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Antidiabetic and antiulcerative potential of *Garcinia lanceifolia* Roxb. bark

Nilutpal Sharma Bora[1,2*], Partha Sarathi Bairy[2,3], Abdus Salam[4] and Bibhuti Bhusan Kakoti[2]

Abstract

Background: *Garcinia lanceifolia* Roxb. has been used by many ethnic communities of Northeast India to mitigate various disorders like dyspepsia, ulcers, diabetes, etc. However, a robust scientific study on its antidiabetic and antiulcer potential is unavailable till date. The aim of this present study is to scientifically validate if the antidiabetic and antiulcer effects reported by the ethnic tribes of Assam has any scientific value or not. The effects were tested in adult Wistar albino rats using approved animal models for preclinical testing of pharmacological activities.

Results: The hydroalcoholic extract of the bark of *Garcinia lanceifolia* Roxb. was prepared and its LD_{50} was calculated. The LD_{50} was determined to be greater than 5000 mg/kg body weight. The extract at doses of 250 mg/kg body weight and 500 mg/kg body weight was found to exhibit a very potent dose-dependent antidiabetic activity. The results were backed by a battery of test including analysis of serum levels of blood glucose, lipid profiles, in vivo antioxidant enzymes, and histopathological studies. Evidence of dose-dependent antiulcer activity of the extract was backed by robust scientific data. It was found that HAEGL induced a significant dose-dependent increase in the ulcer index in both alcohol-induced and acetic acid-induced ulcer models, which was evident from the macroscopic observation of the inner lining of the gastric mucosa and the histological evaluation of the extracted stomach.

Conclusion: The results suggested that the bark of *Garcinia lanceifolia* (Roxb.) has significant antidiabetic and antiulcer potential. Further studies with respect to the development herbal dosage forms and its safety evaluation are required.

Keywords: *Garcinia lanceifolia*, Antidiabetic, Antiulcer, Acute toxicity, Wistar albino rats

Background

Modern world is in great rush pushing everyone to deal with a magnetic and energetic life where each one compromising with their energy balance either knowing or unknowingly. The above circumstances help physiological systems to disturb its own metabolic homeostasis and enzyme balance. Consequences are not safe at all as these conditions leading the mankind into metabolic disorder especially diabetic condition, obesity, hypertension, and dyslipidemia [1, 2].

Diabetes is a metabolic worse condition where living cells are deprived of glucose for energy causing glucose accumulation [3] in blood stream. Insulin, secreting hormone from pancreatic β cell, helps glucose molecule to enter into cell associated with cell surface glucose transporter to produce energy for living body in the form of adenosine triphosphate [4, 5]. However, diabetic condition aggravate as a collective or individual result of inadequate insulin secretion and insulin resistance [6] toward cell surface insulin receptor. It is now listed among the leading five causes of death worldwide indicating the eye opening statistics from American Diabetes Association and World Health Organization (WHO) [7, 8]. On the other context, ulcer or peptic ulcer is a very common gastrointestinal (GI) disorder which affected

* Correspondence: nilutpalsharma3@gmail.com
[1]NETES Institute of Pharmaceutical Science, NEMCARE Group of Institutions, Mirza, Kamrup, Guwahati, Assam 781125, India
[2]Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam 786004, India
Full list of author information is available at the end of the article

more than 10% of the world's population characterized by damage of lower GI tract inner lining either by acid and/or pepsin with *Helicobacter pylori* [9] in the absence of them. Along with these conditions, continuous administration of non-steroidal anti-inflammatory drugs (NSAIDs), stress, smoking, and excessive alcohol consumption are some proven factors that erase the mucosal epithelium layer [10–12]. As collective of those patient faces severe burning sensation in lower abdomen with abdominal pain and passing blood with stool sometimes [9].

Pharmacological interventions are available for both the problems but being as synthetic chemicals, they target some other functions causing unwanted toxicities. Antidiabetic agents like biguanides, sulfonylureas, α-glucosidase inhibitors [13], PPAR-γ agonists, SGLT-2 antagonist, and DPP-IV inhibitors, [6, 14, 15] are controlling the pandemic condition either in single mode or in combination but cardiac and hepatic tissues affected in most of the case. For treating peptic ulcer proton pump inhibitors (PPI), H_2 receptor antagonists, antimuscarinics, sucralfate, and bismuth are employed but they are also having additive effects [16]. So these viewpoints urge the exploration of some herbal products as better and safe alternatives.

Garcinia lanceifolia Roxb [17, 18]. belonging to family Clusiaceae has been a lesser known plant in the pile of huge published literatures for days. In a recent study, Ghosh et al. [19] reported its antihyperglycemic potency of whole plant using the protocols of oral glucose tolerance test (OGTT). Other species of *Garcinia* are reported for antiulcer effect [12, 16, 20] so far with other pharmacological activities. In light of the above survey, an attempt was made to investigate and report the antidiabetic and antiulcerative potential of methanolic extract from *Garcinia lanceifolia* Roxb. bark. using adult Wistar albino rats. The physiological, anatomical, and genetic similarity of Wistar rats with humans and their ease of handling and maintenance make them an ideal choice for the testing of preclinical testing of biological activities.

The objective of this study was to a twofold one. Firstly, in one set of rodents, the antidiabetic activity of the plant was tested in streptozotocin induced diabetic model; and secondly, in another set, the antiulcer activity was tested using two different models, viz., alcohol-induced antiulcer model and acetic acid-induced ulcer model separately. Prior to that, the acute toxicity study was performed as per approved protocols, to calculate the LD_{50} of the extract.

