

Research Article



In-vitro and Ex-vivo Studies on Antiobesity Leptin Activation Effects of *Salvia hispanica*, *Ocimum basilicum* and *Coriandrum sativum* Extracts

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ABSTRACT

In the present years, the major cause of obesity is the rapidly changing lifestyle. The obese individuals are prone to other disorders like hypertension, heart disease, type II diabetics and cancer in severe cases. Obesity has become hazardous to health in the present generation. *In-vitro* and *In-vivo* studies were conducted to determine the anti-obesity effect of the three seed extracts of *Salvia hispanica* L, *Ocimum basilicum* and *Coriandrum sativum*. Various assays were carried out in *in-vitro* and *in-vivo* conditions. Leptin activation was determined in the adipocytes cells of chicken liver induced with the seed crude extracts. The results suggest that the seed extracts can be used to treat obesity.

Keywords: Anti-obesity, Crude seed extract, Adipocyte cells, Leptin activation.

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INTRODUCTION

The increased mass of adipose tissue that has been accumulated causing undesirable effect on health and is one of the world's leading disease conditions is obesity. When the body mass index (BMI) which is derived by the division of a person's height is over 30kg/m² such people are considered as obese. Major risk factor for a plethora of severe diseases, including Diabetes, non-alcoholic fatty liver disease, Cardiovascular disease and Cancer. It is a nutritional problems it increases the risk of morbidity from several pathologies. In the study carried out accumulation of excessive fat is a positive energy balance which is obtained by the interaction among several factors, hormonal status, decreased physical activity, genetic environmental, economic, and cultural factors. The study is carried out to determine the obesity effects of the crude seed extracts, which exhibit no side effects and can be easily incorporated into daily meals and prevent obesity. The aim of the study is activating hormones in the body by which the satiety and overeating is reduced and thereby the obesity. The study deals at estimating the lipase activity which is enhanced and the glucose release exhibited by the chosen seed extracts¹ central player in the pathophysiology of various disease is obesity and particularly cardiovascular disease diabetes mellitus type 2, cancer, obstructive sleep apnea, asthma and osteoarthritis and thereby it reduces the life expectancy by 6 to 7 years and it is estimated to cause

111,909 to 365,000 deaths per year on average in developed and developing countries .

In the body leptin hormones is found in the free form or bound form (with protein) and changes exponentially with fat mass and its levels are higher during midnight and early morning and at night it is lower levels of leptin reduce after short-term fasting, sleep deprivation, starvation, exercise and on the contrary increase by emotional stress, increased insulin levels, increased estrogen levels, increased maltonin levels in presence of insulin. In the lateral hypothalamus, leptin acts on receptors by decreasing the effects of neuropeptide γ to inhibit hunger neuropeptide γ , a hunger promote that is secreted by the cells in the gut and in the hypothalamus, the leptin hormone binds to the neuropeptide γ neurons to decrease the activity of these neurons. Leptin is a 167 amino acid, 16 KDa protein coded by the Ob (Lep) gene that is located on chromosome 7 in humans². The patients have more leptin levels than normal people due to more body mass, high levels of cholesterol, saturates the leptin transporters and hence causes a deficit in leptin³. Decreased levels of leptin alter proteins in the brain which leads to depression, anorexia and Alzheimer's disease.

Research has proven that lipase could be complementary to weight loss since it breaks down fat that is in the body, molecular switches are manipulated make the enzyme work three times harder that could help people struggling with obesity and serious related health problems⁴. Research suggests that regulation of endocannabinoid (EC) metabolism by elevating EC synthesis and reducing EC degradation is failed by insulin-resistant adipocytes. Individuals who are obese, insulin-resistant exhibit higher concentrations of ECs. This leads to excessive accumulation of visceral fat and hence various obesity related diseases and type II diabetes.⁵ Maybe insulin resistance is the basic underlying cause due to type II



diabetes leading to cardiovascular disease and obesity development⁶.

