ANTIOBESITY ACTIVITY OF MARINE ALGAE TURBINARIA ORNATE

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ABSTRACT

Currently obesity is a major threat to the health of people all over the world. Globally, there are more than 1 billion overweight adults, at least 300 million of them are obese. Obesity and overweight pose a major risk for chronic diseases, including type 2 diabetes, cardiovascular disease, hypertension and stroke and certain forms of cancer. The key causes are increased consumption of energy-dense foods high in saturated fats and sugars and reduced physical activity. This study concerned with the use of different extracts of Turbinaria ornate to prevent the development of obesity in rats. Turbinaria ornata was collected from Erwadi in gulf of Mannar, Tamil Nadu. Turbinaria ornata was shade dried at room temperature and reduced to a coarse powder. The algae was extracted with three solvents by cold maceration with increasing polarity in order of cyclohexane, ethyl acetate and methanol. Obesity was induced by feeding them with high fat diet. Thirty Wistar rats were divided into six groups. Group 1 treated as control group, Group 2 Wistar rats fed with a High-fat diet for a period of 6 weeks, Group 3 were given atrovastatin 3 mg/kg/day orally for a period of 21 days along with High fat diet in rats for a period of 6 weeks. The animal of the Group 4 were given methanolic extract 400 mg/kg/day, orally for 6 weeks along with High fat diet in rat for a period of 6 weeks. In Group 5 cyclohexane extract were given at a dose 400 mg/kg/day orally for a period of 6 weeks, Group 6 methanolic extract were given at a dose 400 mg/kg/day orally for a period of 6 weeks after induction of obesity with High fat diet in rats for a period of 6 weeks. All the three extracts were found to be significant at p<0.001 and
inhibit the antiobesity activity. The methanolic extract shows the highest antiobesity activity followed by the ethyl acetate and cyclohexane extracts against the atrovastatin as the standard which showed the highest antiobesity activity.

KEYWORDS: Anti obesity, Seaweeds, Turbinaria ornata, Atrovastatin.

INTRODUCTION

Obesity is an excessive accumulation of fats due to sedentary lifestyle and consumption of high caloric foods. At the cellular level adipocytes or fat cells cell size is increase due to the accumulation of fats in the cytoplasm.\(^1\) Obesity is the state of excess body fat stores, which should be distinguished from overweight (i.e. excess body weight relative to a person’s height),'obesity' may be defined as an illness where the health (and hence life expectancy) is adversely affected by excess body fat. The generally accepted benchmark is the body mass index (BMI). The BMI is expressed as W/h\(^2\), where W = body weight (in kg), h = height (in metres). Although it is not a perfect index (e.g. it does not distinguish between fat and lean mass), the BMI is generally well correlated with other measurements of body fat, and it is widely employed in obesity studies. While there are problems in defining a 'healthy' weight for a particular population, the World Health Organization (WHO) classifies people with a BMI of < 18.5 kg/m\(^2\) as 'underweight', and those with a BMI of 18.5-24.9 kg/m\(^2\) as of 'acceptable' or 'normal' weight. A BMI in the range of 25.0-29.9 kg/m\(^2\) signifies 'grade 1 overweight'. If it is between 30.0 and 39.9 kg/m\(^2\), the patient is deemed to be obese or 'grade 2 overweight', while those with a BMI of > 40 kg/m\(^2\) are said to be 'grade 3 overweight'.\(^2\) Once it was considered that obesity was only in high income countries. But now a day, it has spread dramatically in medium and low income countries.\(^3\) Obesity is linked to a metabolic syndrome which is known to cause hypertension, diabetes and hyperlipidemia.\(^4-7\) BMI obviously depends on the overall energy balance, another operational definition of obesity would be that it is a multifactorial disorder of energy balance in which calorie intake over the long term exceeds energy output.\(^8\) Prevention of obesity is done by medicinal treatment directly from nature and it is better than chemical and surgical treatment.\(^9\)

MATERIALS AND METHODS

Collection and Authentication of Algae

*Turbinaria ornata* was collected from Erwadi in gulf of Mannar, Ramanthpuram district, Tamil Nadu, India on October 10, 2016. This was authentified by Dr. K. Eswaran (Prinicipal Scientist), CSIR - CSMCRI MARINE ALGAL REASERCH STATION, Mandapam Camp-
623519, Tamilnadu. Adhered sand and salts were removed from the algae by washing with sea water and the algae was transported to the laboratory of Pharmacology, I.T.S college of Pharmacy, Muradnagar, Ghaziabad, India.