Methods

Animals

Adult male Wistar Albino rats of uniform weight (180–250 g) were used in this study, which were commercially purchased from the enlisted supplier of the University (M/S Chakraborty Enterprise, Kolkata, West Bengal, India). The animals were free to access food and potable water as they underwent acclimatization under laboratory conditions for 2 weeks prior to the experiment. All experiments were conducted during the day time (between 0900 and 1600 h), after an overnight fasting period. During the entire experimental period, the animals were maintained as per the guidelines of the Guide for the Care and Use of Laboratory Animals, National Institutes of Health (NIH). The rats were hygienically maintained in polypropylene cages in an air-conditioned environment of 22–25 °C, 40–70% humidity with 12 h light-dark cycles, and ventilation of 15–21 air changes/h. The entire study was permitted by the Institutional Animal Ethics Committee (IAEC), Assam, and conducted by following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA); with prior approval number IAEC/DU/60 dated 24/09/2013.

Plant material and extraction

The collection of the bark of *Garcinia lanceifolia* was done from the University campus and neighboring areas of Dibrugarh, Assam India in accordance to the Good Agricultural and Collection Practice (GACP) guidelines. Images of the plant and collected fresh bark are shown in Fig. 1. The plant herbarium was authenticated by Dr. A. A. Mao, Botanical Survey of India, Eastern Regional Centre, Shillong (Letter no.: BSI/ERC/2014/Plant identification/882. A herbarium specimen of the plant was submitted at the Pharmacognosy Research Lab of the Institute for further references. After proper cleaning of the collected bark, it was cut into smaller pieces and dried to constant weight. Thereafter, the dried bark were pulverized in a mechanical grinder and stored in hermetically sealed containers until extraction process commenced.

The hydroalcoholic extract of the stem bark of *Garcinia lanceifolia* (HAEGL) was prepared by using cold maceration process with 1000 mL of water:ethanol (80:20) mixture. The extracts were concentrated by distillation, followed by vacuum drying using a rotary evaporator. Preliminary phytochemical tests were carried out with all the extracts in order to evaluate for the presence of different phytochemical constituents.

Chemicals

All chemicals used in this assay were of analytical grade and obtained from reputed suppliers like SRL Sisco Research Laboratories Pvt. Ltd., Mumbai, India; Roche (Products) Pvt. Ltd., Bayer Diagnostics, Mumbai, India; Himedia Laboratory, Mumbai, India; Loba Chemie, Mumbai, India; Rankem Chemicals, Faridabad, India;

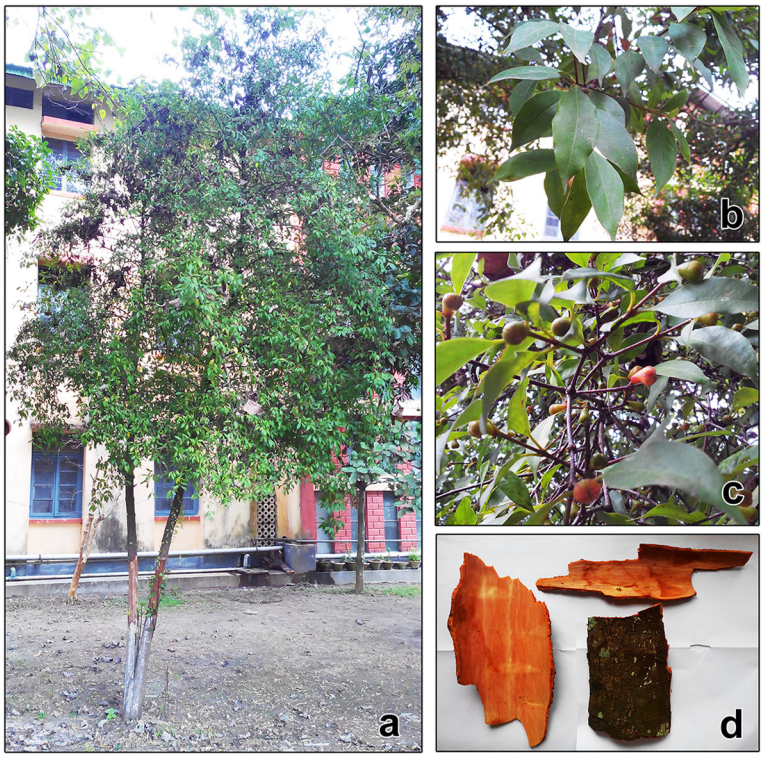

Fig. 1 Images of Garcinia lanceifolia plant. **a** Whole plant. **b** Leaves arrangement. **c** Young fruits. **d** Collected bark

Otto Chemie, Mumbai, India; SRL Sisco Research Laboratories, Mumbai, India; Spectrochem, Mumbai, India; and Beacon Diagnostics Pvt. Ltd; Gujarat, India.

Acute toxicity and LD_{50} calculation of HAEGL

To process the toxicological studies of HAEGL, a series of doses, viz; 175 mg/kg, 550 mg/kg, 1750 mg/kg, 5000 mg/kg body weight were used.

To obtain the LD_{50} of HAEGL, the experiments were planned according to the methods approved by the Organization for Economic Cooperation and Development (OECD). After acclimatization of the environment, rats were administered HAEGL with doses in the sequence listed previously by a single oral gavage. The highest dose limit of 5000 mg/kg for HAEGL was determined by subjecting to a limit test at 5000 mg/kg, as per OECD guideline 425. Each dose was given to a single animal only. If the animal survived the current dose, the second animal received a consecutive higher dose. If the animal does not survive, the second animal received a lower dose. Since HAEGL did not show any toxicity reactions below the regulatory limit doses (i.e., 5000 mg/kg); hence, it was evaluated by a limit test of 5000 mg/kg. The LD_{50} and 95% profile likelihood (PL) of HAEGL were obtained by analyzing the experimental data using the AOT425 program (OECD guideline 425) [21–23].