Chia seeds (*Salvia hispanica L*) is an edible and medicinal plant species and is used for nutritional properties and its beneficial effect on human health. Basil seeds (*Ocimum basilicum*) are boon and is considered as superfood due to its tremendous qualities due to its different chemical constituents it possesses medical uses. Basil seeds are rich sources of many polyphenolic flavonoids, orientin and vicenin been the special one, essential oils like eugenol, citronellol, linalool, limonene, citral and terpineol, high levels of beta carotene, lutein, zeaxanthin, vitamin A and vitamin K: minerals like potassium, manganese, vitamin C and folates. It has properties like weight loss, healthy skin, cooling effect, prevention of acidity, anti-inflammatory, anti-cancer etc. Coriander (*Coriandrum sativum*) is an aromatic herb and the essential oils of the fruits are rich in linalool⁷ and it possess anti-bacterial and antioxidant activity.

The objective of the research is to study the effect of crude extracts of *Salvia hispanica*, *Ocimum basilicum* and *Coriandrum sativum* in leptin activation. To determine the effects of *Salvia hispanica*, *Ocimum basilicum* and *Coriandrum sativum* on lipase activity in the adipocytes. To study anti-obesity properties of seed extracts.

MATERIALS AND METHODOLOGY

Plant Source

Chia seeds (*Salvia hispanica L*), Basil seeds (*Ocimum basilicum*) and Coriander seeds (*Coriandrum sativum*): This was obtained from Vasanth nagar market, Bangalore, Karnataka, India.

Type of Equipment

Centrifuge, colorimeter, magnetic stirrer, homogeniser, PH meter, UV spectrophotometer, dissection kit, water bath and weighing balance.

Chemicals

DMSO (Dimethylsulphoxide), Lipase, BSA (Bovine Serum Albumin), MDA (Malondialdehyde), Lead Acetate, Trypsin enzyme, α -Amylase enzyme, Acetyl Salicylic Acid(ASA), PBS, MTT.

Preparation of Plant Extract

2.5% of methanolic and aqueous extracts of dry powdered seeds were prepared using methanol and water which is first incubated for 24hours in room temperature and then filtered. The filtrate was obtained and used for further analysis.

Antioxidant Activity

Inhibition of Lipid Peroxidation Assay

The stock solution of 1M MDA containing 5mg of MDA dissolved in 5ml distilled water. MDA of different concentration were pipetted out along with 5ml glacial acetic acid and 0.5ml of 0.5% thiobarbituric acid (TBA).The

resultant mixture was then kept in boiling water bath for 45minutes. After incubation, the solution was cooled and 0.05ml of 5M HCl was added and absorbance was read at 535nm against a suitable blank. This was followed for all the samples and the respective percentage was calculated⁸.

DPPH Assay

Ascorbic acid(10mg/ml) was used as the standard to carry out this assay. 200-1000 μ l aliquots of the standard were pipetted out in different test tubes and the volume was made upto 1000 μ l with distilled water. To the mixture 3ml of DPPH(2mg/ml) was added. This mixture was incubated at room temperature for 10 minutes. After incubation, the tube contents were read Spectrometrically at 517nm. DPPH radical scavenging activity (%) was calculated to the formula:

DPPH radical scavenging activity (%)= [(OD of control-OD of the sample)/OD of control]*100⁹.

Determination of Peroxide value

Weigh out approximately 5.0g of fresh oil (or 0.2~0.5g of old oil) in a conical flask. Incubate 5minutes in room temperature. Add 15ml of solvent mixture [Glacial acetic acid: Chloroform (6:4)] solution and swirl to dissolve oil. Add 0.25ml of saturated KI solution by pipette and swirl for 1 minutes (light brown color is observed). Add 15ml of distilled water and titrate with 0.01M Sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) until the solution becomes pale yellow. Add 1ml Starch(1%) indicator solution and continue titration until the blue color disappears(endpoint).Do a blank determination(15ml Glacial acetic acid: Chloroform solution + 0.25ml KI + 15ml distilled water) against 0.01M Sodium thiosulphate.

$$PV = S \times M \div \text{Sample weight (g)} * 100$$

Where S= volume (ml) of sodium thiosulphate solution used in the titration after correction for the blank titration and M= molar concentration of the sodium thiosulphate solution¹⁰.

