The present research work aimed for detailed and systematic phytochemical and pharmacological investigation of some plants *Allium sativum* (Family: Liliaceae; Garlic) and *Nymphaea stellata* (Family: Nymphaeaceae; Blue lotus) for antidiabetic activity. The study started with detailed investigation on phytochemistry and antidiabetic activity of bulbs/peels of three varieties of garlic.

Three varieties of garlic were chosen for the present study viz, Multiple clove garlic (G1), Single clove garlic (G2) and Himalayan garlic (G3). Procured plant materials were authenticated and standardized based on ash values and volatile oil content. The extracts and fractions were prepared from bulbs and peels of all three varieties of garlic. Methanol extracts of fresh garlic cloves and peels were prepared (Garlic Bulb: ME-GB1, ME-GB2 and ME-GB3; Garlic Peel: ME-GP1, ME-GP2 and ME-GP3). Butanol fractions were obtained from methanol extracts of garlic bulb (BF-GB1, BF-GB2 and BF-GB3). Methanol extract of Himalayan garlic peel was further fractionated with acetone and methanol (AF-GP3 and MF-GP3). The physiochemical evaluation was carried out for each extract and fraction in terms of colour, consistency, percentage yield, pH and solubility.

All extracts and fractions of garlic bulb and peel revealed the presence of sulphur compounds, carbohydrates, phytosterols, saponins, flavonoids, tannins, proteins and phenolic compounds. Alkaloids were found to be present in the ME-GB3. Volatile and fixed oils were present in the extracts of multiple and single clove variety, whereas it was absent in the extracts of GB3 variety. Both acetone and methanol fractions obtained from ME-GP3 revealed the presence of phytosterols, saponins, flavonoids, tannins and phenolic compounds.

The antioxidant activity of extracts and fractions of garlic bulbs and peels of different varieties was determined using DPPH assay, TBARS assay, *in vitro* methaemoglobin model and total reducing power assay. Among all the extracts and fractions, ME-GP3 and AF-GP3 exhibited good antioxidant activity as compared to other extracts and fractions. This could be attributed to the presence of high content of phenolics and flavonoids. The possible synergistic effect between garlic and capsicum was established using *in vitro* methaemoglobin model. The combination of ME-GP3 with capsicum extract was found to be more effective in protecting haemoglobin against sodium nitrite induced oxidation.
From the results of the haemolytic assay, garlic extract/fraction at the higher concentrations caused haemolysis of RBC’s and oxidation of haemoglobin due to the presence of saponins and thiosulfinates. But in case of sodium nitrite induced oxidation of haemoglobin, garlic extract/fraction prevented the oxidation of haemoglobin at lower concentrations. From this it confirmed that at lower concentrations garlic acts as an antioxidant and at higher concentrations acts as an oxidizing agent.

Based on the results of antioxidant studies, three extracts and two fractions exhibited good antioxidant activity were chosen for acute toxicity study. Acute toxicity study was carried out as per OECD guidelines 423 and the LD50 of the tested extract/s and fraction/s was higher than 2000 mg/kg. Thus, it is categorized under category 5 of GSH as per OECD guidelines 423. Based on the acute toxicity study, three active extracts/fractions were selected for evaluation of anti diabetic study.

The *in vivo* antidiabetic study of three extract/s and fraction of garlic was carried out by STZ-Nicotinamide and neonatal STZ induced models of type 2 diabetes in rats. The body weights of the rats treated with ME-GB2, BF-GB2 and ME-GP3 maintained their initial body weights but not statistically significant at $p<0.05$ when compared to diabetic control which signifies that the tested extracts and fraction does not have any impact on the body weight gain. The extracts/fraction of garlic exhibited significant lowering of blood glucose levels in STZ-NA diabetic rats on day 7 and 15. It is the first report to show the blood glucose lowering effect of garlic peel.

Hypertriglyceridemia and hypercholesterolemia are two major problems in patients with diabetes mellitus and responsible for vascular complications of diabetes. A marked decrease in triglycerides, total cholesterol, LDL and VLDL was observed, while increase in HDL cholesterol has been observed in diabetic rats treated with extracts which show the possibility of extracts in interfering with cholesterol metabolism. Only garlic bulb methanol extract, showed significant ($p<0.05$) elevation of HDL cholesterol levels when compared with diabetic rats. Treatment with ME-GB2 and double dose of ME-GP3 showed significant ($p<0.05$) reduction in the elevated concentration of LDL cholesterol when compared with diabetic control. The hypolipidemic activity of garlic has been reported by many authors. However, the present study has explained the role of garlic peel in significantly improving lipid profile in diabetic condition. All the extracts and fraction showed good antidiabetic
activity, however garlic extract and garlic peel extract (ME-GB2 & ME-GP3) exhibited promising activity.

