Summary and Conclusions
Garden cress (*Lepidium sativum* L.) is an annual herb, belonging to family Cruciferae (Brassicaceae). It is widely cultivated in temperate climates throughout the world for various culinary and medicinal uses. In India, it is extensively grown in the central and northern states for seeds which are used in a variety of medicinal preparations. Since ancient times, the seeds have been used in traditional medicine. The GC seeds are bitter, thermogenic, depurative, rubefacient, galactogogue, tonic, aphrodisiac, ophthalmic, antiscorbutic, anti histaminic and diuretic. They are useful in the treatment of asthma, coughs with expectoration, and poultices for sprains. Their use in treatment of leprosy, skin disease, dysentery, splenomegaly, dyspepsia, lumbago, leucorrhoea, scurvy and seminal weakness is also documented. Seeds, leaves and roots of GC are of economic importance owing to its medicinal properties. GC seeds exhibit the morphological characteristics of oil seeds and can be used for extracting oil. There are very few studies on the GC seed oil and its constituent fatty acids from India or elsewhere. Scarcity of systematic studies on GCO and its biological efficacy as a source of ALA, was the instigation for the current study. The objectives of the present study was to investigate the physicochemical properties of GCO, bioavailability of bioactive fatty acids and its effect on overall well being in rats. Further, aim of the study was to elucidate the effect of GCO and/or its combination with SFO with varying ratios of $\omega$-6/$\omega$-3 PUFA on functional indices of immune system. The final objective was to assess the anti-inflammatory efficacy of GCO in experimentally induced ulcerative colitis in rats.

The major findings of the study can be summarized as follows:

1. The amount of oil extracted from GC seeds using soxhlet method, supercritical CO$_2$ extraction and cold pressing was 21.54, 18.15 and 12.6 (wt %) respectively. The oil content in GC seeds is comparable to other oil seeds, therefore GC seeds can be classified under the category of oil seeds.

2. Peroxide values of GCO extracted using cold pressing, soxhlet extraction and supercritical CO$_2$ extraction was less than 4.1 meq. peroxides/kg oil and acid values were less than 1.5 % of oleic acid. Amongst the three methods employed for extraction, cold pressed GCO showed lowest peroxide value of 0.7 meq. peroxide/
kg oil and acid value was 0.28% of oleic acid. Overall, the oil extracted from GC seeds was of good quality.

3. Physical properties of GCO, such as viscosity, specific gravity, refractive index and colour was in accordance with the values permissable for vegetable oils.

4. Specifications such as saponification value (178.3 mg KOH/100g), iodine value (125.3 g iodine absorbed/ 100g oil) and unsaponifiable matter (1.4 g %) were comparable to conventional vegetable oils.

5. GCO contains 8 fatty acids viz., palmitic acid (9.7 - 10.0%), stearic acid (2.8 – 2.9 %); oleic acid (22.1 – 22.9 %), linoleic acid (11.1-11.8%), linolenic acid (33.6-35.7%), arachidic acid (3.1%), eicosenoic acid (11.4-12.1%) and erucic acid (3.6-4.4%). Major portion of the fatty acids were constituted by ω-3 PUFA- ALA (35.7%) followed by ω-9 MUFA oleic acid (23%), SFA (15%), MUFA (38%) and PUFA (47%) are present in the proportion of 1:2.5:3. ω-6/ ω-3 PUFA ratio in oil was 0.4. This study ascertains GCO as a good source of PUFA, mainly ALA and modulates ω-6/ ω-3 PUFA ratio of the diet.

6. Major portion of ω-3 PUFA – ALA (42%) in GCO is esterified at Sn-2 carbon of TAG followed by oleic acid (40%) and LA (17%). This finding indicates that the ALA in GCO is highly bioaccessible favoring its accretion in the tissue compartments.

7. Two main classes of antioxidants detected in GCO were tocopherols (119mg /100g) and carotenoids (532µg/ 100g). Principle isomers of tocopherols identified were α, δ and γ tocopherol and the major carotenoids identified were β-carotene, zeaxanthin and lutein. These antioxidants play an important role in the stability of GCO.