Enzymatic assay of Lipase

This is conducted using a reaction mix and blank mix. The reaction mix constitutes 2.5ml distilled water, 1ml of 200 Tris HCl (Ph-7.2) and 2ml olive oil (Substrate).The blank mix is devoid of the substrate.1ml of the crude extracts and 4ml of the lipase enzyme is added to all the test tubes. The tubes were incubated at 37°C for 30 minutes. Then 3ml of Ethanol (95%) is added to all the tubes and were titrametrically estimated against 0.05N NaOH in the presence of Phenolphthalein as an indicator until a pale red colour is acquired.

Lipase activity (units/ml) = Volume of NaOH \times Molarity of NaOH \times 1000 \times 2 \times Dilution Factor \div 1¹.



Anti-Inflammatory Activity Assays

Inhibition of Protein Denaturation

0.5 ml of 1% BSA was used as control. Aspirin(1mg/ml) was used as standard drug that inhibit the protein denaturation process.0.5 ml of BSA was treated with 0.1 ml of different crude extracts and with drugs that were chosen for inhibition of denaturation. It was incubated at 51 °C for 20 min. The solution was cooled and absorbance was read at 660 nm. Percentage inhibition was calculated using the following formula:

Percentage inhibition= (OD of the Standard- OD of the sample)÷OD of the standard*100.

Cell Viability Assay

Cells cultured in DMEM medium had been handled with crude sample extracts at a final concentration of 100µg/ml for 2days, after which were incubated with MTT(5mg/ml) solution (Sigma-Aldrich) at 37°C for 3 hours. After the cells have been dissolved in 0.04N HCl (in Isopropanol), the formazan level was analysed by measuring the optical density at 570nm¹².

Glucose Diffusion Assay

The seeds were homogenised with 20ml of a 0.01M Potassium phosphate buffer(p^h-6.9) stored at 37°C. After homogenization, the samples were rinsed with further 20ml buffer solution. The pH of the samples were decreased to 2.5 with O-Phosphoric acid, after which 1ml of trypsin enzyme was added. The samples were placed in 37°C stirring water bath for 60 minutes. Each buffered samples was returned back to ph 6.8 with KOH and then 2ml of amylase enzyme was added. The whole contents were then transferred into a dialysis bag. The membrane was knotted at both the ends using thread and dipped into a beaker containing 500ml buffer solution. The beakers were kept in the magnetic stirrer and maintained at room temperature. The glucose movement to the external solution was measured at time interval of 30minutes starting from 0 minute to 150 minutes. The glucose in the solution was estimated by DNS method.

Leptin Assay

About 250mg of adipose tissue have been placed inside the cell culture plates containing 1ml buffer solution. The adipose tissue was treated with 0.5ml of vegetable oil and 1ml of the crude seed extracts. The tissue was incubated at 37°C for 2 days.

RIA procedures were carried out using leptin (2.5µg)which was dissolved in 50µl 50mM Na₂PO₄(p^h-8.0), 1% Triton X-100 and iodinated with 0.5mCi Na₁₂₅I using 2µg Iodogen. After 2 minutes, the reaction was stopped by addition of 50µl PBS containing 10mg/ml Tyrosine,10% glycerol and 0.1% xylene cyanol. The reaction was applied to a Sephadex G-50 column equilibrated with PBS containing 0.01% NaN₃. Fractions (300µl) were eluted into tubes containing 1ml Assay buffer (PBS, ph-8.0, containing 0.01% NaN₃ and 1% Bovine Serum Albumin (BSA) radioactivity of

each fraction was determined by gamma counting. Incorporation of radioactivity into bovine leptin was 55-60%, yielding a tracer with a specific radioactivity of 200µCi/µ¹³.