Based on the above study, two bioactive extracts (ME-GB2-250 mg/kg & ME-GP3-50 mg/kg) were selected for detailed evaluation of antidiabetic activity using diabetic model II by neonatal STZ induced model of type 2 diabetes in rats. Treatment with ME-GB2 and ME-GP3 did not improve the body weights of NIDDM rats. Diabetic animals treated with ME-GB2 and ME-GP3 at the dose of 250 mg/kg and 50 mg/kg respectively, for one month exhibited a significant (p<0.05) decrease in fasting blood glucose levels on day 30 as compared to NIDDM control. Our study confirmed the improved glycemic control of NIDDM rats treated with garlic peel at the dose of 50 mg/kg.

Diabetic rats treated with ME-GB2 exhibited significant (p<0.05) reduction in serum insulin and glycated hemoglobin levels when compared to diabetic control. The resultant decrease insulin levels associated with lowering of fasting blood glucose levels could probably be due to the increase in insulin sensitizing activity of extract. Treatment with ME-GB2 and ME-GP3 improved the fasting insulin resistance (FIRI) which further confirmed the insulin sensitizing effect of extract in overcoming insulin resistance.

Hypertriglyceridemia and hypercholesterolemia are significantly improved in the diabetic rats treated with garlic bulb/peel extract. ME-GP3 was found to be better in lowering cholesterol and triglyceride levels than ME-GB2. No significant differences (p<0.05) were observed in case of HDL-c levels between the diabetic control and treatment groups. Treatment with pioglitazone showed decrease in LDL-c levels, even the animals treated with garlic and garlic peel extract exhibited decrease in LDL-c, but not significant statistically at p<0.05.

The elevation of serum urea and creatinine related to renal dysfunction in diabetic hyperglycemia were improved significantly (p<0.05) on treatment with ME-GP3. Treatment with ME-GB2 significantly decreased only the serum creatinine levels. Animals treated with Garlic extract at 250 mg/kg caused statistically significant reduction in the activity of SGOT when compared with diabetic control and standard which signifies the role of extract in overcoming the hepatic damage caused by diabetes. In histopathological analysis of the pancreas of the diabetic rats treated with standard Pioglitazone, ME-GB2 and ME-GP3 were comparable to normal rats in
terms of the overcoming moderate degenerative changes caused by diabetes. In diabetic rats treated with extracts of garlic extract, the liver and kidney architecture were appeared more or less like normal control. The results obtained with ME-GB2 and ME-GP3 in insulin resistant diabetic model II was further confirmed the antidiabetic activity of these extracts.

Methanol extract of Himalyan Garlic peel (ME-GP3) exhibited significant lowering of fasting blood glucose levels and hypolipidemic activity. It also exhibited promising antioxidant activity in various in vitro assays when compared to all the other extracts and fractions. Three phytoconstituents (F1, F2 and F3) have been isolated from this bioactive extract (ME-GP3) by preparative TLC and compound F1 was obtained with highest purity followed by F3. The constituent F2 is the mixture of component F1 and an unknown component.

All the three constituents were of polyphenolics in nature and strong scavengers of DPPH radical. The purity of F1 & F3 was confirmed by HPLC and HPTLC studies. These compounds were subjected to various spectral studies like UV, LC-MS, IR and NMR for its characterization. The isolated compound F1 likely a phenyl propanoid compound that could be having the basic structure of guaiacyl glycerol-β-ferulic acid ether with less than one -CH₂- in the side chain. The isolated compounds F2 and F3 could not be elucidated with the available data.

All the three isolated antioxidant constituents were found to be present in the acetone fraction (AF-GP3) of bioactive extract (ME-GP3). The acetone fraction was found to possess promising antioxidant activity in various in vitro assays when compared with ME-GP3. Acetone fraction was subjected to in vitro antidiabetic target studies viz, α-glucosidase (AGI), Dipeptidyl peptidase IV (DPP IV) and Protein Tyrosine Phosphatase 1B (PTP1B). The acetone fraction exhibited partial inhibition on α-glucosidase and PTP1B and least inhibition of DPP IV which also confirms its partial role in exhibiting antidiabetic activity through these diabetic targets.

Raw garlic and garlic preparations have been widely recognized as agents for prevention and treatment of many disorders. Our study on bioactivity guided extraction and evaluation resulted in beneficial outcome and further adds to the existing therapeutic benefits of garlic. Thus, the present study has opened new avenues for the improvement of medicinal uses of garlic especially garlic peel for diabetes which is a waste product of agro food industry.