8. GCO is stable at refrigerated conditions (4°C) with no antioxidants added. For long term storage (measured as accelerated oxidation of GCO at 60°C for 8 days)
addition of ascorbyl palmitate at 200 ppm serves as an excellent antioxidant offering stability to GCO.

9. Supplementation of GCO to Wistar rats at 2.5, 5 and 10% in the diet did not affect the growth and development. The absorption of GCO was as good as SFO in rats even at 10% level in the diet.

10. GCO favorably modulated lipid profiles in rats. Diet containing GCO significantly reduced serum TAGs by 40% further a downward trend of hepatic total cholesterol, serum total cholesterol, VLDL-C and LDL-C was recorded in rats fed with GCO diets in comparison to those fed on SFO diet. The serum levels of good cholesterol, HDL-C was not affected by GCO diets. These results indicate the efficacy of GCO in reducing the risk factors contributing to CVD.

11. Feeding GCO to rats for 8 weeks resulted in significant accretion of ω-3 PUFAs (ALA, EPA and DHA) in serum, liver, brain, adipose, heart and kidney. A positive correlation between the amount of ALA (in GCO) in diet and amount of ω-3 PUFA in tissue compartments was observed. GCO feeding increased the DHA levels in the brain by 11% of total fatty acids. Adipose tissue recorded the highest amount of ALA which serves as fat reserve for the body. The increase in ω-3 PUFAs by GCO supplementation decreased ω-6 PUFAs in tissue lipids. A negative correlation between amount of ALA in diet and ω-6 PUFA in tissue compartments was observed. These findings clearly indicate that GCO was effective in increasing tissue levels of ALA and LC ω-3 PUFA.

12. Fatty acid composition of total lipids in spleen lymphocytes and peritoneal macrophages can be altered by GCO in rats. The incorporation of ω-3 PUFAs as an effect of GCO supplementation was minimal in immune cells in comparison to the extent of incorporation in tissue lipids of vital organs. But the increment in ω-3 PUFA significantly reduced the levels of ω-6 PUFAs in spleen lymphocyte and peritoneal macrophages. These finding demonstrate that, GCO can modulate the
fatty acid composition of lipids in immunocompetent cells of rats when supplied in the diet.

13. GCO supplementation to rats resulted in significant decrease of T cell proliferation in \textit{ex vivo}. GCO at concentration of 2.5\% in diet with $\omega$-3/$\omega$-6 PUFA ratio of $\sim$5.5 registered 40 and 36\% reduction in proliferation of T-lymphocytes in response to Con A and PHA, respectively. The reduction in B lymphocyte proliferation was not significant in comparison to SFO group. This indicates the modulatory effect of GCO on T cell mediated immune response which could be of importance in treatment or management of T cell mediated inflammatory conditions. The decrease in proliferation without an effect on IL-2 levels indicate the possibility of involvement of membrane factors altering the TcR complex or expression of IL-2 receptors in immune cells as an effect of GCO feeding.

14. Increased levels of $\omega$-3 PUFAs and decreased $\omega$-6 PUFAs in peritoneal macrophage as an effect of GCO feeding resulted in significant decrease in the production of nitric oxide. The $\omega$-6/$\omega$-3 PUFA ratio of $\sim$5.5 was enough to bring about significant decrease in NO production, indicating the importance of $\omega$-6/$\omega$-3 PUFA ratio rather than the absolute amounts of either $\omega$-6 or $\omega$-3 PUFA in the diet. Since macrophages derived NO play an important role in immunopathologies in RA and IBD, supplementation with GCO might offer help in management of such diseases.

15. GCO significantly inhibited the production of LTB$_4$ from activated macrophages in comparison to SFO. Decreased AA levels in peritoneal macrophage lipids resulted in decreased availability of these fatty acids for the production of pro inflammatory, chemotactic agent such as LTB$_4$.