RESULTS AND DISCUSSION

Antioxidant Activity

Inhibition of Lipid Peroxidation Assay

These oxidative effects suggest that obesity problems are related to increase in endogenous lipid peroxides. Statistics display that the indicator of lipid peroxidation MDA falls markedly in association with obesity with orlistat. The demonstration of decreased free radical generation has crucial implications for oxidative mechanism underlying obesity associated issues.¹⁴

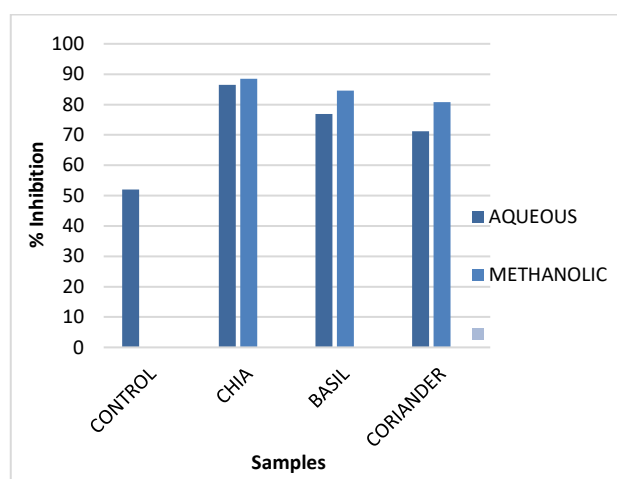


Figure 1: Malondialdehyde (MDA) concentration in control, Methanolic and Aqueous Crude Extracts of Chia, Basil, Coriander seeds

The percentage inhibition in control was found to be 52% where the inhibitory effect of methanolic extract of Chia is having 88.46%, however much less with the case of Aqueous extract of Coriander with 71.15%. This showed that the methanolic and aqueous crude extracts of the seeds have more inhibitory effect when compared to the control. It infers to have Anti-oxidant effect and increase in the concentration of MDA.

The percentage inhibition of lipid peroxidation was shown to be highest in Chia exhibiting greater antioxidant property that is concerned with the scavenging of free radicals. Inflammation leads to the release of lysosomal enzymes, which have a damaging activity on tissues leading to lipid peroxidation in macromolecular biological membranes. When these biological membranes containing polyunsaturated fatty acids are damaged by lipid peroxidation, it indicates the pathological conditions such as heart attacks, septic shocks and arthritis

DPPH Assay

DPPH is a popular method to examine the antioxidant property. DPPH is a stable free radical with red colour and when is mixed with a substance that can donate a

hydrogen atom forms a reduced form giving rise to yellow color when scavenged. Antioxidants with DPPH when reduced forms DPPH-H that causes decrease in absorbance which is read at 517nm.¹⁵

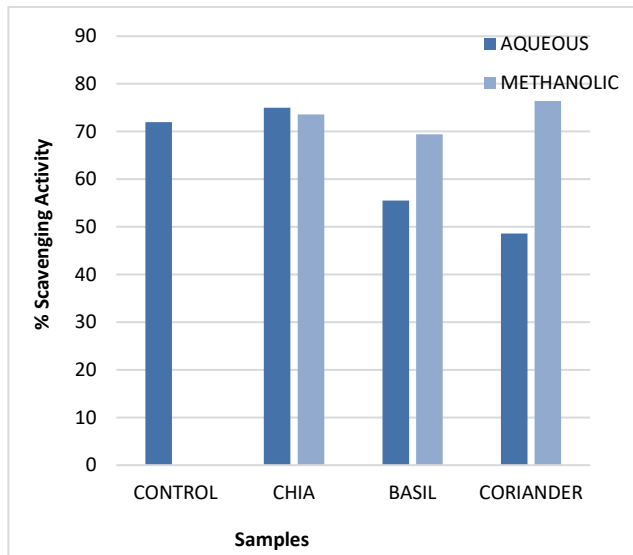


Figure 2: Scavenging Effect of Control, Methanolic and Aqueous Crude Extracts of Chia, Basil, Coriander seeds

The percentage of anti-oxidant property of ascorbic acid was found to be 72% where the percentage of anti-oxidant property of methanolic extract of Coriander was found to be 76.38%, however much less with the case of aqueous extracts of Coriander with 48.61%. It was observed that the methanolic extracts of Chia, Basil and Coriander seeds showed significant anti-oxidant effect when compared to the aqueous extracts. It shows that the aqueous extracts did not remove much of the free radical species.

