selected doses of BULE in paracetamol as well as alcohol induced hepatotoxicity whereas reduced glutathione assay revealed a significant protection at $p \leq 0.001$in paracetamol induced hepatotoxicity treated with 200, 400 mg/Kg doses of BULE while this protection was observed to be significant at $p \leq 0.0001$ in alcohol induced hepatotoxicity treated with 200 mg 400 mg for Kg BW of BULE. The enhancement in the superoxide dismutase activity was found to be insignificant at all doses of BULE in paracetamol as well as alcohol induced hepatotoxic models. BULE exhibited antioxidant activity that offered hepatoprotection against chemical-induced hepatotoxicity, in similar lines aqueous extract of *Artemisia absinthium* L. also showed protection against liver damage which contributed to its antioxidant potential (Amat *et al*., 2010). In addition, in several cases,the strong antioxidant properties of silymarin are considered to be responsible for its hepatoprotective activity (Surai, 2015).

Table 3: Effects of *Biophytum umbraculum* leaf extract (BULE) on antioxidant potential in Paracetamol and alcohol-induced hepatic damage in male Wistar rats

Groups	LPO (U/mg of protein)	GSH (mg/dL)	SOD (U/mg of protein)
Paracetamol induced hepatic damage			
G1: Control	5.67 ± 0.84	48.65 ± 4.44	9.75 ± 0.64
G2: Positive control (Paracetamol 2g/Kg; p.o.)	12.07 ± 1.02	27.73 ± 2.97	3.73 ± 0.56
G3: Paracetamol 2g/Kg; p.o. + Silymarin 100 mg/Kg	7.17 ± 0.52	41.43 ± 2.29****	8.02 ± 1.11
G4: Paracetamol 2g/Kg; p.o. + BULE 100 mg/Kg	10.09 ± 1.20	29.73 ±2.29	5.27 ± 0.69
G5: Paracetamol 2g/Kg; p.o. + BULE 200 mg/Kg	8.55 ± 0.59	38.67 ± 3.43***	7.28 ± 0.60
G6: Paracetamol 2g/Kg; p.o. + BULE 400 mg/Kg	7.90 ± 0.76	40.30 ± 2.65***	8.02 ± 1.11
Alcohol induced hepatic damage			
G1: Control	4.87 ± 0.69	54.08 ± 4.77	10.20 ± 1.13
G2: Positive control (Alcohol 5g/Kg of 25% w/v; p.o.)	12.03 ± 1.11	23.62 ± 3.24	3.48 ± 0.36
G3: Alcohol 5g/Kg of 25% w/v; p.o. + Silymarin 100 mg/Kg	7.05 ± 0.59	42.92 ± 3.85****	8.20 ± 1.24
G4: Alcohol 5g/Kg of 25% w/v; p.o. + BULE 100 mg/Kg	9.55 ± 0.70	30.47 ± 4.49	5.38 ± 0.71
G5: Alcohol 5g/Kg of 25% w/v; p.o. + BULE 200 mg/Kg	9.17 ± 0.38	38.80 ± 3.63****	7.72 ± 0.77
G6: Alcohol 5g/Kg of 25% w/v; p.o. + BULE 400 mg/Kg	8.42 ± 0.73	42.15 ± 3.29****	8.17 ± 1.13

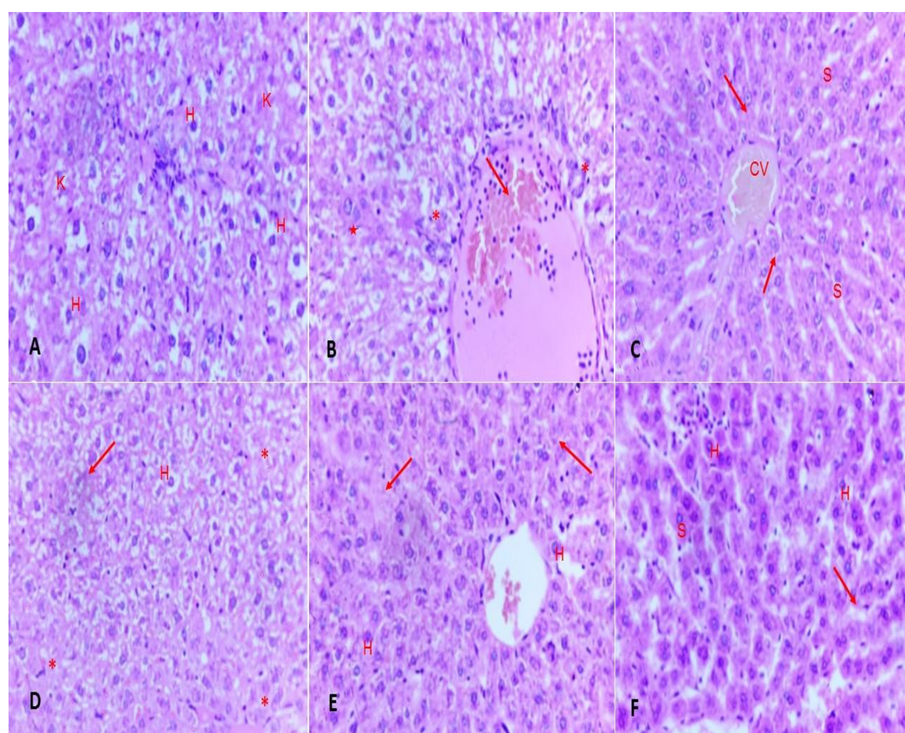
LPO= Lipoxygenase; GSH= Reduced Glutathione; SOD= Superoxide dismutase. BULE= *Biophytum umbraculum* leaf extract. All the values are expressed in Mean ± S.E.M, where N = 5; **** p< 0.0001, *** p< 0.001, ** p<0.01, *p<0.05, ns - non-significant. Treatment groups were compared against the induction control group.

