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Assessment of *Ulva lactuca* Extract on Inflammatory Markers in Fructose Fed Experimental Rats

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Abstract: Adipose tissue controls energy homeostasis. Adipocytes secrete hormones and inflammation-related adipokines in addition to storing fat. Green seaweeds are a typical dietary element in Eastern Asian nations like India. They are rich in proteins, vitamins, minerals, fibre, polyunsaturated fatty acids, and bioactive compounds. This study examined *Ulva lactuca* extract's influence on hormones and anti-inflammatory markers in fructose-fed rats. High fructose-fed rats had elevated blood leptin, IL-6, TNF-, and decreased adiponectin and ghrelin. *Ulva lactuca* extract improved leptin, IL-6, TNF-, adiponectin, and ghrelin in obese rats. *Ulva lactuca* may restore adipose tissue function owing to saponin, steroids, alkaloids, polyphenol, and glycoside.

Keywords: *Ulva lactuca* extract, Hormones, Cytokines, Bioactive substances, IL-6, TNF-, Adiponectin, Ghrelin

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Introduction

Inflammation is a physiological response needed to restore equilibrium after numerous stressors, however, persistent inflammation or an excessive reaction may be detrimental (Wang and Trayhurn, 2006). Low-grade chronic inflammation occurs with overweight and obesity. Recent investigations have shown several intracellular inflammation pathways related with these disorders; studies in mice and people show that foods may rapidly elicit inflammatory responses. Overfeeding is considered to cause inflammation in metabolic areas such as fat, liver, and muscle (Faloia et al., 2012). Obesity’s proinflammatory cytokines are generated by infiltrating macrophages and adipocytes. Many studies include persistently low inflammation in the
relationship between obesity and metabolic problems, since many chronic degenerative conditions, like atherosclerosis, are connected with high blood pressure, which has recently been linked to inflammation (Hotamisligil, 2006; Mohanasundaram et al., 2021).

In recent years, marine resources have been studied for bioactive substances to produce novel medications and health foods. Marine algae are rich in numerous natural compounds. Polyunsaturated fatty acids, polyphenols, flavonoids, sterols, proteins, sulfated polysaccharides, and vitamins have been extracted from marine algae as bioactive constituents (Manikkam et al., 2016; Ruocco et al., 2016; Suleria et al., 2016). These chemicals have antioxidant, anti-diabetic, cancer, anti-obesity, and anti-inflammatory effects (Zhao et al., 2015; Fernando et al., 2016; Suganthi et al., 2020).

Marine algae are a plant resource and useful food source (Senni et al., 2011). This study aimed to determine the impact of Ulva lactuca on inflammatory markers in high fructose-fed rats.

Materials and Methods

Animals:

This study employed 180 to 220 g healthy male Wistar albino rats, procured from Companies in Sri Venkateswara, Bangalore, India. The animals were kept in polypropylene cages with rice. During the test period, the animal chamber was well-ventilated and had a 12-h light/dark cycle (27±2°C). All animals had ad libitum grains and water. They were acclimated for a week before the experiment. The experiment followed the Committee’s criteria for control reasons (JJC/BC/AH/002/2022) and animal testing monitoring (CPCEA), New Delhi, India.

Extraction:

Fresh Ulva lactuca was collected from Tamil Nadu’s south coast, Mandapam, Rameswaram, India. The collected Ulva lactuca was cleaned with distilled water and dried in the shade and powdered. 10 g of coarse powdered material was macerated in ethanol, then filtered and dried at 40°C. The dried extract was stored in a desiccator until required (Sofowara, 1993; Trease and Evans, 1989; Harborne, 1973, 1984).

Experimental design:

Diets were prepared according to Nandhini et al. (2002). Table 1 shows the diet compositions.

Animals were weighed and sorted into 4 groups of 6 animals each. Group 1: Normal rats given control food; Group 2: These rats were given a fructose-enriched diet for 8 weeks. Group 3: HFD-fed animals co-administered ethanolic extract of Ulva lactuca (500 mg/kg body weight) by oral gavage daily for 8 weeks. Group 4: HFD-fed animals given 9 mg/kg Orlistat for 8 weeks.

Table 1: Composition of the experimental diets (g/kg diet)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control diet (%)</th>
<th>High-fructose (HF) diet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>61</td>
<td>-</td>
</tr>
<tr>
<td>Fructose</td>
<td>-</td>
<td>61</td>
</tr>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Groundnut oil</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>9.6</td>
<td>9.6</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>0.2ml</td>
<td>0.2ml</td>
</tr>
</tbody>
</table>

Sampling:

Animals were anaesthetized with thiopentone sodium (50 mg/kg) after the experiment. Anticoagulant EDTA was used to collect blood. Serum was separated and analyzed for various parameters.

