5. SUMMARY AND CONCLUSION

The present study entitled “Effective Utilization of Processing Techniques on Nutritional and Pharmacological Activities of Pumpkin Seeds” is an attempt to assess the differences in physicochemical properties on processing techniques and evaluate the anti depressive and hypocholesterolemic effect of processed pumpkin seeds on animals and human models. Pumpkin seeds are one of the under-utilized agricultural products with high medicinal value. Hence the study was taken up with the following objectives:

- To study the effect of processing techniques on proximate composition, mineral composition, amino acid composition, fatty acid profile and physical properties of pumpkin seeds powder.
- To evaluate the antidepressant effect of processed pumpkin seeds aqueous extracts on selected animal model (by using behavioral animal models).
- To investigate the hypocholesterolemic effect of processed pumpkin seeds powder extract on selected animal model.
- Optimization of germinated pumpkin seeds powder incorporated bread using Response Surface Methodology.
- To assess the antidepressant activity of optimized germinated pumpkin seed bread on selected human subjects.
- To investigate the hypocholesterolemic activity of optimized germinated pumpkin seeds bread on selected human subjects.

The methodology is as follows:

Phase I

- Morphological characteristics of pumpkin seeds were detected.

- The differences in physical and chemical properties like proximate, mineral, amino acids compositions, fatty acids profile and antinutrients on processing techniques such as raw, autoclave, boiling, germination and roasting were analyzed.

Phase II

- Anti depressive activities of processed pumpkin seeds extract in rats were investigated using force swimming test and tail suspension test.
- Hypocholesterolemic activities of processed pumpkin seeds in rats were investigated using force swimming test and tail suspension test.

**Phase III**

- Optimization technique was used to find optimal values of wheat flour ($X_1$), germinated pumpkin seeds ($X_2$) and butter ($X_3$) on the response variables such as weight loss ($Y_1$), porosity ($Y_2$), specific volume ($Y_3$), protein ($Y_4$) and tryptophan ($Y_5$) for the development of germinated pumpkin seeds bread.
- Twenty different formulations of germinated pumpkin seeds bread with varying wheat flour, germinated pumpkin seeds and butter were developed and their physical and nutrient composition was assessed.
- All the germinated pumpkin seeds bread was evaluated for their acceptability by a panel of 10 judges using ballot sheet for bread samples.

**Phase IV**

- Forty moderate depressive adults under the age group of 20-50 years were selected from 360 samples using Beck Depression Inventory (BDI) scale and their background information, health and dietary pattern was collected using interview schedule. Anthropometric measurement was assessed using standard procedure. Effect of supplementation (30 days) of germinated pumpkin seeds bread on depressive subjects was evaluated by assessing the initial and final BDI among the selected subjects.
- Sixty Hypercholesteroleemics from both sex under the age group of 20-50 years were selected for evaluate the effect of supplementation of germinated pumpkin seeds bread on hypercholesteroleemics. Initially the background information, health and dietary pattern were collected using interview schedule. Anthropometric measurement was assessed using standard procedure. The effect of supplementation (30 days) was studied by assessing the initial and final lipid profile among the selected subjects.
The collected data was consolidated, statistically analyzed and the results drawn are summarized here:

Phase I

- The morphological characteristics of the whole seed and seed kernel of the pumpkin seeds revealed that seed length, width, thickness sphericity and thousand grains mass were 0.61±0.01, 0.32±0.04, 0.08±0.01, 2.84±0.01 mm /seed and 299±0.40g respectively in whole pumpkin seeds and 0.51±0.03, 0.27±0.00, 0.07±0.00, 1.96±0.23 mm /seed and 119±0.01 g respectively in pumpkin seeds kernel of dried samples.

- Physical properties of processed seeds powder showed that the bulk density (1.74±.025), water absorption capacity (98.60±.173), retrogradation capacity (1.100±.10) and forming capacity (17.43±0.41) were higher and in germinated pumpkin seeds powder. Oil absorption capacity and form stability was higher in roasting (89.77±0.680%).

