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Hepatoprotective and Antibacterial activity of Ethanolic extract of Neonaucleasessilifolia Bark

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ABSTRACT: This paper corresponds to the investigation of the possible hepatoprotective and antibacterial properties of the crude ethanolic extract of Neonaucleasessilifoliabark. CCL_4induced hepatoprotective activity study was carried out on Wister albino rats at the dose of 250 & 500 mg/kg of body weight. The levels of SGPT, SGOT, ALP, and TB serum enzymes and GSH, SOD and CAT antioxidant enzymes of hepatotoxic and plant extract treated animals were determined as marker of hepatoprotection. In comparison to the control group the hepatotoxin induced rats showed significant rise in the level of SGPT, SGOT, ALP and TB whereas in the bark extract treated rats these enzyme levels were markedly reduced.In addition, in spite of decreased levels of the antioxidant enzymes in CCL_4 induced animals, an increased enzyme levels were identified in the N. sessilifoliabark extract treated groups indicating the capability of this plant to protect against hepatotoxicity. Antibacterial activity was analyzed following disk diffusion method against four bacterial strains. Zone of inhibition was created upon applying the crude ethanolic extract against both gram-positive (Staphylococcus aureus, Bacillus subtilis)& gram-negative bacterias (Salmonella enteridis, Escherichia coli). The ultimate results found provide the scientific basis for the traditional use of this plant in the treatment of liver diseases and bacterial infection.

KEYWORDS:N. sessilifolia, Wister albino, CCL_4, SGPT, SGOT, ALP, TB, GSH, SOD, CAT, gram-positive,

I. INTRODUCTION

Neonaucleasessilifolia is a mountainous plant belonging to the Rubiaceae family grows mostly in the land of dry hill forest in Chittagong, Chittagong hill tracts and Sylhet in Bangladesh. The leaves of this tree are about 12.5-23 cm long, epileptic, opposite, glabrous, coriaceous, nearly sessile, cordate; the solitary white flowers are 3.7 cm in diameter, fragrant, silky, axillary,globose head having stout peduncles; the fruits are distinctively capsule like, 8 mm in length, ovate. The hilly plant was reported to contain different chemical elements Beta Sitosterol, StigmasterolGlucoside, Quinovic and Saponins [1]. Different parts of this medium to large sized evergreen tree isbeing utilized by local tribal peoples as a source of conventional medicines for ages. Bark of this plant retains astringent, tonic, and styptic properties which is used by tribals peoples to heal gingivitis, diarrhea, tuberculosis with hemoptysis, metritis and liver disorders, bowel complaints and fever whereas the fresh leaf paste is known to be used to treat bacterial skin infection. Apart from that, the woods are used by Cambodian people as a tonic and depuration [2-3]. The present study was designed to investigate the hepatoprotective and antibacterial activities of Neonaucleasessilifoliabark.

II. MATERIALS AND METHODS

2.1 Collection and Extraction

Bark of Neonaucleasessilifoliaplant was collected from the Chittagong Hill Tracts range on "October, 2017" with strict prohibition on any kind of adulteration.The plant sample was sent to Bangladesh National Herbarium, Mirpur, Dhakafor identification (Accession No.: DACB45283). The collected bark was separated from unwantedcomponents followed by washing with tap water. After 15 days of shade drying the bark was powderedusing a suitable grinderand extracted according to cold extraction method. About 400 gmof powdered bark was submerged in 2000 mL ethanol in a glass container and kept for 10 days with regular shaking and stirring. The bark extract was then filteredusing a piece of clean, white cotton clothfollowed byevaporation in a rotary evaporator. After a few days of waiting, the concentrated extract (approx. yield value 3.8 %)

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was transferred into a small beaker and stored in a cool, dark and dry place.

2.2 Animals

Young Wister-albino ratsof either sex weighing between 75-120 grams were used for hepatoprotective experiment. Afterpurchasing, from the animal house of Jahangirnagar University, Savar, Dhaka-1342 the rats were kept in the animal house of the Pharmacy Discipline, Khulna University, Khulna (Animal ethics approval no. - KUAEC-2020/12/24). The rats were exposed to ideal ambient laboratory conditions {relative humidity (55-60) %, room temperature (25 ± 2)^{0}C} and natural day night cycle for 14 days prior to the experiment. The animals were fed standard laboratory food and tap water.