Antidiabetic assay

Oral glucose tolerance test

Initial screening of the extract for the hypoglycemic activity was done in healthy rats by conducting oral glucose tolerance test (OGTT). The OGTT was performed for two different doses of HAEGL (250 and 500 mg/kg of bodyweight per orally) and blood glucose level was measured by one touch glucometer (ACCU-CHEK®, Roche India Pvt. Ltd., Mumbai, India). The blood glucose levels were estimated at the intervals of 0th, 30th, 60th, 90th, and 120th min after the administration of extract [24].

Induction of non-insulin dependent diabetes mellitus

Following overnight fasting, type II diabetes was stimulated in the experimental animals by an intraperitoneal (i.p.) inoculation of streptozotocin (Spectrochem, Mumbai, India) dissolved in 0.1 M cold citrate buffer (pH 4.5), at a dose of 65 mg/kg of bodyweight; followed 110 mg/kg of nicotinamide via the i.p. route (Spectrochem, Mumbai, India). The control rats were administered with the vehicle alone. The induced rats were tested for the presence of elevated blood glucose levels after 7 days and the rats with moderate hyperglycemia (blood glucose range of above 250 mg/dl) were utilized for the experiment [25].

Table 1 Results of limit test for HAEGL conducted using AOT425 Statistical Program

Test sequence	Animal ID	Dose (mg/kg)	Short-term result	Long-term result
1	HAEGL1	5000	O	O
2	HAEGL2	5000	O	O
3	HAEGL3	5000	X	X
4	HAEGL4	5000	O	O
Legend: (X = died, O = survived)				
Summary of long term results				
Dose		Animals survived	Animals died	Total
5000 mg/kg bw		3	1	4

Statistical estimation suggested that the LD_{50} is greater than 5000 mg/kg

Experimental design

The rats were segregated into five groups and for each group five animals were taken as follows:

Group 1: Normal control rats administered with 0.5% carboxymethylcellulose (CMC) vehicle 5 mL/kg bodyweight per orally.
Group 2: Streptozotocin (STZ)-induced diabetic control rats.
Group 3: Diabetic control rats treated with HAEGL (250 mg/kg body weight/day) dissolved in aqueous solution of 0.5% CMC per orally for 15 days.
Group 4: Diabetic control rats administered orally once a day for 15 days with HAEGL (500 mg/kg body weight/day) dissolved in aqueous solution of 0.5% CMC. Group 3 and 4 were served as test drug treated groups.
Group 5: Diabetic control rats treated orally once a day for 15 days with the standard drug metformin (Yarrow Chem Products, Mumbai, India) at a dose 10 mg/kg of bodyweight which served as standard control.

Blood was sampled from the tail vein of the overnight (12–15 h) fasted rats and fasting blood glucose levels along with the average body weights were closely monitored on 0th, 5th, 10th and 15th day. On the 15th day, all the animals underwent euthanasia via cervical dislocation under moderate anesthesia and evaluated for various biochemical parameters, histopathology and in vivo anti-oxidant status [26].

Biochemical investigation

Total cholesterol (TC), triglycerides (TG) in EDTA plasma, and aspartate transaminase (SGOT), alanine transaminase (SGPT), alkaline phosphatase (ALP) levels in serum were measured colorimetrically using specific kits (Beacon Diagnostics Pvt. Ltd; Gujarat, India) as per the manufacturers' instructions.

Histological studies

After euthanizing the animals, the pancreas of the experimental animals were collected and analyzed for

Table 2 Results of acute toxicity studies (LD_{50} calculation) of HAEGL conducted using AOT425 Statistical Program

Test sequence	Animal ID	Dose (mg/kg)	Short-term result	Long-term result
1	HAEGL1	175	O	O
2	HAEGL2	550	O	O
3	HAEGL3	1750	O	O
4	HAEGL4	5000	X	X
5	HAEGL5	1750	O	O
6	HAEGL6	5000	O	O
7	HAEGL7	5000	O	O
8	HAEGL8	5000	O	X
Legend: (X = died, O = survived)				
Summary of long term results				
Dose (mg/kg bw)		Animals survived	Animals died	Total
175		1	0	1
550		1	0	1
1750		2	0	2
5000		2	2	4
All doses		6	2	8

Statistical program suggested that estimated LD_{50} = 5000 (the one dose with partial response). 95% PL confidence interval is 2045 to Greater than 20,000

Table 3 Effect of HAEGL on oral glucose tolerance test in Wistar albino rats

Animal group	0 min	30 min	60 min	90 min	120 min
Normal control	77.0 ± 0.54	141.4 ± 0.92	152.6 ± 1.12	162.8 ± 0.96	181.6 ± 1.43
HAEGL (250 mg/kg)	78.2 ± 1.31	103.2 ± 0.91**	100.8 ± 1.49**	94.0 ± 0.54**	83.82 ± 2.83**
HAEGL (500 mg/kg)	79.0 ± 0.83	100.6 ± 1.47**	101.8 ± 2.31**	41.6 ± 0.97**	81.0 ± 0.54**

Values are expressed as mean ± SEM (n = 5), statistical significance: *p < 0.05, **p < 0.01, compared with normal control group

histological changes. The skin samples were fixed in a fixative mixture of fixative (picric acid, formaldehyde 40%, and glacial acetic acid) for 24 h and thereafter embedded in paraffin with a melting point of 55–57 °C. Sections of 6 μm were obtained and stained with hematoxylin and eosin (H&E) stain to assess any structural alterations in the pancreas. All stained specimens were observed using optical light microscope and photographed using a camera.