Determination of Peroxide value

Reducing the peroxide value is considered to represent the initial protective response required for adjusting the hydrogen peroxide concentration under normal physiological conditions as well as after oxidative insult. Optimum level of antioxidants is required in body which in turn potentiates peroxidase activity to stay healthy.¹⁶

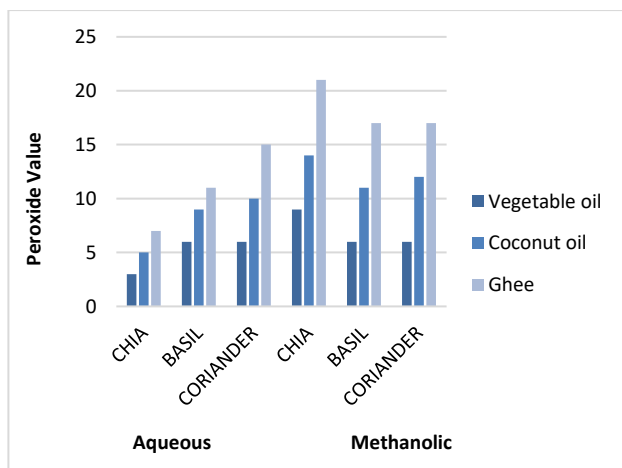


Figure 3: Peroxide Values of Methanolic and Aqueous Crude Extracts of Chia, Basil, Coriander seeds

The peroxide value of the vegetable oil, coconut oil and ghee was found to be 15,7,42 respectively. Both the methanolic and aqueous crude extracts have shown to reduce peroxide value of the lipid samples, which in turn reduces its effects of oxidative stress exhibited in the body.

Peroxidase activity within the body or that exhibited by food sources is an important indicator of level of oxidative stress. Lowering of the peroxide value of lipid sources is important to protect the organism from oxidative damage by free radical.

Enzymatic assay of Lipase

Obese patients have improved adipose tissue lipoprotein lipase activity in line with fat cell at the same time as in comparison with lean patients. This enzyme, which is in charge for the uptake and storage of lipoprotein triglyceride in adipose tissue, the unique position of the enzyme AT-LPL with regards to fat storage indicates that it has a function inside the development of human obesity and/or the safety of the obese state. AT-LPL activity consistent with cellular has been tested to be extended in weight issues and is definitely correlated with fats cell size similarly to relative weight.¹¹

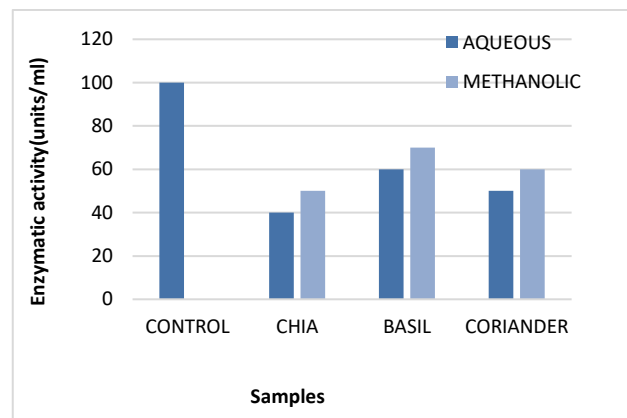


Figure 4: Lipase Activity of Control, Methanolic and Aqueous Crude Extracts of Chia, Basil, Coriander seeds

The methanolic seed extracts of Basil was found to show anti-oxidant and lipooxygenase enhancing effects. It is observed to exhibit the highest hypolipidemic effect by enhancing the activity of lipoprotein lipase in fat tissue and heart resulting in higher absorption of triglycerides hence reducing obesity. A pronounced activity induced by methanolic and aqueous seed extracts of Basil was followed by Coriander and Chia.