Adiponectin, leptin, and ghrelin:

Radioimmunoassay (RIA) was used to evaluate serum adiponectin, leptin, and ghrelin. Adiponectin test limit was 2 ng/ml. Leptin and ghrelin detection limits were 0.8 ng/ml.

TNF- and IL-6 serum quantification:

IL-6 (BMS634) and TN- (BMS622) were measured using ELISA kits from the Bender Med system. 50 µl ELISA diluents were pipetted into wells coated with anti-IL-6 and TNF- antibodies, followed by 100 µl of each standard and 50 µl serum. The plate was sealed and incubated at room temperature for
2 h. After incubation, the wells were washed three times. After the last wash, 100 µl of detection solution was added, covered, and incubated for 1 h. After washing three times with washing solution, 100 µl substrate reagent was added and incubated for 30 min. The colour development was stopped by adding 100 µl stop solution, and absorbance was measured at 570 nm. Values are expressed as pg/ml.

Statistical analysis:
The mean value for six rats in each group was used to assess statistically significant differences between the groups, followed by the DMRT test for many comparisons (Harvey and Paige, 1998). P <0.05 was selected for significance by Graphpad Instat Software Version 3 (San Diego, CA, USA).

Results and Discussion
The ethanolic extract of Ulva lactuca was analysed for secondary metabolites. Saponin, steroids, alkaloids, polyphenols, and glycosides were found in Ulva lactuca ethanolic extract. It has been reported that polyphenolic substances may suppress pancreatic lipase and lipoprotein lipase (Moreno et al., 2003; Moreno et al., 2006).

Visceral fat is enlarged in metabolic syndrome. Pektas et al. (2015) revealed that dietary fructose combined with omental mass expansion raises plasma insulin and triglyceride levels, referring to metabolic syndrome and visceral obesity in male and female rats. Dietary fructose-induced visceral fat storage led to increased expression of genes involved in insulin signal transmission, affected endocrine function, and activated pro-inflammatory and anti-inflammatory markers in male and female rats’ adipose tissue (Pektas et al., 2016).

Ghrelin and leptin hormones regulate energy balance (Popovic and Duntas, 2005), hence, measuring their plasma levels might suggest an animal’s vulnerability to HFD-induced weight gain. Leptin, an adipocytic hormone, regulates energy balance, food intake, and weight reduction. It may function as a negative feedback loop from fat tissue to the hypothalamus (Fig. 1). Alternatively, stomach-secreted ghrelin is a fast-acting brain gut peptide that stimulates growth hormone release and hunger as part of a positive feedback loop to the hypothalamus. In the current investigation, rats given HFD for 8 weeks demonstrated a drop in orexigenic hormone ghrelin and an increase in anorexigenic hormone leptin, similar with other results (Dogan et al., 2007; Klok et al., 2007). High-fructose diets may dysregulate ghrelin and leptin, compromising homeostasis and causing obesity. Ulva lactuca increases ghrelin and leptin. De Melo et al. (2009) have also reported similar findings.

Adipose tissue may produce adiponectin. In this research, HFD-fed rats had lower plasma adiponectin than control rats. According to Hajer et al. (2008), adiponectin production is decreased in obesity, insulin resistance, metabolic syndrome, and type-2 diabetes. Studies have established a negative link with body weight, notably abdominal visceral fat. Adiponectin has an insulin-sensitizing characteristic, and low plasma adiponectin levels are connected with insulin resistance (Yamauchi et al. 2001) and PPAR, a nuclear factor that controls the expression of critical genes involved in lipid and glucose metabolism and adipocyte development. Diabetes agonists like rosiglitazone enhance adiponectin production, which increases insulin sensitivity (Aprahamian et al., 2009; Doss et al., 2016; Mohanasundaram et al., 2016).

Pro-inflammatory cytokines have a key role in obesity-related inflammation and its molecular processes. Higher levels of inflammatory cytokines in obese people contribute to insulin resistance. In obesity, adipose tissue produces anti-inflammatory cytokines. Adipocytes and macrophages produce them. After weight reduction, blood cytokine levels drop (Faloia et al., 2012). TNF and IL-6 cause persistent inflammation (Gregor et al., 2011). Two key cytokines, tumour necrosis factor- (TNF-) and interleukin-6 (IL-6), are overexpressed during IR. Dysregulated IL-6 and TNF- decrease hepatic
insulin receptor activation and downstream insulin signalling \textit{in vivo}, impairing insulin’s cellular activity. TNF- causes cell injury by overproducing oxidants that destroy cellular components.