- Proximate composition of processed pumpkin seeds powder revealed that compared to other techniques, germination increased protein (31.7± .030), fat (52.9±1.00) and energy (631.3±0.98) while raw seeds (3.5±0.05) contained high amount of fibre.

- Processing significantly (p<0.05) decreased the calcium, magnesium and manganese content. Among the processing techniques, germinated pumpkin seed contained high level of phosphorus (3.7±0.06%) and iron (485.1±5.10ppm) and copper 944.4±1.80ppm).

- Amino acid composition of processed pumpkin seed powder stated that Duncan’s multiple range test results showed significant difference (p<0.05) between all the processed samples for the amino acid content. Methionine, thronine, tryptophan and serine were higher in germination technique than other processing.

- Fatty acid profile of processed pumpkin seeds revealed that among different processing techniques, total unsaturated fatty acid content in germinated pumpkin seeds extract was high (99.16%) followed by boiled (98.47%) and roasted 85.62%. The lowest total unsaturated fatty acid content was observed in raw seeds.
extract. In autoclaved pumpkin seeds, all the compounds were not identified but 100% of identified compound was unsaturated fatty acids. Linoleic and oleic acids were the major fatty acids presented into the seeds.

- Results of phytochemical screening revealed that the phytoconstituent available in pumpkin seeds are flavanoids, alkaloids, tannin, phenols and steroids/terpenoids and proteins. Steroids/terpenoids were only identified in aqueous extract of germinated and raw sample.

- The results of tannin content revealed that higher reduction of tannin was observed in the autoclaved (164.0±1.00) pumpkin seeds extract followed by roasting (171.00±6.55) when compared to raw sample.

- Result about total phenol revealed that processing of pumpkin seeds showed reduced total phenols in roasting (34.0±1.00) followed by boiling (35.66±0.577), autoclave (36.00±1.73) when compared to raw sample. The highest total phenol content next to raw pumpkin seeds was observed in germinated pumpkin seeds.

- Yielding recovery of processed pumpkin seeds powder showed that autoclave pumpkin seeds had the high yield recovery than other processing techniques.

- Storage stability of processed pumpkin seed powder in Duncan’s multiple range tests showed that moisture content of all the processed pumpkin seeds increased significantly at 5% level during the storage period of 150 days.

**Phase II**

- Result on effect of processed pumpkin seeds extract in Force Swimming Test in depressed animal model revealed that mean duration of immobility was significantly reduced by Imipramine (group III) as compared to the depressed control (p<0.01). Group-I (normal) shows significant (p<0.05) increase in the immobility time after 30 days of supplementation. Group III (Imipramine), Groups-IV (raw), Group-V (autoclaved), Group VI (boiled), Group-VII (germinated) and Group-VIII (roasted) animals showed highly significant (p<0.01) decrease in the duration of immobility compared with the initial analysis. Among the processed samples germinated pumpkin seeds supplementation shows highly effective in reducing the depression.
Results on the effect of processed pumpkin seeds extract on Tail suspension Test in depressed animal model reported that there was no significant difference in Group-I & Group II (Normal & Depressed control) animals compared to initial immobility time. Group-III (Imipramine), Group IV (Raw), Group-VI (Boiled), Group VII (Germinated) and Group-VIII (Roasted) showed one percent significant and group V showed 5% significant reduction of immobility time in tail suspension test on supplementation.

Hypo cholesterolemic activity of processed pumpkin seeds extract on animal model showed that, more reduction in total cholesterol and LDL-C was observed in group-VII rats supplemented with germinated pumpkin seeds powder extract. Regarding triglycerides level and VLDL-C more reduction was observed in group IV treated with raw pumpkin seeds powder extract followed by group- V treated with autoclave pumpkin seeds powder extract. Good increase in HDL-C was observed in germinated pumpkin seeds powder extract supplemented group followed by both group VIII and group IV. Duncan’s multiple range test results showed significant difference (p<0.05) between all the group of animals for total cholesterol, triglyceride, LDL-C, HDL-C and VLDL-C.