2.3 Drugs and Reagents

In thehepatoprotective activity study Silymarin(Brand name- Radisyl 140 mg capsule) was used as standard which was collected from local pharmacy.Other reagents namely Carbon-tetra-chloride (CCl4), chloroform, olive oil, ethanol, distilled water, agar media, powdered ciprofloxacin, nutrient broth, bacterial strains were provided by the pharmaceutical laboratory of pharmacy discipline, Khulna University.

2.4 Phytochemical Screening

Preliminary phytochemical group test was carried out on the crude bark extract prior to pharmacological activity tests to identify the presence of major functional groups. The analysis was performed based on the method described previously [4-6].

2.5 Acute Toxicity Study

Acute toxicity test of the plant extract was carried out according to the method described by previously with minor modifications [7]. Experimental animals were divided into four groups containing three Wister rats in each group namely control group and three treated groups. The control group received 2% Tween 80 in water whereas the treated groups were administered ethanolic bark extract at the dose of 500 mg/kg, 1000 mg/kg and 2000 mg/kg (0.01× body weight).The animals were observed first for short period (4 h) followed by long period (72 h), for any physiological and behavioral changes.

2.6 Hepatoprotective Activity Study

Hepatoprotectiveactivity test of the plant extract was carried outfollowing the method described previously with minor modifications [8]**.** Twenty-five young Wister albino ratsweighing between 75-120 gmwere divided into five groups containing five animals in each group namely control group, hepatotoxic group, standard group and two extract treated groups. Control group received distilled water, hepatotoxic group also received distilled water, standard group received the standard drug silymarin and the extract treated groups were administered two doses of plant extract (250 mg/kg and 500 mg/kg) at the dose of (1 mL/kg body weight, p.o.) for 9 days. On the 7th day after 12 hour fasting each of the animals were given CCL_4 except control group. Animals were sacrificed under mild chloroform anesthesia to collect blood samples for assaying marker enzymes such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and bilirubin with help of a blood analyzer. Kidney and liver were also dissected out and stored in 10% neutral buffered formalin for histopathological examination [9].

Catalase (CAT), Superoxide Dismutase (SOD) and reduced glutathione (GSH) was determined in the histopathological study. Tissue homogenate was prepared according to the method described previously [10]. The tissue (Liver) was weighed and 10% (w/v) tissue homogenate was prepared from part of the sample in 0.15 M Tris-HCL (pH 7.4) prior to centrifugation at 2500 rpm for 10 minutes. The supernatant obtained thereafter was used to estimate CAT, SOD, and GSH.For measurement of GSH mixture of 0.5 ml of the tissue homogenate and 0.1 ml of 25 % TCA was kept on ice for a few minutes followed by centrifugation at 3000 rpm for few minutes. 0.3 ml of supernatant was separated and mixed with 0.7 ml of 0.2 M sodium phosphate buffer (pH 8) and freshly prepared 2 ml of 0.6 mM DTNB (pH 8) resulting in a yellow colored solution.After 10 minutes the color was estimated at 412 nm against a blank containing 0.1 ml of 5 % TCA and a standard containing 100 n-moles/ml glutathione [11]. For determining SOD 300 µl of the tissue homogenate was mixed with 1.8 ml of 50 mM carbonated buffer (pH 10.2) followed by addition of 0.1 ml of 3×10-4 M epinephrine and 1 ml of EDTA solution (pH 10.2). Temperature was maintained at 30^{o}C and the increased absorbance was estimated at 480 nmagainst blank [12]. CAT was reckoned by composing 0.3 ml of the tissue

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homogenate with 5.7 ml of phosphate buffer and 3 ml of 5 mM H_2O_2 in buffer. After one minute the absorbance of the solution was measured at 240 nm against blank [13].