TNF- plays a crucial function in inflammation, immune system development, apoptosis, and lipid metabolism, and it affects adipose tissue lipid metabolism and insulin signalling. Obesity increases circulating TNF- and weight reduction decreases it. TNF- increases IL-6 release and decreases adiponectin. TNF- increases insulin resistance by inhibiting insulin receptor substrate 1 signalling (Wang and Trayhurn \textit{et al}., 2006).

IL-6 affects acute phase responses, inflammation, hematopoiesis, bone metabolism, and cancer development. IL-6 influences energy balance, inflammation, hunger, and energy intake at the hypothalamus level. It deregulated in obesity, insulin resistance, inflammatory bowel illnesses, inflammatory arthritis, and sepsis, causing chronic inflammation (Naugler and Karin, 2008).

High fructose intake leads to obesity and metabolic syndrome (Johnson \textit{et al}., 2007). Table 2 and Figure 2 show that HFD-fed rats had higher blood leptin, IL-6, and TNF- and lower adiponectin and ghrelin. Hormones were restored in Orilstat-treated animals. Our conclusion corresponds with previous publications (Lira \textit{et al}., 2011; Kumar and Sharunetha, 2018; Suganthi, 2020).

Insulin resistance in obesity is directly associated to adipose tissue inflammation (Khan \textit{et al}., 2020), when higher TNF expression in obese mice and humans was observed (Hotamisligil \textit{et al}., 1995). Animal and human research revealed the increased expression and/or production of TNF, IL-1, and IL-6 in obese adipose tissue (Madan
Table 2: Effect of Ulva lactuca on Hormones and inflammatory markers in high fructose-diet fed experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/ml)</td>
<td>0.53±0.09\textsuperscript{a}</td>
<td>1.76±0.07\textsuperscript{b}</td>
<td>0.57±0.05\textsuperscript{a}</td>
<td>0.55±0.05\textsuperscript{a}</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>1.24±0.12\textsuperscript{a}</td>
<td>0.43±0.05\textsuperscript{b}</td>
<td>1.15±0.15\textsuperscript{a}</td>
<td>1.19±0.11\textsuperscript{a}</td>
</tr>
<tr>
<td>Ghrelin (ng/ml)</td>
<td>0.92±0.09\textsuperscript{a}</td>
<td>0.25±0.03\textsuperscript{b}</td>
<td>0.85±0.07\textsuperscript{a}</td>
<td>0.89±0.06\textsuperscript{a}</td>
</tr>
<tr>
<td>TNF-(\alpha) (pg/ml)</td>
<td>3.56±0.39\textsuperscript{a}</td>
<td>23.74±1.28\textsuperscript{b}</td>
<td>3.46±0.41\textsuperscript{a}</td>
<td>3.49±0.38\textsuperscript{a}</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>33.29±2.18\textsuperscript{a}</td>
<td>90.74±2.93\textsuperscript{b}</td>
<td>35.89±2.54\textsuperscript{a}</td>
<td>34.81±2.85\textsuperscript{a}</td>
</tr>
</tbody>
</table>

et al., 2006). In this study HFD-fed rats had higher inflammatory marker levels than controls.

Fructose as a single carbohydrate supply (60/100 g) induces IR and related problems in rats. High-fructose-fed animals experience oxidative damage and inflammation. Cytokines (TNF-\(\alpha\) and IL-6) are greater in fructose-fed hamsters than chow-fed counterparts. In high-fructose diet-induced metabolic syndrome rats, dietary phenolic acids restore insulin resistance, hyperglycemia, dyslipidemia, inflammation, and oxidative stress (Mohanasundaram et al., 2021). Ulva lactuca reduced TNF-\(\alpha\) and IL-6 in HFD-fed rats, indicating that it regulates proinflammatory cytokine release in adipose tissue. The current study agrees with observations of de Melo et al. (2009) and Suganthi et al. (2020) and reveal that Ulva lactuca extract therapy inhibited inflammation and offers promise in reducing inflammation.

**Conclusion**

Adipose hormones and cytokines are dysregulated in HFD-fed rats. Ulva lactuca preserves adipocyte activity in HFD-fed rats through regulating hormones and cytokines. Ulva lactuca restores adipose tissue function by including phytochemicals such as saponin, steroids, alkaloids, polyphenols, and glycosides.

**References**


Lira FS, Rosa JC, Cunha CA, Ribeiro EB, do Nascimento CO, Oyama LM and Mota JE. (2011) Supplementing alpha-tocopherol (vitamin E) and vitamin D3 in high fat diet decrease IL-6 production in murine epididymal adipose tissue and 3T3-L1 adipocytes following LPS stimulation. Lipids Health Dis. 10(1): 1-5.


Senni K, Pereira J, Gueniche F, Delbarre-Ladrat C,


