The present study clearly showed that 50% hydroethanolic extracts of *Barleria cristata* L. and *Rubia cordifolia* Linn. plants had significant antioxidant, hypoglycemic, hypolipidemic and antimicrobial activity. All these activities could be contributed by their phytoconstituents.

The preliminary phytochemical screening of crude extract of *Barleria cristata* L. leaf, stem and *Rubia cordifolia* Linn. root samples revealed the presence of various bioactive components. Of which alkaloids, flavonoids, saponins and tannins were the most prominent phytoconstituents.

The following quantification studies showed some differences in the level of constituents in these two plants tested. Flavonoids, saponins, phenol and protein were present in higher amount in *Barleria cristata* L. whereas alkaloids and tannin content were more in *Rubia cordifolia* Linn. root powder. Both extracts were rich sources of vitamins C, E and contain consumable level of vitamins Thiamin and Riboflavin.

Free radicals were generated as by product of biological reactions or from exogenous factors. Free radicals were highly reactive and damage the cell leading to cell inactivation and disease conditions. A potent scavenger of free radicals might serve as a possible preventive measures for the diseases. The extract of the plants exhibited different extent of antioxidant activity in a dose dependent manner.

The 50% hydroethanolic stem extract of *Barleria cristata* L. showed higher activity than the leaf of *Barleria cristata* L. and root of *Rubia cordifolia* Linn. in scavenging DPPH, SOD, H$_2$O$_2$, NO free radicals. The radical scavenging capability of both plant extracts were denoted in the order of BC$_{stem}$ > BC$_{leaf}$ > RC$_{root}$. This might be related to the higher amount of Total phenol, Tannin, Total carotenoids, Reduced
glutathione, Vitamin C and Vitamin E content which were natural antioxidants present in this plant extracts.

HPTLC analysis of Barleria cristata L. coarse leaf powder revealed the presence of 5 different saponin compounds in the extract. Further through HPLC study using brahmi tablet as a standard, Bacopa saponin structure related four saponin compounds were identified in crude and in 50% hydroethanolic leaf powder extract.

Acute toxicity study of 50% hydroethanolic extracts of both plants did not show any significant sign (or) symptoms of toxicity in normal rats proving their high safety for long term oral treatment.

Alloxan was one of the usual substances used for the induction of diabetes mellitus. It has a destructive effect on the β cells of the pancreas. Glibenclamide was an oral sulphonylurea antidiabetic preparation and widely used as standard drug in antidiabetic study.

In the hypolipidemic, hypoglycemic study of Barleria cristata L. leaf extract in alloxan induced diabetic rats, significant increase in serum fasting blood glucose and lipid components – TC, TG, VLDL – C, LDL – C with decrease in body weight and HDL – C levels were observed. On the other hand, leaf extract treatment to high lipid diet fed animals produced a dose related hypolipidemic and hypoglycemic effects.

The increased blood glucose level was brought down and gain in body weight was seen. The serum lipid components – TC, TG, VLDL – C, LDL – C levels were reduced significantly. The protective cholesterol, HDL – C (responsible for cholesterol transport) levels were increased significantly.

In the antidiabetic study of plant extracts separately and in combination manner, alloxan effectively induced diabetes in normal rats that are reflected by elevated levels of blood glucose, glycosylated Hb and reduced levels of body weight, liver glycogen,
insulin of the injected animals. Treatment with standard drug glibenclamide and 50% hydroethanolic extract of *Barleria cristata* L. stem, leaf and root of *Rubia cordifolia* Linn. in separate and combined manner reversed these conditions.

While comparing plant extracts for antidiabetic study, 50% hydroethanolic extract of *Barleria cristata* L. stem showed better activity than the leaf extract. In combined plant extract antidiabetic studies, 50% hydroethanolic extract of *Barleria cristata* L. stem and leaf resulted in better antidiabetic activity than the combined *Barleria cristata* L. stem and *Rubia cordifolia* Linn. root extracts.

Decrease in glycogen content of liver observed in diabetic control rats were due to the leakage of insulin in diabetic state. Prevention of glycogen depletion in the liver following administration of plant extract (in separate and combined) and standard drug could have been achieved by stimulation of insulin release. The highest improvement was recorded in 400 mg/kg b.w. dosage of *Barleria cristata* L. (stem and leaf) combined extract treated animals.