In vivo antioxidant assay

About 400 mg of liver tissue was samples from each experimental animal subject, rinsed in normal saline, and blotted with filter paper. The tissues were then homogenized in 1.15% potassium chloride (KCl) and centrifuged at 1200 rpm at 40 °C for 10 min. The supernatant was collected which were again centrifuged at 10,000 rpm at 40 °C for 10 min. Again the supernatant were collected and centrifuged at 14,000 rpm for 60 min at 40 °C. The microsomal fraction were taken, suspended in KCl, and stored at − 20 °C.

Thereafter, the processed samples were analyzed separately to determine the levels of lipid peroxidation (LPO), reduced glutathione (GSH), and catalase activity (CAT) as per methods described earlier, with trivial modifications [27–29].

Antiulcer assay

Absolute alcohol induced ulcer model

The study was carried out as per the methods described by Umamaheswari et al. 2007 and Deore et al. 2011 [12, 30]. The rats were divided into five groups and for each group five animals were taken as follows:

Group 1: Normal control rats administered with 0.5% carboxymethylcellulose (CMC) vehicle 5 mL/kg bodyweight per orally.
Group 2: Rats with ethanol-induced ulcers (1 mL absolute alcohol per orally).
Group 3: Ulcer-induced rats administered orally once a day for 5 days with HAEGL (250 mg/kg body weight /day) dissolved in aqueous solution of 0.5% CMC.
Group 4: Ulcer-induced rats administered orally once a day for 5 days with HAEGL (500 mg/kg body weight/ day) dissolved in aqueous solution of 0.5% CMC.
Group 3 and 4 were served as test drug treated groups.
Group 5: Ulcer-induced rats treated orally once a day for 5 days with the standard drug ranitidine (Yarrow Chem Products, Mumbai, India) at a dose 50 mg/kg of bodyweight which served as standard control.

On the 6th day, the animals were sacrificed by cervical dislocation under mild anesthesia and stomach was incised along the greater curvature and examined for ulcers. The ulcer index was counted, by taking into account the product of length and width of the ulcers observed in the glandular section of the stomach (square millimeters per rat). The summation of the lengths (mm) of all lesions for each stomach sample salvaged was termed as the ulcer index (UI)

$$\mathrm{UI} = (n \text{ lesion } I) + (n \text{ lesion } II) + (n \text{ lesion } III)$$

where UI = ulcer index; I = presence single, submucosal, punctiform hemorrhages along with of edema and hyperemia; II = presence of hemorrhagic submucosal lesions with minor erosions; III = evidence of profound ulcers with invasive lesions and erosions.

Table 4 The deviation of body weight of the animals treated with HAEGL during 15 days of treatment

Animal group	Day 0	Day 5	Day 10	Day 15
Normal control	190.6 ± 2.80##	191.6 ± 2.80##	192.6 ± 0.39##	195.6 ± 2.20##
Diabetic control	182.4 ± 1.47#	181.2 ± 1.59*	176.6 ± 2.33**	174.4 ± 2.20**
HAEGL (250 mg/kg)	196.2 ± 4.12##	193.00 ± 3.39##	192.6 ± 3.62##	195.8 ± 4.32##
HAEGL (500 mg/kg)	179.2 ± 1.55*	176.4 ± 1.12**	177.20 ± 1.15**	179.00 ± 1.87**
Standard	170.60 ± 2.80**	171.00 ± 3.67**	174.00 ± 4.00**	174.8 ± 4.30**

Values are expressed as mean ± SEM (n = 5), statistical significance: *p < 0.05, **p < 0.01, compared with that of normal control group; #p < 0.05, ##p < 0.01, compared with standard group

Table 5 The effect of HAEGL on fasting blood glucose level on streptozotocin-induced diabetic rats during 15 days of treatment

Treatment	Day 0	Day 5	Day 10	Day 15
Normal control	92.53 ± 0.44	102.00 ± 0.54$^{\#\#}$	102.60 ± 1.07$^{\#\#}$	96.40 ± 0.67$^{\#\#}$
Diabetic control	242.6 ± 1.12**	264.80 ± 1.46$^{**\#\#}$	270.00 ± 2.23$^{**\#\#}$	270.00 ± 0.54$^{**\#\#}$
HAEGL (250 mg/kg)	244.80 ± 1.28**	177.40 ± 1.12$^{**\#\#}$	173.80 ± 1.59$^{**\#\#}$	135.20 ± 1.46$^{**\#\#}$
HAEGL (500 mg/kg)	241.40 ± 1.10**	160.80 ± 1.85**	132.00 ± 0.89$^{**\#\#}$	123.80 ± 2.03$^{**\#\#}$
Standard drug	240.00 ± 2.73**	164.00 ± 1.87**	132.60 ± 1.12**	113.20 ± 1.20**

Values are mean ± SEM (n = 5), statistical significance: *p < 0.05, **p < 0.01, compared with normal control group; #p < 0.05, ##p < 0.01, compared with standard group

The percentage inhibition was estimated by utilizing the following formula;

$$\%\text{inhibition} = \left(\frac{UI_{\text{control}} - UI_{\text{treated}}}{UI_{\text{control}}}\right) \times 100$$

Acetic acid induced ulcer model

The rats were segregated into five groups and for each group five animals were taken as follows:

