SUMMARY AND CONCLUSION

“Bioactive compounds” are important constituents that typically occur in small quantities in foods. They are being extensively studied to evaluate their effects on health. Black rice originates in China and it is popular in oriental countries. Though it is grown in Manipur and Keelapoongudi village of Tamil Nadu, no systematic studies are available on the nutraceutical potentials of black rice in India. Hence the present study entitled “Nutraceutical Potentials of Black Rice (*Oryza sativa* L.) and Its Hypoglycaemic Activity in Streptozotocin Induced Diabetic Rats” was conducted with the specific objectives of determining the physico-chemical properties, nutrient and nutraceutical potentials of the black rice and white rice and also to study the *in vitro* and *in vivo* hypoglycemic potentials of black rice in streptozotocin induced diabetic rats. Black rice (Kavun rice (raw)) and white rice (Ponni raw rice) were collected from the village of Keelapoongudi, Karaikudi district, Tamil Nadu, India.

The raw samples of black rice and white rice were powdered separately in a mixer grinder for two minutes and stored in the air tight plastic pouches. One hundred gram of white rice and black rice were mixed with deionized water in the ratio of 1:4 and 1:2 (W/V), cooked for 45 and 20 min at 15 psi in a pressure cooker. One hundred gram of white rice and black rice were mixed with deionized water in the ratio of 1:7 and 1:5 (W/V), cooked for 55 and 38 minutes by conventional method. The cooked samples were dried in a hot air oven at 30°C and powdered finely and stored in the air tight plastic pouches.

In phase I, the physico-chemical characteristics of raw rice grains of black rice and white rice were studied using standard techniques. Characteristics studied include thousand kernel weight, hundred kernel weight, length/breadth ratio, bulk density, porosity, volume expansion, optimal cooking time, amylose, elongation ratio and index, equilibrium moisture content on soaking and density in rice samples.
The powders of raw and cooked samples of black and white rice were analysed for themacronutrient and micronutrients like total moisture, carbohydrates, crude fiber, protein, fat, ash, amino acid composition, fatty acid composition, thiamine, riboflavin, niacin, iron, sodium, calcium, phosphorous, magnesium, selenium, zinc and potassium using standard protocols in phase II.

In Phase III, it was thought of interest to develop traditional recipes using black rice. A group of semi-trained panelists were used for sensory evaluation of recipe using seven point hedonic rating scale. The attributes studied in sensory evaluation are appearance, colour, flavor, taste, texture and overall acceptability. Acceptability trial was carried out for black rice and white rice in traditional recipes like breakfast, lunch and snack items namely idli, dosa, adai, aapam, paniyaram (karam and sweet), idiyappam, roti, sweet and karam kolukattai, puttu, pongal, sweet pongal, laddu, kali, boiled rice (pressure cooked, conventionally cooked), vegetable biriyani, tamarind rice, mint rice, coconut rice, lemon rice, tomato rice, khanji, vadagam, murukku, sweet balls and kolukattai (Karam, Sweet and Milk).

In phase IV, nutraceutical potential of black rice was evaluated by the qualitative phytochemical screening of black rice and white rice in raw and cooked forms in different solvents. Quantitative phytoconstituents (total phenols, flavonoids and tannins), enzymatic antioxidants (superoxide dismutase, Glutathione peroxidase, Glutathione- S- transferase, peroxidase, catalase, polyphenol oxidase, ascorbate oxidase) and non-enzymatic antioxidants (Reduced glutathione, Vit E) were evaluated for the raw and cooked samples of black rice and white rice. In vitro radical scavenging assays (DPPH radical scavenging activity, reducing power assay, FRAP, ABTS* radical scavenging activity, hydrogen peroxide scavenging activity and hydroxyl radical scavenging assay) were carried out for the ethanolic and aqueous extract of black rice and white rice in raw and cooked forms. Further, total anthocyanin content, total and individual polyphenolic content were measured for the raw and cooked samples of black rice and white rice.