The histopathological evidence of the liver in general was concluded by cellular rearrangements, infiltration, inflammation, necrosis etc. In this study,the liver sections with paracetamol induction showed hydropic degeneration, congestion, and focal necrosis while alcohol induction showed significant necrosis and inflammatory infiltrations. However, treatment with BULE after paracetamol induced toxicity registered mild signs of necrosis at 200 mg/ Kg and no signs of necrosis at 400 mg/Kg as shown in figure 5. Likewise, treatment with BULE after alcohol induced toxicity registered mild cell infiltrations at 200 mg/ kg and no signs of necrosis at 400 mg/ kg as shown in Fig. 6.



A: Control Liver section with sinusoidal and central vein dilatation (→), vascular congestion (*), hepatocytes (H), stellate Kupffer cells (K); B: Liver section of paracetamol (2 g/kg) treated rats with hydropic degeneration (→), congestion and focal necrosis (*), centrilobular congestion (*); C: Liver segments of rats administered paracetamol, and silymarin (200 mg per kg, p.o) with histological appearance of central vein (→), no signs of necrosis; D: Liver section of rat treated with paracetamol+ *Biophytum umbraculum* leaf extract (100 mg per kg; p.o.) with mild hydrophobic lesions (*), congestion and mild signs of necrosis (→); E: Liver section of rats administered with paracetamol + *Biophytum umbraculum* leaf extract of 200 mg/kg; p.o.) mild hydrophobic lesions (*) with congestion and mild signs of necrosis (→); portal vein (PV); F: Liver section of rat treated with paracetamol + *Biophytum umbraculum* leaf extract of 400 mg per kg; p.o.) exhibiting normal histological presentation with no signs of necrosis; intact hepatocytes (H); hepatic artery (HA); Central Vein (CV); stellate Kupffer cells (K).

Fig. 5. Liver sections depicting the effect of *Biophytum umbraculum* leaf (BUL) extract on paracetamol induced hepatic damage in male Wistar rats.



A: Control Liver section with intact hepatocytes (H); stellate Kupffer cells (K); B: Liver section of Alcohol (5 g/kg) treated rats with significant necrosis and inflammatory infiltration (→); pericellular infiltrations (*) ; hepatic cell disruptions (*); C: Liver segments of rats treated with Alcohol + silymarin (200 mg per kg, p.o) with normal appearance with no signs of necrosis; central vein (CV); intact hepatic cell along the central vein (→); Sinusoids (S); D: Liver segments of rats treated with Alcohol+ *Biophytum umbraculum* leaves extract (100 mg/kg; p.o.) mild inflammatory infiltration (→); mild cellular disruptions (*); hepatocytes (H); E: Liver segments of rats administered Alcohol+ *Biophytum umbraculum* leaf extract of 200 mg/kg; p.o.) mild inflammatory cell infiltration (→); intact hepatocytes (H); F: Liver segments of rats treated with Alcohol+ *Biophytum umbraculum* leaf extract of 400 mg per kg; p.o.) with normal appearance with no signs of necrosis; intact hepatocyte strands along the central vein (→); liver sinusoids (S); hepatocytes (H).

Fig. 6. Liver sections depicting the effect of *Biophytum umbraculum* leaf (BUL) extract on alcohol induced hepatic damage in male Wistar rats.

CONCLUSIONS

The results in the present study have specified that the leaf extract of *Biophytum umbraculum* has antioxidant potential that offers significant protection against paracetamol induced and alcohol-induced hepatotoxicity in male Wistar rats and elaborative research on the mechanisms at the molecular level would be conclusive to justify the hepatoprotective action of *Biophytum umbraculum* leaf extract (BULE).

FUTURE SCOPE

The 70% hydroalcoholic leaf extract of *Biophytum umbraculum* exhibited hepatoprotective activity, and the same can be checked utilizing other solvent extracts; as well as the active chemical constituents responsible for the activity could be explored further.

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Conflict of Interest. None.

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