Group 1: Normal control rats administered with 0.5% carboxymethylcellulose (CMC) vehicle 5 mL/kg bodyweight per orally.
Group 2: Rats with acetic acid-induced ulcers (by injection of 0.05 ml of 10% acetic acid into the subserosal layer present in the glandular region of the anterior barrage of the stomach via a midline gastric incision performed under light anesthesia).
Group 3: Ulcer-induced rats administered orally once a day for 20 days with HAEGL (250 mg/kg body weight /day) dissolved in aqueous solution of 0.5% CMC.
Group 4: Ulcer-induced rats administered orally once a day for 20 days with HAEGL (500 mg/kg body weight/ day) dissolved in aqueous solution of 0.5% CMC.
Groups 3 and 4 were served as test drug-treated groups.
Group 5: Ulcer-induced rats treated orally once a day for 20 days with the standard drug famotidine (Yarrow Chem Products, Mumbai, India) at a dose 20 mg/kg of bodyweight which served as standard control.

All treatments were administered orally 1 day post-surgery for 20 days, and on the 21st day all the animals underwent euthanasia by cervical dislocation under moderate anesthesia, stomachs were salvaged, and the healing progression of the ulcers were evaluated. Gastric lesions were assessed by investigating the inner gastric plane using a dissecting-binocular microscope. Thereafter, the ulcer area (mm^2) and curative rate (%) were determined [30]. Thereafter, the stomach were immersed in 10% formalin and processed for histopathological evaluation. If presence of ulcerated tissue was detected, the center part of the damaged tissue was taken and dissected in half along the long diameter. In case of undamaged tissue, the sections from the basal part were taken.

Statistical analysis

All statistical analysis was performed by utilizing the GraphPad Prism software version 5.0 (GraphPad Software, La Jolla, CA, US). One-way ANOVA followed by various post-hoc tests was used to analyze the difference among multiple dosage groups. All values are expressed as mean ± SEM. A p < 0.05 value, evaluated at 95% level of confidence, was considered as statistically significant; unless otherwise indicated in the results.

Results

Acute toxicity and LD_{50} calculation of HAEGL

For the limit test of HAEGL, the Acute Oral Toxicity (OECD Test Guideline 425)—AOT425 Statistical Program was used and each animal after exposure to the

Table 6 The effect of HAEGL on biochemical parameters in streptozotocin-induced diabetic rats on the 15th day of treatment

Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	SGOT	SGPT	ALP
Normal control	158.2 ± 0.91$^{\#\#}$	207.6 ± 1.12$^{\#\#}$	52.60 ± 1.12	40.80 ± 0.96$^{\#\#}$	104.20 ± 0.37$^{\#\#}$
Diabetic control	228.2 ± 0.91$^{**\#\#}$	300.2 ± 0.91$^{\#\#**}$	105.00 ± 2.2$^{**\#\#}$	82.40 ± 0.74$^{**\#\#}$	172.60 ± 1.12$^{**\#\#}$
HAEGL (250 mg/kg)	207.4 ± 1.12$^{**\#\#}$	256 ± 1.87$^{\#\#**}$	72.80 ± 0.96 $^{**\#\#}$	65.80 ± 0.96$^{**\#\#}$	133.2 ± 1.20$^{**\#\#}$
HAEGL (500 mg/kg)	177.8 ± 1.28$^{**\#\#}$	237.4 ± 1.12**	54.00 ± 1.34$^{\#\#}$	52.40 ± 1.07$^{**\#\#}$	125.20 ± 1.46**
Standard drug	165.8 ± 0.96**	236.4 ± 1.56**	51.80 ± 0.91	47.40 ± 0.74**	120.40 ± 0.74**

Values are mean ± SEM (n = 5), statistical significance: *p < 0.05, **p < 0.01, compared with normal control group; #p < 0.05, ##p < 0.01, compared with standard group

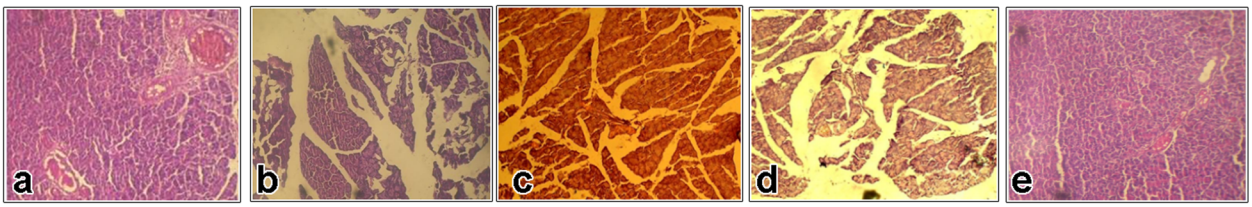

Fig. 2 Representative images for histopathological evaluation of pancreas of Wistar albino rats subjected to STZ-induced diabetic model at × 100 magnification. **a** Normal control group. **b** Diabetic control. **c** HAEGL (250 mg/kg). **d** HAEGL (500 mg/kg). **e** Standard

hypothesized limit dose (5000 mg/kg body weight) and the results as listed in Table 1. Thereafter, mortality in each unique animal was observed and recorded. LD_{50} value of the test sample was hence calculated. The limit test was found to be complete when 3 animals survived the hypothesized limit dose administered in the study.

Thereafter, for the estimation of acute toxicity, the dose progression suggested by AOT425 Statistical Program was used. Only one animal was exposed to one dose followed by observations for signs of toxicity or death. The test was found to be complete when three animals survived at the limit dose as calculated in the LD_{50} calculation study previously. The test results are displayed in Table 2.