In phase V, In vitro inhibitory potential of black rice and white rice in raw and cooked forms against α-glucosidase and α –amylase enzyme inhibiting activity were
evaluated to find their role in controlling blood glucose level. *In vitro* glycaemic index of raw and cooked samples of black rice and white rice were measured. Then, *In vivo* hypoglycaemic activity of black rice in streptozotocin induced diabetic rats were evaluated. Diabetes mellitus was induced in overnight-fast of 12 hours in male albino wistar rats by single intraperitoneal injection of streptozotocin (STZ 45mg/kg body weight). The experimental rats were divided into 5 groups of 6 animals in each group. A total of 30 rats (18 diabetic rats and 12 normal rats) were used. Streptozotocin was given to induce diabetes in rats. The rats were divided into five groups after the induction of diabetes. The formulated black rice pellets were given to experimental groups (group III and V) for a period of 90 days. After 90 days of hypo-glycaemic study, all rats from each group were sacrificed, the blood was withdrawn from each rat by retro orbital plexus method and the biochemical estimations were carried out in blood, liver and pancreas samples of control and experimental rats in each group and alterations occurring in this rats were assessed by the following biochemical parameters: derived parameter, hematological parameter, glycemic profile, renal markers, lipid profile, hepatic marker enzymes, protein levels, lipid peroxidation, enzymatic antioxidants, non-enzymatic antioxidants, membrane bound enzymes, glycoproteins, carbohydrate metabolizing enzymes, TCA cycle markers and histopathology of liver, kidney and pancreas were assessed.

The data was consolidated and subjected to statistical analysis with appropriate tools using the SPSS version 16.0 version. Descriptive statistics (mean and standard deviation), Two way ANOVA were used to interpret the mean acceptability of recipes in product development phase. Results are expressed as the Mean ± SD. Statistical significance was evaluated by One way analysis of variance (ANOVA) using SPSS version16.0 and the individual comparisons were obtained by the Duncan Multiple Range Test (DMRT) was applied and interpret the biochemical parameters in control and experimental groups to determine the significant difference between the groups.
The salient findings of the study are summarized below:

- The 1000 kernel weight was found to be 18.28±0.04g and 10.66±0.09g for black and white rice. The 100 kernel weight of black and white rice was found to be at 1.67±0.05g and 1.38±0.12g respectively. Length and width ratio for black rice and white rice samples were 2.95±0.02 and 2.67±0.03 which denoted the shape and size of the grains. Both samples belonged to quasi slender varieties. The highest length / breadth ratio was found in black rice. Bulk density of samples in the present study was found to be 0.77±0.00 and 0.83±0.00 g/ml for black and white rice respectively. Density and porosity of black rice and white rice were 1.32±0.04 g/ml, 27.57±0.47 per cent and 1.24±0.09g/ml, 30.07±0.48per cent respectively. The results of gel consistency for 30 and 60 minutes in both samples were similar. The optimal cooking time for black rice and white rice were found to be at 68 mins and 28 mins. The elongation ratio and elongation index of blackrice and white rice were 2.16±0.01, 1.13±0.05 and 2.03±0.0, 1.27±0.06 respectively. The amylose content of black and white rice were found to be 6.76±0.83 per cent and 19.49±0.56 per cent.

- The moisture content of black rice and white rice raw ranged between 10.54 to 11.02 per cent. The percentage of moisture content had significantly increased due to pressure cooking and conventional cooking. The total carbohydrate content of all samples was higher than 75 per cent. The protein and fat contents in black rice and white rice samples ranged from 9.56g to 7.43g; 2.65g to 1.89g and 7.71g to 3.65g; 0.73g to 0.32g, respectively. The results of fibre showed that the high amount of fibre content was recorded in the pressure cooked sample of black rice (6.49g) among all other samples. The ash content was found to be higher in raw sample of black rice as 1.75 among all the samples and 0.37 in white rice raw among the white rice samples. Calcium content in raw sample of black rice was found to be high as 19.56mg. Iron content of black rice raw found to be higher as 3.17mg/g and was subjected to loss of iron in pressure cooked and conventionally cooked samples of black rice as 2.14 and
Sodium, potassium and zinc content of black rice ranged from 23mg to 10.97mg, 404 to 173mg and 19.56 to 11.87mg respectively. Magnesium and phosphorous were found to be higher in raw sample of black rice as 155mg to 412 mg and white rice raw sample exhibited 71mg to 263mg of magnesium and phosphorous levels which found to be higher in white rice samples. The ratio of EAA to non-EAA of rice samples ranged from 0.59–1.24 and their ratios of EAA to total amino acids was in the range of 0.37 – 0.53. The high amount of TAA (9.35mg/g) present in pressure cooked sample of black rice and found to be very low in white rice raw sample (5.46mg/g). Black rice samples contained high amount of monounsaturated and polyunsaturated compared to saturated fatty acids, when compared to other samples and black rice samples contained more unidentified fatty acids.