16. The decrease in proinflammatory cytokines by GCO was very minimal in comparison to the effect of SFO in rats.

17. The activity of 5-lipoxygenase, lysosomal phosphatase enzyme activity and H$_2$O$_2$ release was not significantly affected by GCO in comparison to SFO when supplied through diet.
18. The clinicopathologic disease state equivalent to human ulcerative colitis was induced in female Wistar rats by administering dextran sulphate sodium (DSS; 35-50 kd) in drinking water at 3 and 2% for 14 days. The disease condition ensued with clinical signs of significant weight loss, diarrhea, bloody feces and increased colon weight per unit length in colitic rats in comparison to normal rats. Further the UC was characterized by increased infiltration of neutrophils with corresponding elevation in myeloperoxidase and alkaline phosphatase activity and inflammatory mediators such as NO and TNF-α in colons of colitic rats. This model was used to assess the anti-inflammatory activity of bioactive lipids in GCO.

19. ALA which is the principle fatty acid in GCO significantly increased the LC derivatives (EPA and DHA) in colon lipids. The increase of ω-PUFAs in colon was linear with the amount of ALA in GCO the diets.

20. The severity of ulcerative colitis was less in rats when GCO was supplemented at 5 and 10 % in diet with ω-6/ω-3 PUFA ratio of ~2 and ~0.4 respectively in comparison to 10% SFO and 2.5% GCO in the diet with ω-6/ω-3 PUFA ratio of 110 and ~5.5 respectively.

21. GCO content of greater than 5% and ω-6/ω-3 PUFA ratio of < 2 in diet significantly reduced the macroscopic indices of UC such as weight loss, diarrhea, bloody feces in comparison to SFO diets of colitic rats. This result is an indication of protective effect of GCO diets against the inappropriately activated immune response by DSS in rats.

22. The protective effect of GCO was further demonstrated by reduction in colon weight per centimeter length in comparison to SFO diet fed to colitic rats. In UC rat model, the weight of the colon significantly increases due to increased immune cell content, edema, and vasculature, therefore the decrease in the weight of colon is an indication of protective effect of GCO in colon of colitic rats.

23. The histological features in colon sections taken from colitic rats fed with GCO 10 and GCO 5 diets with ω-6/ω-3 PUFA ratio of 0.3 and 2 respectively, showed
significant improvements such as intact epithelial lining, lamina propria, reduction in number of immune cells in sub mucosal, inter and intra epithelial spaces. In comparison to the above observations, colon sections taken from colitic rats fed with SFO and GCO2.5 with ω-6/ω-3 PUFA ratio of > 150 and ~ 5 respectively, showed typical histological features of colon inflammation such as ulceration, epithelial damage, crypt dilation, mixed cell infiltration and granulocytes. Therefore, it is plausible to say that GCO supplementation to rats can modulate the fatty acid composition of colon lipids leading to the generation of less inflammatory mediators, culminating in protection against acute inflammation of colon in rats.

24. Increased myeloperoxidase activity in colon homogenates of colitic rats is a biochemical marker of neutrophil infiltration and increased alkaline phosphatase activity is correlated to colon injury in rat model of UC. GCO content of greater than 5% and ω-6/ω-3 PUFA ratio of <2 in diet significantly reduced the activities of myeloperoxidase and alkaline phosphatase in colons of coltic rats in comparison to SFO diet. This outcome confirms the reduction in extent of infiltration of neutrophils and colon injury by GCO diet.

25. Nitric oxide (NO) and reactive nitrogen species (RNS) derived by the interaction of NO with oxygen and/or reactive oxygen species is reported to participate in the development of oxidative tissue/cellular damage. GCO supplementation reduced NO content in the colons of colitic rats in comparison to SFO diet. This indicates that GCO supplementation offers protection against UC in rats by reducing the oxidative stress on one hand and NO mediated inflammatory response on the other.

26. GCO diets decreased TNF –α concentrations in colon of colitic rats in comparison to the ones fed with SFO diet. This outcome is an indication that the components in GCO affect the production of inflammatory cytokines, where the mechanism involved needs to be investigated.

27. Feeding GCO induced the restoration of GSH levels in colons of colitic rats when compared to SFO supplementation in diet. The restoration of GSH is an indication
of reduced oxidative stress in the colons of colitic rats fed with GCO. GCO contains a good amount of natural antioxidants and ω-3 PUFAs that are incorporated in the colon tissue as a result of GCO feeding could have played a synergistic effect in restoring colon GSH levels. Since UC is a highly oxidative stress condition, strengthening the antioxidant defense by GCO suppresses the tissue injury and overall severity of the disorder.