**Animals**

Wistar rats of either sex (150-200 g) were used for experimental purpose. The animals were housed in hygienic cages (6 rats / cage) under standard conditions of temperature (25±2)°C, relative humidity (45±20) % and (light) 12h: (dark) 12h cycle. The rats were fed with standard pellet diet (Amrut feeds, Chakan) and water. The animals were allowed to acclimatize to experimental conditions by housing them for 8-10 days prior to the experiments. The Formalin experimental design and research plan along with animals handling and disposal procedure were approved by Institutional Animal Ethical Committee. Registration no1044/PO/Re/S/07/CPCSEA, 27 feb, 2007, Project proposal no. ITS/07/IAEC/2013.

**Chemicals**

Atrovastin, Ethyl Alcohol, Solvents like methanol, ethyl acetate, cyclohexane, chloroform, petroleum ether, acetone, di-ethyl ether were of analytical grade (AR). Serum Cholesterol kit, Serum Triglyceride kit, Serum HDL Cholesterol kit, Serum LDL-Cholesterol kit, Serum VLDL Cholesterol kit, Formaldehyde (40%), High fat diet (33% standard chow, 33% Nestlé, condensed milk, 7% saccharine and 27% water).

**EXTRACTION AND PHYTOCHEMICAL INVESTIGATION**

**Drying of Algae**

*Turbinaria ornata* was shade dried at room temperature and reduced to a coarse powder with the help of the grinder, 1000 gm of the *turbinaria ornata* coarsely powderd obtained after drying, before extraction powdered material was passed through a sieve to assure easy successive cold maceration.

**Extraction Procedure**

The plants of *Turbinaria ornata* was dried in shade and powdered. Dried algal powder (1000 g) was extracted three times each by cold maceration at room temperature. In cold maceration whole powdered plant drug is kept in contact with the solvent in a stoppered container for 48-72 hours with frequent agitation until soluble matter is dissolved. This method is best suitable for use in case of the thermo labile drugs by Tiwari P. *et al.* (2011). For extraction, the
powder was taken in round bottom flask and macerated for 72-48 hours at room temperature with all aerial defatted completely by Handa, S. S., et al. (2008). The algae was extracted with three solvents with increasing polarity in order of cyclohexane, ethyl acetate and methanol respectively at the room temperature three times each. The extracts thus obtained were concentrated by distilling off the solvent under reduced pressure by using Rota vacuum Evaporator (Buchi, Germany) Suffness M., and Douros J., (1978). The defatted marc thus obtained was air dried and then the percentage yields of all three extracts i.e. cyclohexane, ethyl acetate and methanol of *Turbinaria ornata* was calculated.

**Extraction with cyclohexane: successive cold maceration**

1000 gm of powdered algae was cold macerated thrice for 72 hr with the cyclohexane, for the first time the extract was macerated with 800 ml cyclohexane and then filtered, again for the second time extract was macerated with 700 ml cyclohexane and then filtered, same procedure was to be followed for the third time and the filtered extract were dried and the dried obtained extract yield was 2.4 gm., percentage yield was determined using following formula

\[
% \text{ yield} = \frac{\text{weight of extract in gram}}{1000} \times 100
\]

**Extraction with ethyl acetate: successive cold maceration**

1000 gm of powdered algae was cold macerated thrice for 72 hr with the ethyl acetate, for the first time the extract was macerated with 800 ml ethyl acetate and then filtered, again for the second time extract was macerated with 700 ml ethyl acetate and then filtered, same procedure was to be followed in the third time and the filtered extract were dried and the dried obtained extract yield was 3.97gm. Percentage yield was determined using formula.

**Extraction with methanol: successive cold maceration**

1000 gm of powdered algae was cold macerated thrice for 72 hr with the methanol for the first time the extract was macerated with 800 ml methanol and then filtered, again for the second time extract was macerated with 700 ml methanol and then filtered, same procedure was to be followed in the third time and the filtered extract were dried and the dried obtained extract yield was 12 gm. percentage yield was determined using formula.

**Phytochemical Analysis of *Turbinaria ornata* Extracts**

The extract were subjected for phytochemical investigations by using the standard procedures according to the methods described by Khandelwal and Kokate. Different extracts of the
*turbinaria ornata* shows the presence of phytoconstituents like alkaloids, terpenes, phenols, tannins, saponins, flavonoids, quinones, proteins, sugars, carbohydrates, alkaloids, coumarins, steroids terpenoids and cardiac glycosides.

**ACUTE TOXICITY STUDIES**

The acute toxicity was performed according to OECD guidelines (OECD 423). The selected wistar rats were used for toxicity studies. The animals were divided into four groups of three in each. The animals were fasted overnight prior to the acute experimental procedure. Extract was given orally to rats at the graded dose like 1000, 2000 and 4000 mg/kg Body Wt. immediately, after dosing, the animals were observed continuously for first four hours for behavioural changes and for mortality at the end of 24 h and daily up to 14 days for any behavioural change or mortality.[12]

**Induction of obesity**

The Wistar rats were given high fat diet which was with regular laboratory pellets supplemented with (33% standard chow, 33% Nestlé, condensed milk, 7% saccharine and 27% water). Body weight was measured once in every two days. to compare the difference in weight over the period of experimentation.