In both the experiments, symptoms exhibited by the experimental animals after exposure to the test formulations were observed during for a 14-day period. During this period, all the animals that survived did not show any signs of toxicity; like loss of appetite, diarrhea, and vomiting or passive behavior, hypopnea, tremor, and arching of back. It was found that HAEGL is non-toxic as per the Hodge and Sterner scale and AOT425 Statistical Software with a LD_{50} value of 5000 mg/kg body weight and 95% profile likelihood (PL) of 2045 to > 20000.

Antidiabetic assay

Oral glucose tolerance test

In oral glucose tolerance test (OGTT), HAEGL, from 30 min onwards exhibited significant decrease in plasma glucose levels (Table 3). Initially, the induction of diabetes was ascertained by the presence of a high fasting blood glucose levels in the experimental rats. It was evident from the study that the experimental animals were responsive toward the particular OGTT protocols.

Analysis of serum glucose and body weight levels

The effects of different doses (250 mg/kg and 500 mg/kg of bodyweight) of the HAEGL on the fasting blood level were investigated in the streptozotocin-induced diabetic Wistar albino rats using metformin hydrochloride as standard drug (10 mg/kg of bodyweight). The divergence of body weights and study reports of oral glucose tolerance test (OGTT) of the animals were noted in Tables 4 and 5 respectively, which were noted on the 0th, 5th, 10th, and 15th day of treatment.

Biochemical investigation

The levels of TC, TG in EDTA plasma and SGOT, SGPT, ALP levels in serum were evaluated on the 15th day; i.e., the final day of treatment using HAEGL. It was observed that both the doses of HAEGL were able to mitigate the deviation in the biochemical markers which were affected in streptozotocin-induced diabetic Wistar albino rats. The results are tabulated in Table 6.

Histological studies

The results of the histopathological studies on the pancreas of the streptozotocin-induced diabetic Wistar albino rats are shown in Fig. 2. The pancreas plays a major role

Table 7 Effect of HAEGL on liver in vivo antioxidant enzymes in streptozotocin-induced diabetic rats on the 15th day of treatment

Treatment	Lipid peroxidase (nM of MDA/mg of protein)	Glutathione (nM/mg of protein)	Catalase (nM of H_2O_2 decomposed/min/mg of protein)
Normal control	$15.41 \pm 0.24^{\#\#}$	$50.80 \pm 0.80^{\#\#}$	$14.20 \pm 0.80^{\#\#}$
Diabetic control	$35.60 \pm 0.40^{**\#\#}$	$23.80 \pm 1.02^{**\#\#}$	$5.60 \pm 0.40^{**\#\#}$
HAEGL (250 mg/kg)	$25.20 \pm 0.48^{**\#}$	$31.80 \pm 0.73^{**}$	$5.40 \pm 0.40^{**\#\#}$
HAEGL (500 mg/kg)	$23.20 \pm 0.80^{**\#\#}$	$42.20 \pm 0.97^{**\#\#}$	$7.60 \pm 0.24^{**}$
Standard drug	$27.40 \pm 0.40^{**}$	$35.20 \pm 0.80^{**}$	$8.40 \pm 0.24^{**}$

Values are mean ± SEM ($n = 5$), statistical significance: *$p < 0.05$, **$p < 0.01$, compared with normal control group; #$p < 0.05$, ##$p < 0.01$, compared with standard group

Table 8 Effect of HAEGL on alcohol-induced and acetic acid-induced gastric ulcer on rats

Treatment	Alcohol-induced gastric ulcer index	Acetic acid-induced gastric ulcer index
Normal control	–	–
Ulcer control	34.67 ± 2.60	42.00 ± 1.73
HAEGL (250 mg/kg)	12.67 ± 1.76**	16.67 ± 0.88**
HAEGL (500 mg/kg)	11.33 ± 1.20**	14.67 ± 1.45***
Standard drug	4.00 ± 0.58***	4.67 ± 0.88***

Values are mean ± SEM (n = 3), statistical significance: *p < 0.05, **p < 0.01, when compared with ulcer control group

in the regulation of blood sugar levels due to the secretion of insulin by the β cells of the islets of Langerhans. It was observed that the various detrimental effects observed in the sections of the pancreas extracted from diabetic rats were significantly ameliorated in the HAEGL-treated groups. Effects like shrunken cell size, architectural damage, absence of islets cells, fatty layer degradation, loss of cellular integrity, irregular gap junctions, and lymphocyte infiltration were evident in the diabetic control group. Whereas, in the treated groups, these effects were very nominal and the sections were very much comparable to the standard drug treated group. A normal control group was also observed for comparative analysis.

In vivo antioxidant assay

Oxidative stress induced by streptozotocin was evaluated by analyzing various in vivo markers. LPO, GSH, and CAT enzymes were estimated in all the experimental groups and the results are displayed in Table 7. It was observed that due to occurrence of diabetes, there was a significant increase LPO levels along with depletion of GSH and CAT enzymes in the diabetic control group. These effects were markedly decreased in the HAEGL-treated groups, and the results were found to be statistically significant when compared with the diabetic and normal control groups.