Phase III

The optimum condition for wheat flour (X$_1$) 70g, germinated pumpkin seed powder (X$_2$) 35g, and butter (X$_3$) 5g respectively. Corresponding to these values of process variables, the value of weight loss (Y$_1$) 9.80%, porosity (Y$_2$) 46.15, specific volume (Y$_3$) 2.54 cm$^3$/g, protein (Y$_4$) 19.24gm and tryptophan (Y$_5$) 1032.91mg.

Organoleptic evaluation of germinated pumpkin seeds bread shows that variation IV had (70g wheat flour, 35g germinated pumpkin seeds powder and 5g of butter) high score in all attributes except crust colour.

The nutrient content of the standard bread was energy 341k.cal, protein 12.1g, fat5.75g carbohydrate 69.4g and iron 4.9 (mg), calcium and phosphorus 48 (mg) and 355 (g), magnesium 132 mg and zinc 2.2 mg. The optimized germinated
pumpkin seeds bread contained energy 496.10k.cal, protein 19.84g, fat 23.75 g carbohydrate 51.06g and iron 5.12 (mg), calcium and phosphorus 44.1 (mg) and 1543.5 (g), magnesium 582.4 mg and zinc 2.12 mg.

- Phytosterol analysis revealed that three compounds namely Campesterol, Stigmasterol and Sitosterol were analyzed in both standard and optimized germinated pumpkin seeds bread. The results showed that high amount of campesterol, stigmasterol and sitosterol were present in optimized germinated pumpkin seeds bread than standard bread. The amount of total phenol was 4.02 mg/gm of germinated pumpkin seed bread and 2.77mg/gm of standard bread.

- The analysis of shelf life of the bread revealed that 3 days of storage didn’t show any specific change in the sensory parameters of bread but Duncan’s multiple range test results showed the significant difference ($p<0.05$) on sensory parameters except taste and aroma in optimized germinated pumpkin seeds bread on storage.

Phase IV

- Three hundred and sixty subjects were screened for selecting moderately depressed subjects. The prevalence of depression among the participants based on BDI scale was 60% of them had minimal depression, 25% of them had mild depression, 11% of them had moderate depression and only 4% of them had severe depressed range.

- Among the 40 moderate depression subjects, 40% of them were male and 60% of them were female. The BDI was high in female. More than half of the (62.5 %) subjects were in the age group of 40- 50 years. Twenty five percent of them were 30-40 years and remaining 12.5% were 20-30 years. High score of BDI (26.6±2.68) was found in age between 20-30 years. Results on type family of the selected subjects reported that 30% of them were from nuclear family and 10% of them were from joint family. But 60% of the selected subjects were from institution. The present study revealed that institutional subjects had high BDI than other groups.
Among the selected subjects, 55% of them were married, 10% of them were widow/widower and 12.5 percent of them were divorced/separated. BDI showed that divorced/separated (27.40±2.07) had higher score than other subjects. The result of education levels in present study revealed that 47.5% of subjects had up to high school and a total of 42.5% of them had been graduates or post graduates. Twenty percent of families had low monthly income level of Rs.4000, Rs.4000 to 8000 was noticed among 56% of the families and only 10% had high income level i.e. Rs.10,000 and more. The types of activity done by subjects revealed that 30% male and 27% female were moderate workers and 5% male and 7.5% female were heavy workers.

Frequency of consumption of different food items by the subjects found that food items like cereals, fats and oils, spices and condiments were used daily by all the subjects. Forty percent of the subjects used pulses occasionally in their diet. Green leafy vegetables were consumed by 75% of subjects occasionally. About 38% of subjects consumed fruits once in a week. Sixty three percent of the subjects used egg, 83% used chicken and 44% used nuts and oil seeds occasionally. Forty seven percent subjects incorporated beef in their diet once in a week and 85% of them never used mutton in their diet.

Food intake of selected depressed male subjects revealed that there existed highly significant differences (p<0.01) between mean intake and recommended dietary allowances (RDA) except fats and oils, cereals and millets. In the case of fats and oils there was not any difference between daily intake and RDA. Food intake of selected depressed female subjects showed that there was a highly significant difference (p<0.01) between mean intake and recommended dietary allowances (RDA) except fats and oils and cereals and millets. In the case of fats and oils RDA and actual intake was equal.