- Results showed that idli, dosa, pongal, khanji, idiyappam and kali prepared using white rice showed one per cent significant difference (p<0.01) between the recipes. Idiyappam prepared using white rice, appearance, flavor and colour scored the best attributes among the recipes. Results revealed that all the lunch recipes (boiled rice (pressure cooked, conventionally cooked), tamarind rice, tomato rice, mint rice, lemon rice, coconut rice, vegetable biriyan) prepared using white rice scored higher (p<0.01) significant level. The snack items prepared both from black rice and white rice were equally acceptable.

- The results of phytochemical screening exhibited the presence of maximum secondary metabolites (alkaloids, tannin and phenolic compounds, flavonoids, amino acids and proteins and carbohydrates) in ethanol and aqueous extracts of raw and cooked samples of black rice and white rice. The ethanolic extract of black rice raw sample showed a highest yield of about 3.32 g/ 100g, whereas the aqueous extract of conventionally cooked sample of white rice had the lowest yield of 0.45 g/ 100g. The results revealed that black rice raw sample exhibited higher values of all the enzymatic and non – enzymatic antioxidants and the levels decreased when
the black rice was subjected to cooking. Low amount of enzymatic and non enzymatic antioxidants were recorded in raw and cooked samples of white rice.

- The ethanolic extract of black rice, raw exhibited 569.67 mg/100g of phenols followed by the ethanolic extract of pressure cooked sample of black rice and ethanolic extract of conventionally cooked sample of black rice 531.78mg/100g and 520.34 mg/g respectively. The ethanolic extract of black rice raw established higher content of flavanoid (144.67mg/100g) than the pressure cooked samples of black rice (131.23mg/ 100g). Losses were found in the aqueous extract than the ethanolic extract of all the rice samples and further losses were seen in the cooked rice varieties.

- All the rice extracts exhibited reducing power activity, the dose dependent trend has been maintained in all the assays but the Black rice extracts showed higher activity than the white rice. Like reducing power activity similar dose dependent trend was adopted in the hydroxyl radical scavenging activity, superoxide radical scavenging activity, hydrogen peroxide radical scavenging activity and DPPH radical scavenging activity. All the extracts resulted in higher inhibiting activity but the Black rice extracts showed higher activity in lower concentration than the White rice. The highest ABTS cation radical scavenging activity was registered in the Black rice sample extracts than the white rice sample extracts. White rice exhibited low radical scavenging activity and the total antioxidant activity. Consequently we found that the pressure cooked samples of Black rice and White rice showed less activity than the raw samples followed by conventional cooked samples. In the raw and cooked samples of Black rice, Red rice and White rice, the methanolic extracts showed higher activity than the water extract. Total anthocyanin content of black rice raw exhibiter higher value 317mg/100g followed by the black rice pressure cooked sample and conventionally cooked sample (269 and 148 mg/100g). Black rice raw contains higher value of total polyphenolic content of 76.54 followed by the pressure cooked and conventionally cooked sample of black rice
Nutraceutical Potentials of Black Rice (Oryza sativa L.) and Its Hypoglycaemic Activity in Streptozotocin Induced Diabetic Rats

(10.61 and 6.28 GAE mg/100g). White rice samples exhibited the total polyphenolic content from the range of 2.13 to 1.51 GAE mg/100g in raw and cooked samples. The individual polyphenolic content of black rice Protocatechuic acid, 4-OH Benzoic acid, Vanillic acid, Syringic acid, Caffeic acid, P-Coumaric acid and Ferulic acid were found to be high in black rice raw samples when compared to cooked samples of black rice.

• *In vitro* hypoglycaemic potential of rice varieties were expressed in terms of IC50 value of α-amylase and α-glucosidase inhibiting activity of black rice raw aqueous extract were 109 and 130 µg/ml respectively. *In vitro* glycaemic index of black rice raw and cooked samples falls under the category of medium GI whereas white rice raw and cooked samples comes under the category of high GI. Gradual increase in GI was observed in cooked forms when compared to raw sample in both rice.