Anti-Inflammatory Activity Assays

Inhibition of Protein Denaturation

The study shows that treatment by the crude seed extracts resulted in significant improvement in lipid parameters by exhibiting anti-inflammatory properties as expressed by the results. The crude seed extracts thus may also have anti-atherogenic hypocholesteremic and immune modulator effects which were probably mediated by unsaturated fatty acids like α linolenic acid present in the seeds.¹⁷

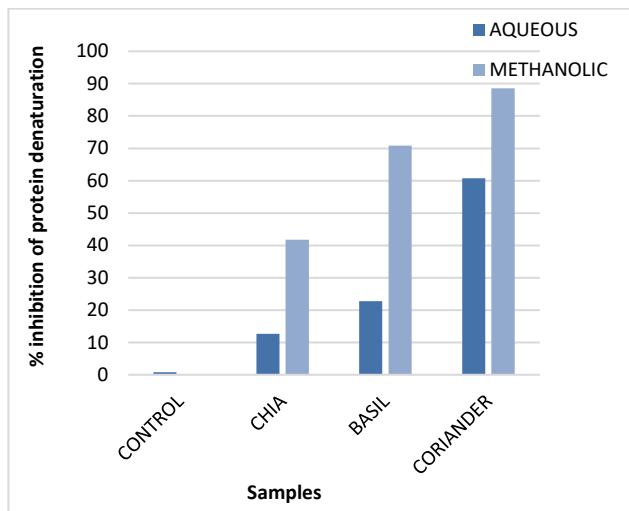


Figure 5: Percentage Inhibition of Protein Denaturation of Control, Methanolic and Aqueous Crude Extracts of Chia, Basil, Coriander seeds

The percentage inhibition of protein denaturation was found to be directly proportional to prevent inflammation. Maximum inhibition of protein denaturation was shown by Coriander, Basil and Chia seeds comparatively.

Cell Viability Assay

Excess Nutrients end in mitochondrial disorder, which leads to obesity and associated pathologies, mitochondria play valuable roles in ATP production, energy expenditure, and disposal of ROS. High energy substrates result in mitochondrial disorder with consequential effects on lipid and glucose metabolism. Adipocytes help to preserve the right stability among energy storage and expenditure and retaining this stability requires normal mitochondrial function.¹²

After incubation period with MTT, the adipose tissues took up a dark violet-pink color indicating formazan precipitation while as compared to the control cells that had been untreated. The amount of color developed is directly proportional to the viability of the fat cell.

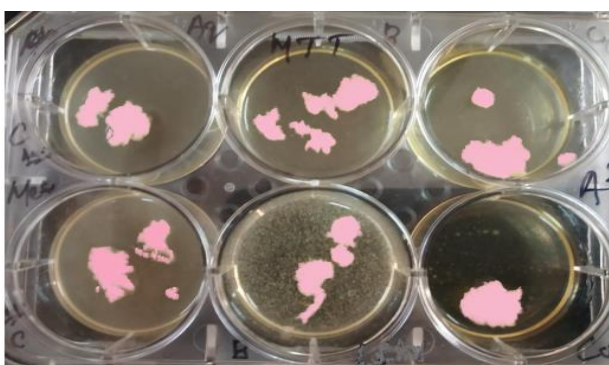


Figure 6: Adipose cells treated with MTT for Cell Viability

The treatment of adipose tissues with the three crude seed extracts one at a time in the culture plate, *ex-vivo* conditions, rendered near dark violet-pink coloration in comparison to the control cells that were untreated as seen in Fig.6. The methanolic crude extract of Chia showed

maximum color intensity while as compared Basil and Coriander crude extract with adipose tissue, proving that these seed extracts have the potential to increase mitochondrial activity and lower cell cytotoxicity. This concludes that green coffee can help to maintain energy homeostasis(ATP production) for an extended period.

Glucose Diffusion Assay

Hypoglycemic plant extracts (50 g/l) showed reduced glucose diffusion from dialysis bag and their AUC (area under curves) in comparison to control, after 24 h. Aqueous extract of grape seeds at 50g/1 concentration prevented 57% of glucose diffusion from dialysis bag.The results of the study showed that the aqueous extract of *Eucalyptus globules* had a lower viscosity than other examined extracts and the highest glucose diffusion property.¹⁸