The plant extracts and standard drug resulted in reduction of blood glucose (due to increase in glycolysis) and increase in hexokinase activity after treatment period. In diabetic condition the activity of glucose-6-phosphatase was enhanced whereas after treatment with the liver extract glycogen content was increased and the activity of glucose-6-phosphatase reduced suggesting utilization of glucose-6-phosphatase enzyme in glycogenesis.

Oxidative stress in diabetes resulted in disruption of cellular function. An increased level of lipid peroxidation was observed in diabetic rats. Lipid peroxidation leads to liver necrosis which was detected by measuring the activities of liver function marker enzymes *viz.* Phosphatases, LDH, Transaminases which were released into the blood from damaged cells. Their increased level in diabetic control rats indicated liver damage.
Increased transaminase level as a result of decreased insulin level in diabetics were responsible for the increased gluconeogenesis and ketogenesis observed in diabetic control rats. On oral administration of plant extract in separate and combined forms to diabetic rats, their levels were brought to near normal level and comparable with standard drug glibenclamide.

This confirms the hepatoprotective effect of plant extracts. *Barleria cristata* L. stem extract (separately and in combined form with its leaf) treatment had resulted in better healing effect than other two, *Rubia cordifolia* Linn. root extract separately and *Barleria cristata* L. stem with root of *Rubia cordifolia* Linn. combined extracts.

In diabetic rats, the SOD, CAT, GPx, GST (enzymic antioxidants), vitamin E and vitamin C (non-enzymic antioxidants) levels were reduced when compared with normal rats. This result proved the important adaptive response of the cell to the increased peroxidative stress in diabetic conditions. After the standard drug and plant extract treatments significant increase in their levels were observed at different test doses. This clearly indicated their ability to restore the antioxidant status of diabetic conditions.

Diabetes is associated with hyperlipidemia. The level of lipid components - TC, TG, VLDL, LDL-C were elevated in diabetic rats and their levels were reduced significantly in extract and drug treated animals. The highest depletion was recorded in 400 mg/kg b.w. dosed rats. These effects might be due to reduced TG synthesis or lipolysis which might be the result of increased insulin level in standard drug and plant extract treated animals.

The standard drug glibenclamide and plant extract inhibited the alloxan induced renal toxicity as observed from decreased serum urea and creatinine levels in the plant extract treated animal groups. The recovery was more effective in combined extract than in separate extract administration suggesting renal protective effect of the plant extracts.
Antibacterial and antifungal activity of pure saponin fractions of both plant extracts were checked in vitro over the most often isolated clinical pathogens. The results revealed that *Barleria cristata* L. leaf extract had more growth inhibitory activity than *Rubia cordifolia* Linn. root extract. This study further confirms that microbial growth arresting activity increases significantly with increase in saponin concentrations.

Histological studies on Liver, Kidney, Pancreatic tissue sections of *Barleria cristata* L. stem extract treated diabetic rats showed restoration of damaged β cells in pancreas (which collaborate with increased serum insulin level), absence of necrosis and less vacuole formation in liver and kidney cells. Overall improved cell architecture with almost normal cell architecture was observed which proves the protective effect of the extracts. Thus from the histological studies, it is clear that 50% hydroethanolic extract of *Barleria cristata* L. stem could significantly protect from the toxicity induced by alloxan.
CONCLUSION

- Preliminary phytochemical study proved the presence of various bioactive components like Flavonoids, Phenols, Saponins, Glycosides in the plant extracts.

- Both plants are rich sources of natural antioxidants.

- Antibacterial activity of pure saponin fractions were higher with increase in saponin content of the plant extract and demonstrated for the first time the antibacterial activity in these two plant extracts.

- *Barleria cristata* L. plant extract produced optimum activity at 400 mg/kg body weight in separate and combined preparations.

- Both plant extract could be a potential hypoglycemic and hypolipidemic agents which will be of great advantage.

- In combination both plant extracts produce superior effect explaining that both plant extracts act synergistically against alloxan induced diabetic abnormalities.

- The results obtained from this study are comparable with standard drug glibenclamide. The plants had high medicinal value and thus may be recommended as cheap food supplements in treating diabetes, atherosclerotic conditions and hyperlipidemia after a thorough review, research and development in that aspect.