Nutrient intake of depressed male subjects showed that there existed highly significant differences (p<0.01) between mean intake and recommended dietary allowances except for protein and thiamine. Nutrient intake of depressed female subjects showed that there existed highly significant differences (p<0.01)
between mean intake and recommended dietary allowances for energy, fat, calcium, niacin and ascorbic acid. The difference was significant at 5% level between daily intake and RDA in the case of protein, iron, riboflavin and thiamine.

- Life style pattern of depressed subjects showed that about 12.5% the male was alcoholics, 15% male had given up the habit of smoking. From the total number of subjects only 50% of the subjects had the habit of exercise. Only 5% of male and 10% of female was participated the stress management methods like yoga.

- Health status of depressed subjects revealed that 7.5% male and female had heart problems. Among the selected subjects 17.5% male and 27.5% female had hypertension, 10% of female suffering from gastrointestinal diseases, 12.5% male and 27% female had cholesterol treatment, 10% male and 15% female suffer from thyroid problems, 12.5% male and 22.5% female had heart burn while 10% male and 32.5% female had diabetes mellitus.

- Anthropometric measurements of the depressive subjects indicated that, mean height and weight of the male was lower when compared to NCHS standard value. Compared with standard all age group of male subjects’ height was significant at 1% level. The mean height and weight of the female was lower when compared to NCHS standard value. BMI of the selected depressed subjects found that 23% of male and 21% of the female had normal BMI. Twelve percent of male and 18% of female had overweight and 5% of male while female were obese grade I category.

- Effect of supplementation on depression revealed that supplementation of optimized germinated pumpkin seeds reduced the symptoms of depression at 1% level in both male and female subjects but in was placebo group it reduced at 5% level on female and no significant difference was observed in male.

- Results of the demographic characteristics of selected hypercholesterolemic subjects revealed that 30% of them were male and 70% of them were female. Among the selected subjects 45% of them were in the age group of 41-50 years.
and 35% were in the age group of 31-40 years and 25% of them were in the age
group between 20-30 years. Nuclear family system (75%) was mostly observed
than joint family (25%) system. Seventy three percent of the subjects were
married and 15% of them were widow/widower. Activity of the
hypercholesterolemic subjects found that 67% of them were sedentary workers,
28% of them were moderate workers and 5% of them were heavy workers. The
educational levels of 10% subjects were elementary level, 73% had studied up to
high school and 17% of them were graduates or post graduates. Nine percent of
them had monthly income of Rs.4000, Rs.4000 to 8000 was noticed in 13% of
the families of the subjects, 48% of them had the income in the range of Rs.8000
to 10000, and 30% were in the high income level i.e. Rs.10000 and more.

- Results on frequency of use of different food items by the selected
hypercholesterolemic subjects found that fifty three per cent of them used pulses
occasionally in their diet. Green leafy vegetables were consumed by 80% of
subjects occasionally. Fruits were consumed by 38% of subjects once in a week.
Seventy five percent of the subjects used egg, 35% used fish, 83% used chicken
and 60% used nuts and oil seeds occasionally. Fifty four percent subjects
included beef in their diet once in a week.

- Food intake of selected hypercholesterolemic male subjects showed that there
have highly significant differences (p<0.01) between mean intake and
Recommended Dietary Allowances (RDA) for green leafy vegetables and roots
and tubers. Intake of other vegetables and meet/fish/poultry showed significant
difference at 5% level. Intake of cereals and millets, pulses, fruits, milk and milk
products, fats and oils and sugar and jaggery showed there haven’t any
significantly difference between daily intake and RDA. Food intake of selected
hypercholesterolemic female subjects showed that there was highly significant
differences (p<0.01) between mean intake and Recommended Dietary
Allowances (RDA) for cereals & millets, pulses, green leafy vegetables and roots
and tubers and fruits. Intake of other vegetables and Sugar and Jaggery showed
significance at 5% level. Intake of milk and milk products, meet/fish/poultry, fats
and oils showed there was no significant difference between daily intake and RDA.