2.7 Antibacterial Activity Study

Disk diffusion method was used to analyze antibacterial activity of the N. sessilifolia bark extract in two different doses; 25 mg/ml and 50 mg/ml [14]. The first culture was prepared by pouring sterilized agar media into Petridishes to make agar slants onto which two gram-positive and gram-negative attenuated bacterial strains were transferred under aseptic condition using a laminar air flow hood followed by incubation at 37ºc for 16-18 hours to assure the growth of test organisms. The second culture was prepared in the same way except instead of attenuated bacterial strains, the test organisms from the first culture was transferred onto the agar slant using an inoculating loop.Third culture media was prepared by transferring test organisms from second culture to the vials containing 3 ml sterilized nutrient broth media which was readied by adding water to powdered nutrient agar. When turbidity is visualized in the vials after incubating at 37°C for 2-3 hours 10 µL solution from each vial was transferred to the test tubes containing 10 ml autoclaved agar media using micropipette. The test tubes were shaken and rotated to get a uniform suspension which were poured onto sterile petridishes aseptically to get the bacterial strain ready for the experiment. These were marked, divided and labeled into four portions. 25 mg and 50 mg of plant extract were weighed and mixed with 50% ethanol with the help of a sonicator. 10 µl ethanol was used as a negative control solution and positive control solution was made by adding 3 mg of ciprofloxacin powder in 1ml ethanol [15]. The plant extract solution of both doses, negative and positive control solutions were applied onto sterile filter paper discs which were then placed into the respective labeled portions of the seeded test plates and finally incubated upside down at 37°C for 16-18 hours. After completion of incubation the diameters of the zone of inhibitions were measured in terms of millimeter with a calibrated scale.

III. STATISTICAL ANALYSIS

For determination of the significant differences between the control group and treament group Student's t-test was used.

IV. RESULTS

4.1 Phytochemical Screening

Preliminary phytochemical screening of the crude bark extract showed the presence of Tannins, Flavonoids, Saponins, Alkaloids, Glycosides, Proteins and Terpenoids.

4.2 Acute Toxicity Study

No mortality was observed among the animals after consumption of the plant extract. Apart from that, any drug related changes in behavior, breathing, skin effects, water consumption, impairment in food intake and temperature were absent in the experimental rats.

Table (1) The acute toxicity study of the ethanolic extract of N.sessilifoliain rats

OBSERVATION	CONTROL	500 mg/kg	1000 mg/kg	2000 mg/kg
No. of dead rats	0	0	0	0
Food intake	Normal	Normal	Normal	Normal
Skincolor	Normal	Normal	Normal	Normal
Drowsiness	Not present	Not present	Not present	Not present
Sedation	Not present	Not present	Not present	Not present
Eye color	Normal	Normal	Normal	Normal
Diarrhoea	Not present	Not present	Not present	Not present
Coma	Not present	Not present	Not present	Not present

4.3 Hepatoprotective Activity Study

Compared to the normal control group the CCL_4 treated group showed a significant increase in the mobility of serum enzymes SGOT, SGPT, SALP and TB and a decrease in CAT, SOD and GSH enzymes. On the contrary, in the plant extract treated groups a significant reduction in SGOT, SGPT, SALP and TB serum enzymes and a rise in the levels of CAT, SOD and GSH were noticed.

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Table (2) Effect of Silymarin and N.sessilifoliaextract on serum SGPT, SGOT, ALP and TB levels in CCl_4 induced hepatotoxicity.

TEST GROUP	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	TB (mg/dl)
Normal Control	72	159	142	0.1
	62.2	152	168	0.4
	48.4	167	184	0.1
	52.7	172	195	0.2
	78.2	148	158	0.1
Statistical	62.7±5.619	159.6±4.48	169.4±9.36	0.18±0.058
CCL_4 control	94.5	254	452	1.0
	106.2	242	434	1.6
	121	238	395	1.3
	135	258	472	1.6
	98.4	263	448	1.2
Statistical	111.02±7.517***	251±4.75***	440.2±12.83***	1.34±0.117***
Standard + CCL_4	40.7	98	188	0.2
	52.5	116	193	0.4
	68.3	85.2	220	0.1
	56.3	122	205	0.3
	49.1	102	172	0.1
Statistical	49.1±4.533***	104.64±6.555***	195.6±8.08***	0.22±0.058***
Extract 250mg + CCL_4	68.4	195.1	256	0.4
	74.6	186.5	279	0.1
	80.9	205	310	0.1
	83.2	228	283	0.4
	78.9	178	302	0.5
Statistical	77.2±2.615**	198.52±8.626**	286±9.46***	0.30±0.084***
Extract 500mg + CCL_4	58	187	197	0.4
	54.2	162	220	0.3
	47.2	182	252	0.2
	62.1	158	184	0.2
	65.8	176	236	0.1
Statistical	57.46±3.219***	173±5.62***	217.8±12.41***	0.24±0.051***

Values are expressed as Mean± SEM; ***P< 0.0001, **P< 0.001, *P< 0.05; and n=5. CCl_4 treated control group was compared with normal control group, whereas all other groups were compared with CCl_4 treated control group.