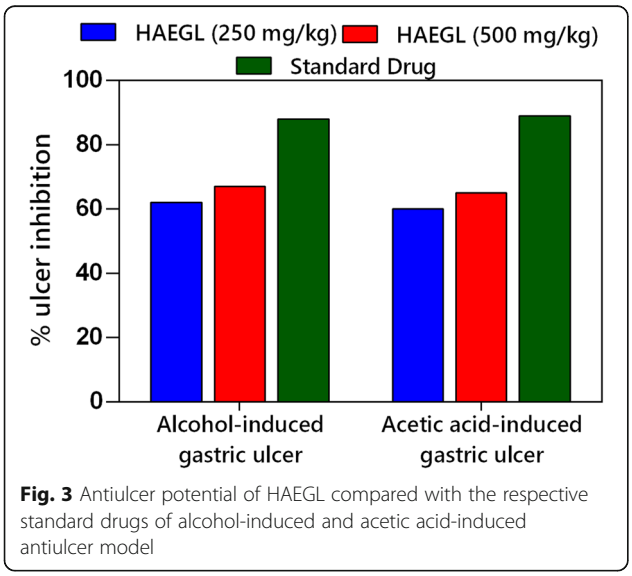

Fig. 3 Antiulcer potential of HAEGL compared with the respective standard drugs of alcohol-induced and acetic acid-induced antiulcer model

Antiulcer assay

Both the studies conducted in the absolute alcohol-induced ulcer model and the acetic acid-induced ulcer model confirmed that HAEGL had a good potential for reduction of gastric ulcer in Wistar rats (see Table 8). In the absolute alcohol-induced ulcer model, rats treated with HAEGL at doses of 250 mg/kg body weight and 500 mg/kg body weight showed a significant reduction in the ulcer index (p < 0.01). After 5 days of treatment, HAEGL (250 mg/kg) and HAEGL (500 mg/kg) showed a reduction of 62% and 67% respectively, while the standard ranitidine showed a protection index of 88% (Fig. 3). Similarly in the acetic acid-induced ulcer model, it was observed that HAEGL (250 mg/kg) and HAEGL (500 mg/kg) showed a reduction of 60% and 65% respectively, while the standard famotidine showed a protection index of 89%.

The results of the histopathological studies, on the inner lining of the extracted stomachs of the experimental animals, are shown in Fig. 4. Fig. 4a–e displays a representative image of each of the groups of the absolute alcohol induced ulcer model and Fig. 3f–j shows the results of the acetic acid induced ulcer model. It was observed that the detrimental effects of both the ulcer models like loss of gland architecture, erosion of the epithelial layer, edema, infiltration by inflammatory cells, etc. were effectively ameliorated in the HAEGL-treated groups. The results were comparable with the standard groups.

Discussion

Garcinia lanceifolia is a well-known medicinal plant used extensively in the northeastern part of India. It is also used in edible culinary preparations and eaten as a fruit. Traditionally, this plant is known to have many medicinal properties like antiulcer, anthelmintic, and anti-inflammatory properties. It is known to contain many bioactive agents like xanthones, bioflavonoids, benzophenones, benzoquinones, and triterpenes which contribute to its beneficial effects [31, 32]. The present study is aimed at the exploration of antidiabetic and

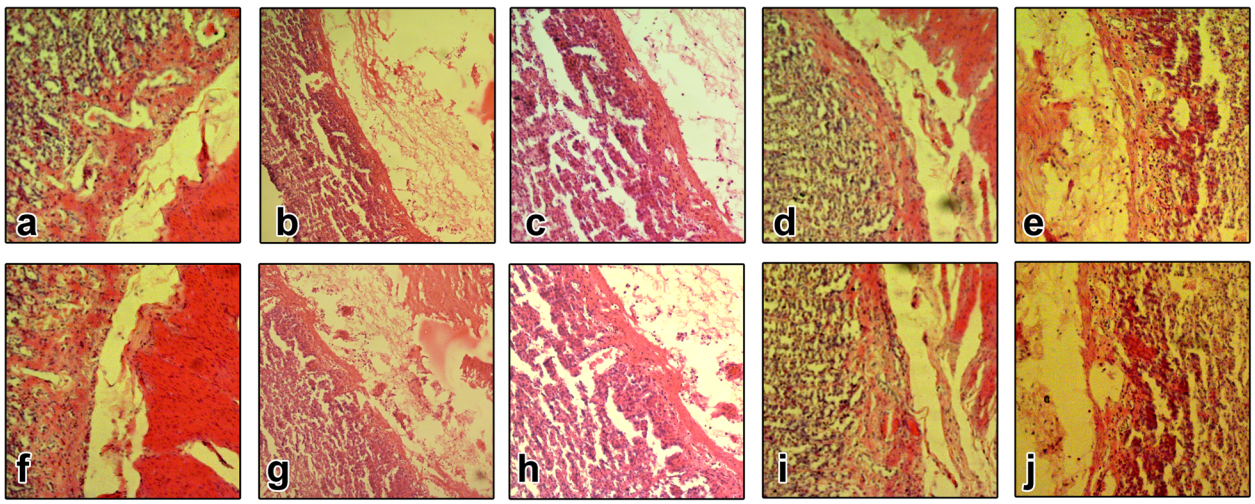

Fig. 4 Representative images for histopathological evaluation of inner gastric surface of Wistar albino rats subjected to alcohol-induced (plate '**a**' to '**e**') and acetic acid-induced antiulcer model (plate '**f**' to '**j**') at × 100 magnification. **a** Normal control group. **b** Diabetic control. **c** HAEGL (250 mg/kg). **d** HAEGL (500 mg/kg). **e** Standard

antiulcer potential of *G. lanceifolia* bark extracts. These traditionally claimed effects were tested using scientifically backed animal models and established protocols.