• The hypoglycaemic activity of black rice revealed that the streptozotocin induced diabetic rats exhibited a significant (p<0.05) decrease in the final body weight when compared with other groups. Treatment of black rice significantly (p<0.05) altered these variations and restored the levels to near normal. In terms of glycaemic values, blood glucose and glycosylated haemoglobin levels were increased in diabetic rats and came to near normal values when the group treated with black rice. Urea, uric acid and creatinine levels were significantly increased (p<0.05) in diabetic group and on treatment with black rice for 90 days significantly reduced these values to near normal. Similar effect was observed in glicizide treated group. Black rice treated rats showed similar effect like that of control rats. The level of protein in serum was decreased in streptozotocin induced diabetic group when compared to normal control group. Results revealed that lowered levels of serum albumin and globulin levels were restored significantly in black rice and standard drug treated diabetic rats.

• A significant elevation in serum lipids was observed in diabetic rats when compared with control rats. On administration of black rice to diabetic rats for 90 days significantly (p<0.05) reversed these values to near normal.
The comparison was done with standard drug glipizide treated group. The black rice alone treated group did not show any significant changes when compared to diabetic control rats. The activities of serum marker enzymes had significantly increased (p<0.05) in diabetic rats. Black rice treated groups significantly reversed these values to normal. Similar effect was observed in the group rats which were treated with the standard drug glicizide. The black rice alone treated rats showed similar effect like that of control rats. In liver and kidney tissues of diabetic rats, lipid peroxidation (LPO) levels were elevated significantly as compared to that of control rats. After administration of Black rice for 90 days, the elevated values restores back to near normal level significantly (p<0.05). Black rice alone treated rats did not show any significant difference when compared to normal rats.

- Black rice administered group showed significant elevation (p<0.05) of all the enzymatic antioxidants and reached near normal values. There was no significant difference in black rice alone treated rats and their values were similar to control rats. A significant (p<0.05) reduction in the non-enzymatic antioxidants like glutathione (GSH), vitamin C and E were observed in diabetic rats when compared with control rats. On oral administration of black rice for 90 days showed significant elevation in all the non-enzymatic antioxidants values and reached near normal values. There was no significant difference in black rice alone treated rats and their values were similar to control rats.

- Na+K+ ATPase and Mg2+ ATPase activities had significantly decreased (p<0.05) in streptozotocin induced diabetic rats when compared to the control rats. Whereas the enzyme activities were found to be significantly increased in black rice treated diabetic rats similar to that of glicizide treated rats when compared to the diabetic rats. Ca2+ ATPase activity was found to be significantly increased (p<0.05) in diabetic group when compared with the control group whereas the enzyme activity of Ca2+ ATPase was found to be significantly increased in black rice treated diabetic group when compared with diabetic rats. Hexokinase and hexosamine were found to
besignificantly increased (p<0.05) in diabetic animals when compared to the control animals in both liver and kidney. The glycoprotein levels got restored to normal level in animals treated with black rice when compared when diabetic animals. In the glicizide treated animals also the levels reverted back to normal whereas in the group treated with black rice alone there were no significant changes.

- The activity of phosphoglucoisomerase was significantly decreased, whereas the activity of aldolase was significantly increased in diabetic rats, when compared with control rats (p<0.05). Administration of black rice for 90 days significantly reversed these values to normal. The activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase were significantly increased in diabetic rats, when compared with control rats (p<0.05), the administration of black rice for 90 days significantly reversed these values to near normal. The activities of malate dehydrogenase and isocitrate dehydrogenase were significantly decreased in diabetic rats, when compared with control rats (p<0.05). Administration of black rice treated groups significantly reversed these values to normal.

From the salient findings summarised, black rice was found to possess high nutritional value and nutraceutical potentials when compared to white rice. Black rice exhibited promising results in vitro and in vivo hypoglycaemic studies. Further studies on human volunteers would throw more light on hypoglycaemic potentials.

**Recommendations for further research:**

- Studies on bioavailability of anthocyanins from black rice.
- In depth studies on nutraceutical potentials of black rice.
- Studies on evaluating glycaemic index of black rice in human volunteers.
- Conduct human clinical trials on efficacy of black rice in Type 2 diabetics.
- Nutrigenomic response studies using black rice on genetic polymorphism in diabetes.
- Gene – nutrient interactions in diabetes in animal models.
- Nutrition education to masses on usage of black rice in traditional recipes.