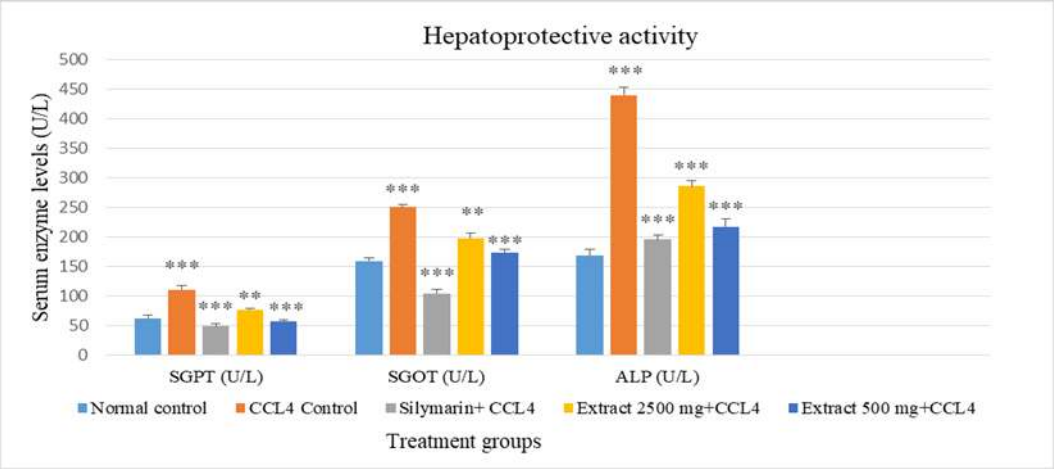

Figure (1) Serum enzyme levels in different group of animals

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Table (3) Reduced glutathione (GSH), Superoxide Dismutase (SOD) and Catalase (CAT) concentration in different groups of experimental animals

GROUPS	GSH		SOD		CAT	
	ABSORBANCE	REDUCED GSH CONCENTRATION (nmol/g TISSUE)	% INHIBITION	CONCENTRATIION OF SOD (U/g TTISSUE)	ABSORBANCE CHANGE, ΔA	CAT ACTIVITY (U/min)
Normal control	0.122	89.9	82.33	54.89	0.206	20.6
	0.119	86.9	87.66	58.44	0.238	23.8
	0.112	79.9	75.66	50.44	0.227	22.7
	0.115	82.9	84.33	56.22	0.247	24.7
	0.124	91.9	72.33	48.22	0.266	26.6
Stastical Analysis	86.3 ± 2.205		53.64±1.8825		23.68±1.001	
CCl4 control	0.045	12.9	36.67	24.44	0.142	14.2
	0.058	25.9	42.33	28.22	0.112	11.2
	0.049	16.9	27.67	18.44	0.138	13.8
	0.046	13.9	34.33	22.89	0.108	10.8
	0.054	21.9	32.33	21.55	0.119	11.9
Stastical Analysis	18.3 ± 2.462 ***		23.108±1.615***		12.38±1.537***	
Silymarin 100 mg/kg+ CCl4	0.114	81.9	72.33	48.22	0.173	17.3
	0.108	75.9	76.67	51.11	0.202	20.2
	0.106	73.9	67.66	45.10	0.181	18.1
	0.112	79.9	71.33	47.55	0.194	19.4
	0.117	84.9	75.67	50.45	0.186	18.6
Stastical Analysis	79.3± 1.99***		48.48±1.076***		18.72±0.503***	
Extract 250 mg/kg + CCl4	0.092	59.9	55.67	37.11	0.148	14.8
	0.086	53.9	53.33	35.55	0.156	15.6
	0.084	51.9	47.67	31.78	0.161	16.1
	0.094	61.9	57.66	38.44	0.143	14.3
	0.083	50.9	51.33	34.22	0.139	13.9
Stastical Analysis	55.7 ± 2.2***		35.42±1.155***		14.94±0.406*	
Extract 500 mg/kg + CCl4	0.097	64.9	65.67	43.78	0.182	18.2
	0.103	70.9	62.33	41.55	0.153	15.3
	0.106	73.9	57.66	38.44	0.168	16.8
	0.094	61.9	63.33	42.22	0.164	16.4
	0.101	68.9	61.33	40.88	0.172	17.2
Stastical Analysis	68.1 ± 2.131***		41.374±0.8772***		16.78±0.476**	

Values are expressed as Mean± SEM; ***P< 0.0001, **P< 0.001, *P< 0.05; and n=5. CCl_4 treated control group was compared with normal control group, whereas all other groups were compared with CCl_4 treated control group.