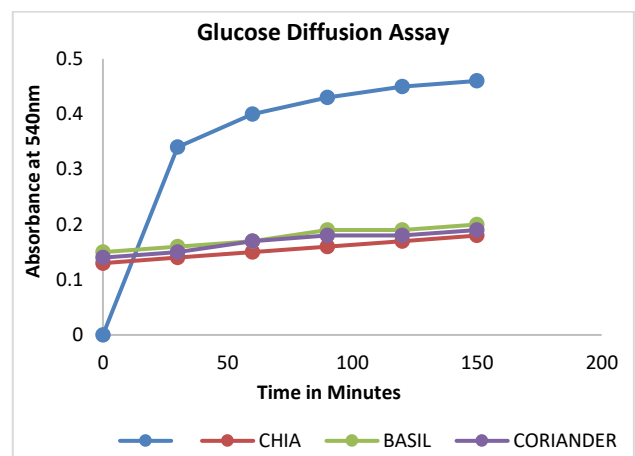


Figure 7: Glucose Diffusion Assay of Chia, Basil, Coriander against the blank

The three crude seed extracts were found to show glucose diffusion. When compared to the glucose diffusion in the control, the diffusion by the crude seed extracts were found to be reduced.

Leptin Assay

Serum levels of the adipocyte hormone leptin are increased in proportion to body fat stores as a result of accelerated production in enlarged fats cells from overweight subjects. The elevated leptin expression discovered in obesity could result from the chronic hyperinsulinemia and expanded cortisol turnover. Fasting leads to a gradual decline in serum leptin that is probably attributable to the decline in insulin and the capacity of catecholamines to decrease leptin expression, as found in both in vivo and in vitro research.¹⁹

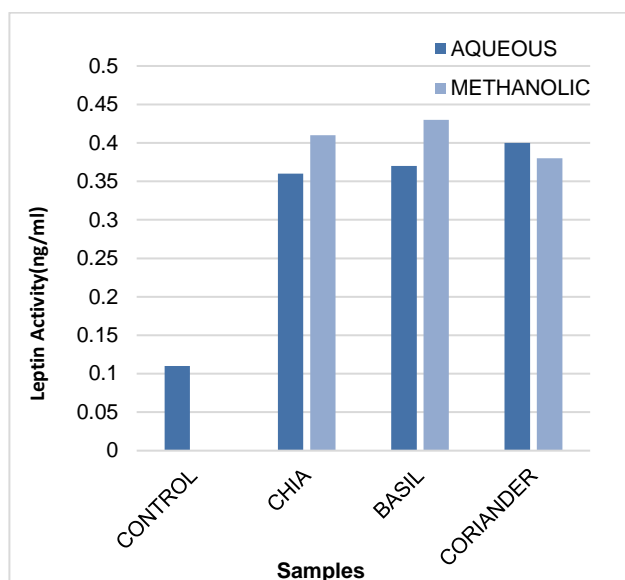


Figure 8: Leptin activity of Control, Methanolic and Aqueous Crude Extracts of Chia, Basil, Coriander seeds

The methanolic crude seed extract of Basil showed the highest ability to activate Leptin and stimulate the adipose tissues to release leptin followed by the methanolic crude seed extracts of Chia and Coriander respectively. It is observed that the Aqueous crude seed extracts had less ability to activate Leptin as compared to the Methanolic extracts.

CONCLUSION

The result suggested that the three crude extracts can reduce oxidative stress, oxidative rancidity, inflammatory effects, and to an appreciable level increase mitochondrial and metabolic activity, lipase enzyme activity for lipolysis and also induce the adipocytes to produce leptin-satiety hormone, all these aspects combined could provide a synergistic effect and carb obesity and its related problems increasing life expectancy and quality.

Optimum level of antioxidants are required in body which in turn potentiates peroxidase activity to stay healthy. Reduced production of leptin with reference obesity may produce low sensitivity to leptin. The extent of leptin that was produced from with the adipocytes was determined by RIA studies, signifying its potential in stimulation and activation of leptin. The interpretation is much likely used in research of insulin and the pathogenesis of type I and type II diabetes. Hence it is important to understand that there is relationship between the insulin sensitivity i.e., glucose release and leptin levels.

The anti-obesity effects of the seed extract were studied in order to determine if the bio-active compounds in the sample had the anti-obesity activity. An array of assays was carried out to study the antioxidant property of the samples. The regulation of the obesity is primarily depending on the diet. The experiments carried out confirmed that the above seeds can be incorporated in the dietary diet as they contain anti-obesity property and has negligible side effects.

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