The acute toxicity studies of HAEGL were tested using Acute Oral Toxicity (OECD Test Guideline 425)—AOT425 Statistical Program. It was found that the LD_{50} value of HAEGL was 5000 mg/kg body weight. There were no visible signs of treatment related adverse effects or mortality in the experimental animals. Changes in behavior or metabolism were not experiential in short period (24 h) and long period observations (14 days). The antidiabetic potential of HAEGL was tested using streptozotocin-induced diabetic model. It was observed that the animals treated with HAEGL displayed a dose-dependent decrease in the serum blood glucose levels along with significant improvements in other symptoms related to diabetes. Hepatic overproduction and decreased utilization of glucose in the body tissues is a characteristic feature of diabetes. These symptoms were found to be ameliorated in the experimental animals treated with 500 mg/kg body weight of HAEGL, at the end of the 15-day treatment period. The blood glucose readings were taken on the 0th, 5th, 10th, and 15th days and HAEGL-treated groups observed significantly lower blood sugar as compared to the diabetic control group (Table 5). The results were not as pronounced as the standard drug (metformin) treated groups but were comparable. HAEGL may be inducing these effects by increasing the effects of insulin in plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form [25]. Levels of cholesterol and triglycerides which were found to be increased in cases of diabetic rats were also control in the HAEGL-treated groups in a dose-dependent manner. Loss of body weight which is a characteristic feature of diabetes was also found to be improved in a dose-dependent manner [33, 34]. STZ is known to produce free radicals in the body and oxidative stress has shown to play a major role in the pathogenesis of types I and II diabetes by causing pancreatic injury. As a result, antioxidants from plant material may play a vital role in the amelioration of these effects [35, 36]. Our study demonstrated that treatment with HAEGL significantly improved the serum levels of SGOT, SGPT, and ALP along with improvements of the in vivo antioxidant enzymes. This may be due to the presence of phenolic compounds, flavonoids, and triterpenoids in HAEGL, as reported in our previously published articles [18, 31]. Histopathological examination of the sections of pancreas extracted from the experimental animals revealed that the gross changes in histopathological architecture along with other structural damage to the cells that were observed in the diabetic rats were not present in the HAEGL-treated groups. The cytoprotective action that was observed in the HAEGL-treated groups was minute and suggested that this protective action may have resulted in the improvement of the diabetic conditions of the rats.

The antiulcer activity of HAEGL was tested using two different animal models; viz. absolute alcohol-induced ulcer model, and the acetic acid-induced ulcer model. The results suggested that HAEGL was able to significantly reduce the ulcer caused in both the experimental models. Ethanol-induced gastric ulcers are widely used in antiulcer

animal model. Metabolism of ethanol in the body releases superoxide anion and hydroperoxy free radicals, which are found to be a part of the mechanism of acute as well as chronic ulceration in the gastric mucosa. Acetic acid-induced chronic ulcer model produces gastric lesions restricted to the glandular stomach which are identical to chronic gastric ulcers of human. Reactive nitrogen species (RNS) have been found to be involved in the gastric mucosal damage [30]. The antioxidant content of *G. lanceifolia* have been reported earlier [18], which may be the postulated reason for the antiulcer activity of this plant.

Conclusion

These studies suggested that the traditional use of *G. lanceifolia* as an antiulcer and antidiabetic agent is backed by scientific data now. It may be hypothesized that continuous consumption of this plant may result in reduced occurrences of diabetes and gastric ulcer in humans. However, further studies regarding the development of a drug which contains these activities are required in the future. Moreover, studies related to the isolation of active constituents and its molecular pathway studies are also warranted.

Abbreviations

ATP: Adenosine triphosphate; WHO: World Health Organization; NSAIDs: Non-steroidal anti-inflammatory drugs; OGTT: Oral glucose tolerance test; IAEC: Institutional Animal Ethics Committee; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; HAEGL: Hydroalcoholic extract of the stem bark of *Garcinia lanceifolia*; OECD: Organization for Economic Cooperation and Development; LD: Lethal dose; i.p.: Intraperitoneal; CMC: Carboxymethylcellulose; STZ: Streptozotocin; mg: Milligram;; kg: Kilogram; h: Hours; TC: Total cholesterol; TG: Triglycerides; SGOT: Aspartate transaminase; SGPT: Alanine transaminase; ALP: Alkaline phosphatase; H&E: Hematoxylin and eosin; min: Minutes; rpm: Rotations per minute; LPO: Lipid peroxidation; GSH: Reduced glutathione; CAT: Catalase activity; UI: Ulcer index; mm: Millimeter

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Details of plant sources

Garcinia lanceifolia (Roxb.): Neighboring areas of University Campus (Voucher specimen No: DU/NSB/01.

Plant authentication

The plant herbarium was authenticated by Dr. A. A. Mao, Botanical Survey of India, Eastern Regional Centre, Shillong (Letter no.: BSI/ERC/2014/Plant identification/882. A herbarium specimen of the plant was submitted at the Pharmacognosy Research Lab of the Institute for further references.

Authors' contributions

All authors have read and approved the manuscript. NSB designed and executed the work, plant collection, extraction, and animal experiments; PSB conducted the serum analysis and extraction of animal organs; AS participated in the histopathological studies; BBK edited the manuscript and gave final permission for publication.

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Availability of data and materials

All data and material are available upon request.

Ethics approval and consent to participate

The experimental procedures relating to the animals were authorized by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals); vide approval number IAEC/DU/60 dated 24/09/2013.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]NETES Institute of Pharmaceutical Science, NEMCARE Group of Institutions, Mirza, Kamrup, Guwahati, Assam 781125, India. [2]Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam 786004, India. [3]School of Pharmacy, Graphic Era Hill University, Bell Road, Clement Town, Dehradun, Uttakhand 248002, India. [4]Gupta College of Technological Sciences, Ashram More, G.T. Road, Asansol, West Bengal 713301, India.

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