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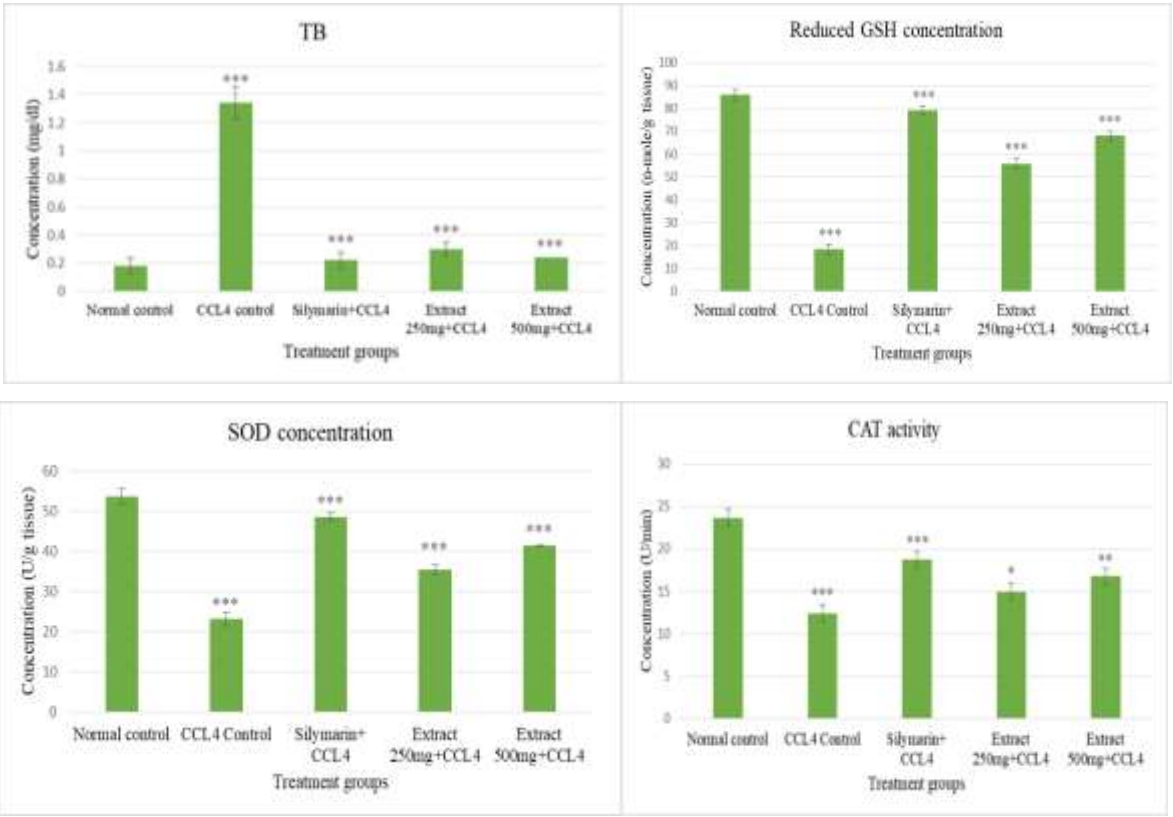

Figure (2) Reduced glutathione (GSH), Superoxide Dismutase (SOD) and Catalase (CAT) concentration in different group of animals

4.4 Antibacterial Activity Study

In comparison with the standard antibiotic discs of ciprofloxacin the crude ethanolicextract of the Neonaucleasessilifolia showed zone of inhibition against two Gram-positive (Staphylococcus aureus, Bacillus subtilis) and two Gram-negative (Salmonella enteridis, Escherichia coli) bacterial strains.

