Title: Study of anti-hyperglycemic property of traditionally used medicinal plants of Kokrajhar district Assam

Abstract

The prevalence of diabetes mellitus has significantly increased in the last few decades. Indians are more susceptible to this metabolic syndrome and its complications than the white population. Managing diabetes with current commercial drugs often comes with various side effects. Novel interventions using readily available resources and techniques aim to revolutionize patient care to a greater extent. Kokrajhar district in Assam, India, is well known for its diverse endemic flora and fauna. Being remote from towns and cities, residents face difficulties accessing modern healthcare due to the non-availability of doctors and the high cost of modern medicine. Therefore, in the complex equation of healing, plants and their compounds continue to be an important source for treating illnesses. This strong belief in medicinal plants persists in this part of India, where the practice dates back millennia. Knowledge of medicinal plant use is preserved through written formats or passed down from generation to generation.

A survey was conducted in the Kokrajhar district of Assam among elderly people and local healers of the Bodo tribes. The survey utilized semi-structured interviews and a ready-made questionnaire. Approximately 20 villages were considered as one single cluster, and information was collected from each cluster. The names of the plants, parts used, modes of use, and traditional formulations were noted. The collected plant samples were then processed for taxonomic identification. Parameters such as frequency of citation, relative frequency of citation, and family importance value were analyzed. Plants with no literature record were processed for phytochemical analysis. The crude extracts of 11 plants were tested for the presence of protein, carbohydrate, phenol, flavonoids, tannins, saponins, glycosides, and alkaloids following standard protocols. The crude plant extracts were quantified for protein, carbohydrate, phenol, and flavonoid content. The antioxidant potency of the plants was assessed using five antioxidant studies: DPPH free radical scavenging activity, ABTS free radical scavenging activity, TBARS assay, FRAP, and TAC. The crude plant extract was also analyzed for heavy metals content using atomic absorption spectroscopy. To identify the probable compounds in the plant samples, GC-MS analysis was performed. The crude plant extract was studied for in vitro α-amylase and α-glucosidase activity. The best plant was further fractionated using hexane, diethyl ether, ethyl acetate, and methanol solvents. The

fractions were also studied for their enzyme inhibitory properties. The best fraction was then tested in an in vivo system.

The toxicity study of the fraction was conducted following OECD guidelines in Wistar male rats. To assess toxicity at higher doses, acute toxicity tests were performed. Rats were fed with 500 mg, 1000 mg, and 2000 mg doses of plant extract per kg bodyweight of rats. Subacute toxicity was conducted for 28 days at three different doses of 100 mg/kg bw, 200 mg/kg bw, and 500 mg/kg bw of the best fraction. Biochemical tests such as serum ALP, ALT, ALKP, total bilirubin, albumin, creatinine, total cholesterol, LDL, VLDL, HDL, and triglycerides were conducted using the Diatek kit on the AVH analyser. Haematological parameters such as total RBC, WBC, haemoglobin, platelet count, lymphocyte, haematocrit, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentrations were measured using the Sysmex XP 300 haematology analyser. Thirty healthy rats were selected for a diabetes study. Diabetes was induced with a single dose of intraperitoneal injection of streptozotocin. All biochemical tests in the serum were repeated, and the kidneys and liver were collected for histological study. Histology was performed using H&E staining. Several antioxidant marker enzyme studies, including CAT, GST, and MDA from the tissue, were evaluated using standard modified protocols.

It is observed that the study area, Kokrajhar district, possesses a rich ethnomedicinal knowledge system that serves as a primary healthcare resource for rural and impoverished communities. Despite the advancements in modern healthcare facilities, the practice of ethnomedicinal healing remains highly popular and widely accepted. The study revealed a total of 37 medicinal plants used in various formulations to prevent hyperglycemia. Apocynaceae was found to be the most popular plant family followed by Moraceae, Combretaceae, and Myrtaceae. The most cited plant was a *Hodgsonia heteroclita* followed by *Rauvolfia tetraphylla*. Among the plant parts, leaves were the most used for traditional herbal formulations. Decoction was the most common method for preparing herbal medicine. The 11-plant crude extract showed the presence of the tested phytochemicals. *Alstonia scholaris* and *Phlogacanthus thyrsiformis* showed the absence of saponins. Tannin was absent in *Alstonia scholaris, Rauvolfia tetraphylla*, and *Clerodendrum infortunatum*.

The protein content ranged from 30.45±7.68 to 401.82±11.68 µg/mg extract, with the highest content observed in *Alstonia scholaris*. The highest carbohydrate content was seen in *A. scholaris* (384.29±14.05 µg glucose/mg plant extract). The highest phenolic and flavonoid content was found in *Phlogacanthus thyrsiformis*, with 123.68±2.95 µg GA/mg extract and 45.85±1.26 QE/mg extract, respectively. For antioxidant assays, the highest TAC was seen in

P. thyrsiformis, while the lowest was seen in *Andrographis paniculata*. The highest FRAP was observed in *Oroxylum indicum* (745.15±10.03 μg FE/mg plant extract), followed by *Ficus racemosa* (432.02±6.26 μg FE/mg plant extract). *Phlogacanthus thyrsiformis* showed the most potent DPPH and ABTS scavenging activity with IC50 values of 23.34±0.33 μg/mg plant extract and 27.13±0.151 μg/mg plant extract, respectively. In the TBARS assay, *Oroxylum indicum* showed the most potent antioxidant activity with an IC_{50} of 27.28±2.11 μg/mg plant extract. The plants also showed negligible amounts of heavy metals, with levels below the WHO permissible limits.

Among the eleven plants, *Ficus racemosa* exhibited significant α-amylase and α-glucosidase inhibitory properties, surpassing the standard chemical acarbose. Regarding solvent fractions, the diethyl ether fraction of *F. racemosa* displayed the most potent antioxidant and enzyme inhibitory properties for α-amylase and α-glucosidase. This specific fraction was selected for further in vivo study. Notably, the animal model study demonstrated no signs of toxicity, even at a dosage of 2 g/kg body weight (bw) in rats. Furthermore, in terms of subacute toxicity, all three doses of the crude extract showed no significant alterations in serum liver function tests and kidney profiles compared to the control group. After induction of diabetes with STZ, blood glucose levels rose from 80-110 mg/dL to 400-500 mg/dL. The higher dose (200 mg/kg bw) of the *F. racemosa* diethyl fraction led to a significant reduction in glucose levels, with no discernible difference from the normal control rats (P=0.05). The higher dose of the fraction resulted in the restoration of biochemical and histochemical profiles, surpassing even the effects of the standard chemical glibenclamide when compared to the diabetic control group. Additionally, the group treated with the plant extract showed no significant differences compared to the normal control group across most blood parameters. Histological examination of liver and kidney cells in diabetic rat groups revealed numerous pathological alterations compared to the normal control rats. However, the group treated with the plant fraction exhibited signs of healing, including the restoration of normal liver architecture and the normalization of parietal and visceral layers of the glomerulus in the kidney.

The substantial phytochemical content and antioxidant properties of the *F. racemosa* fraction, coupled with its effective reduction of glucose levels in diabetic rats, scientifically validate the traditional healers' claim of the plant's antihyperglycemic property. Furthermore, the healing potential of the plant may be attributed to its active compound, which could serve as an antidiabetic agent, effectively lowering blood glucose levels. Additionally, the fraction may have the ability to regenerate β-cells or act as an insulin analogue, thereby improving the diabetic biochemical and histochemical profile of rats to nearly normal levels. However, further isolation and characterization of the compound are necessary to fully comprehend its exact mode of action.