- Mean nutrient intake of male hypercholesterolemic subjects showed that there existed highly significant differences (p<0.01) between mean intake and recommended dietary allowances for calcium. Intake of iron, riboflavin and niacin the difference was noticed as significant at 5% level. Mean nutrient intake of female hypercholesterolemic subjects showed that there existed highly significant differences (p<0.01) between mean intake and recommended dietary allowances for energy and riboflavin. Intake of fat and iron was found to be less than RDA and it was significant at 5% level.

- Life style habits of the selected subjects found that before clinically diagnosed as hypercholesterolemic subjects, 67% of male were alcoholics and 72% were smokers. But 11% of the male are still alcoholics while the remaining had given up the habit of alcohol consumption after diagnosis. Seventeen percent of them continued smoking even after the diagnosis of hypercholesterolemia. Of the total number of subjects only 22% male and 63% of female do not have the habit of exercise before diagnosis of the disease, but after diagnosis of the disease most of them were engaged in exercise. Only 12% of male and 20% of female were using the stress management methods like yoga only after diagnosis of the hypercholesterolemia.

- Health status of the subjects revealed that 12% male and 20% female had heart problems. Among the subjects 15% male and 35% female had hypertension, 15% suffer from nerves problems and gastrointestinal diseases, 5% male and 7% female suffer from liver and kidney problems, 10% male and 7% female suffer from respiratory problems, 3% male and 7% female had skin disease and 12% male and 28% of female had heart burn.

- Anthropometric measurement of the hypercholesterolemic clearly indicated that, mean height and weight of the male were lower when compared to NCHS standard value. BMI of the selected hypercholesterolemic subjects revealed that
58% of them were normal, 22% of them were overweight, and 13% of them were obese grade one.

- Effect of supplementation of germinated pumpkin seeds bread on hypercholesterolemics found that after 30 days, there was decreased plasma lipid profile as a result of supplementation with germinated pumpkin seeds bread compared to placebo. Lipid profiles like total cholesterol, triglyceride, LDL-C, VLDL-C was significantly (p<0.01) decreased and HDL-C was significantly increased by optimized germinated pumpkin seeds bread supplementation compared to initial analysis. In the placebo group there haven’t any significant reductions for lipid profile.

**Conclusion**

Pumpkin seeds are nutritional powerhouse. Processing of the seeds improves the nutritional quality. Germination technique is used to activate the chemical compound and enhance the palatability. Germination increased the protein, L-tryptophan and unsaturated fatty acids present in the seeds when compared to other processing techniques. Germination process has more antidepression and hypocholesterolemic effect in animal models than other processing techniques. Pumpkin seeds contain L-tryptophan, a natural alternative to traditional antidepressants, works by increasing the levels of serotonin in the brain, which in turn reduces the symptoms of depression in human. Hundred grams of optimized germinated pumpkin seed bread which contains 1 gm of tryptophan supplemented daily for the period of one month, reduced the symptoms of depression than placebo sample. The most dynamic component in pumpkin seeds is phytosterol which lower hypercholesterolemia. Two hundred gram (per day) of optimized germinated pumpkin seed bread (provide 804 mg phytosterol daily) was reduced the hypercholesterolemia. Hence the study concludes that food trends may come and go but the excellent natural nutrient source of pumpkin seeds are ever powerful weapon for fighting diseases in adults and enhances the brain function of children.
**Recommendations for Further Studies**

1. Morphological changes and nutritional quality of the seeds in different processing techniques can be assessed.
2. Phytochemical constituents of the pumpkin seeds can be assessed in different processing techniques.
3. The underutilized seeds should be explored for their extensive food and pharmacological use.
4. Impact of pumpkin seeds supplementation on other disease such as prostrate and bladder problems, osteoporosis and arthritis can be conducted.

**Limitations of the Study**

1. The behavior tests such as Force Swimming Test and Tail Suspension Test was used in the study to assess the depression in animal model, therefore the result may not be generalized to other biochemical parameters like activity of reduced glutathione (GSH), superoxide dismutase and catalase, lipid peroxidation in brain and serum of rats that measure the antidepressant activity.
2. Beck depression inventory scale was used in this study to assess the level of depression; therefore the result may not be generalized to serum tryptophan which is converted to serotonin, an important neurotransmitter involved in depression.