Table (4) In vitro antibacterial activity of Neonaucleasessilifolia bark extract

BACTERIAL STRAINS	TYPE OF BACTERIAL STRAINS	DIAMETER OF ZONE OF INHIBITION IN mm			
		STANDARD (CIPROFLOXACIN)	EXTRACT 25 µg/DISC	EXTRACT 50 µg/DISC	NEGATIVE CONTROL
Salmonella enteridis	Gram (-)	35	24	25	0
Staphylococcus	Gram (+)	30	22	24	0
Escherichia coli	Gram (-)	43	21	26	0
Bacillus subtilis	Gram (+)	29	17	19	0

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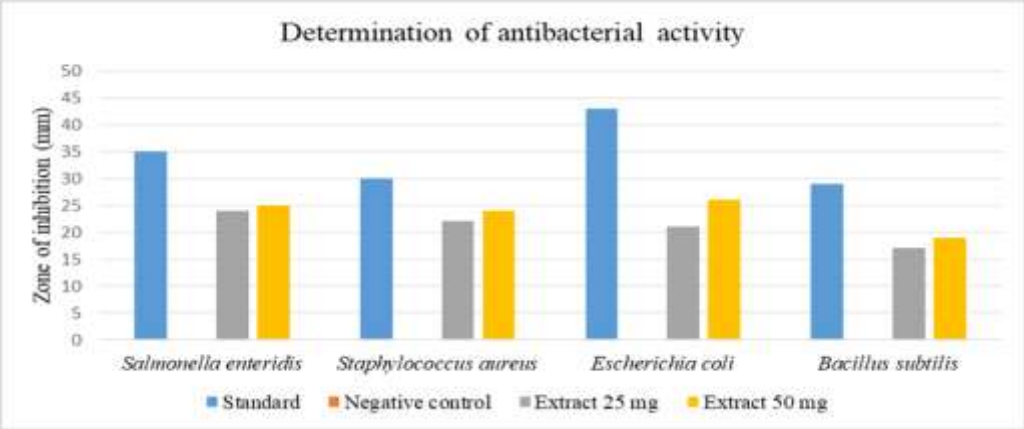

Figure (3) Antibacterial activity of *Neonaucleasessilifolia*bark

V. DISCUSSION

Ethanolic bark extract of Neonaucleasessilifoliawas screened for hepatoprotective and antibacterial properties through CCL_4 induced and disk diffusion method respectively. On phytochemical screening the crude bark extract showed the existence of several secondary metabolites such as Tannins, Flavonoids, Saponins, Alkaloids, Glycosides, Proteins and Terpenoids, responsible for a number of therapeutic and biological activities. In acute toxicity test no mortality or physiological and behavioral changes was visible proving the safety of the plant for pharmacological study.

Carbon tetrachloride (CCL_4), a renowned hepatotoxic agent induces fatty liver and cell necrosis promptingtriacylglyceral accumulation, attenuation of GSH, enhanced lipid peroxidation, membrane disruption, depression of protein synthesis and reduced of enzymatic activity [16-17]. Various hepatic marker enzymes (SGPT, SGOT and ALP) the levels of total bilirubin indicated hepatocytic damage. These enzymes leak into the bloodstream from liver tissue and produce significantly elevated serum levels and total bilirubin [18].In the hepatoprotective study the experimental animals in the hepatotoxic group exhibited an expansion in the serum enzymesSGOT, SGPT, SALP and TBwhereas the ethanolic bark extract treated group showed the opposite. In addition, CCL_4 induced Wister- albino rats revealed a reduction in the antioxidant enzymes, CAT, SOD and GSH. On the contrary, administration of the bark extract represented enhanced levels of those antioxidant enzymes indicating the potential ofNeonaucleasessilifoliabark to prevent hepatic disorders. In the antibacterial study, upon application of the plant extract on four bacterial culture media, zone of inhibition was observed against four bacterial strains alluding to the antibacterial properties of the plant. Thesepharmacological activities may be exhibited due to the presence of flavonoid, tannins, terpenoids, saponins, steroids and alkaloids [19-21].

VI. CONCLUSION

N. sessilifolia is a hilly plant having a number of conventional medicinal uses. The present study showed that the plant bark contains hepatoprotective and antibacterial activities which is possibly due to the presence of several fundamental phytoconstituents. However, further extensive study is required to isolate and identify these compounds to determine the exact mechanism of action relating to these properties.

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