**Experimental design**

Thirty Wistar rats were divided into six groups, five in each group.

(I) **Group**: Normal control group.

(II) **Group**: High-fat diet for a period of 6 weeks.[13]

(III) **Group**: atrovastatin were given 3 mg/kg/day, orally for a period of 21 days along with High fat diet in rats for a period of 6 weeks (14)

(IV) **Group**: methanolic extract 400 mg/kg/day, orally along with High fat diet for a period of 6 weeks.

(V) **Group**: cyclohexane extract were given at a dose 400 mg/kg/day; orally along with High fat diet in rats for a period of 6 weeks

(VI) **Group**: methanolic extract were given at a dose 400 mg/kg/day; orally along with High fat diet in rats for a period of 6 weeks.

**Biochemical parameters**[15-18]

At the end of treatment blood samples were collected from all the groups of the animals through the retro-orbital sinus without the use of anti-coagulant. The blood sample was
Centrifuged using centrifuge at 2000 rpm for 30 min to get serum for study of various biochemical parameters. The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer’s instruction manual provided in the kit.

**MEASURE THE PHYSICAL PARAMETERS**

**Percentage change in body weight**

1. Body wt. of individual animal were taken for each group and record were maintained.
2. Body wt. Were taken daily from the starting day of the study till the last dosing was done before sacrificing the animal.
3. If death of any animal occurs in between the study time, its weight were taken.
4. Any change in the body wt. of the animal were record.

**Histopathological examination of fat pad:**

**Isolation of fat pads**

Three regions of adipose tissue were carefully dissect:

1. The periovarian fat, ovaries were taken out by gentle squeezing from the peripheral fat and then by horizontal cut from all sides fat was isolated; care has been taken that too much traction was avoided on ovaries and fat.
2. The retroperitoneal, by first separating the perirenal fat and then dissecting the retroperitoneal pad in to.
3. The mesenteric, all fat found along the mesentery starting at the lesser curvature of the stomach and ending at the sigmoid colon was considered mesenteric fat.

The periovarian fat of each group were excised and rinsed in 0.9% saline blotted dry of saline and excess blood. They were fixed in 12% formalin for 24 h. The stained slides were observed (×200) in research microscope and photographed.[19]

**Statistical analysis**

For multiple comparisons, one-way analysis of variance (ANOVA) (One way ANOVA followed by Dunnett’s test were used. In case ANOVA were shown significant difference, post hoc analysis were performed. \(P< 0.01\) were considered being statistically significant.
RESULTS AND DISCUSSION

Table No. 1: Percentage yields of extracts.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Extracts</th>
<th>Amount of extract (in gms)</th>
<th>Percentage yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cyclohexane extract of <em>Turbinaria ornata</em></td>
<td>2.4</td>
<td>0.24%</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl acetate extract of <em>Turbinaria ornata</em></td>
<td>3.97</td>
<td>0.397%</td>
</tr>
<tr>
<td>3</td>
<td>Methanolic extract of <em>Turbinaria ornata</em></td>
<td>12</td>
<td>1.2%</td>
</tr>
</tbody>
</table>

ACUTE TOXICITY STUDY

The dose selected for the study was **400 mg/kg** of each of the three extracts.

Table no. 2: Phytochemical Analysis of *Turbinaria ornata* Extracts.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Phytoconstituents Present</th>
<th>Cyclohexane extracts of <em>Turbinaria ornata</em></th>
<th>Ethyl acetate extract of <em>Turbinaria ornata</em></th>
<th>Methanol extract of <em>Turbinaria ornata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids (+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>2</td>
<td>Cardiac glycosides (+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>3</td>
<td>Tannins (+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>5</td>
<td>Reducing Sugars (-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>6</td>
<td>Anthraquinones (-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>7</td>
<td>Steroids (-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>8</td>
<td>Proteins (-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>9</td>
<td>Flavonoids (+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>10</td>
<td>Saponins (+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>11</td>
<td>Phenols (+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

(+) indicates presence (-) indicates absence

TOTAL FLAVONOID CONTENT

Table no. 3: Total flavonoid content of different extracts.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (μg/ml)</th>
<th>Absorbance of extracts</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Methanol</td>
<td>Ethyl acetate</td>
<td>Cyclohexane</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>0.207±0.057</td>
<td>0.269±0.02</td>
<td>0.194±0.06</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>0.417±0.055</td>
<td>0.283±0.055</td>
<td>0.233±0.050</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>0.537±0.017</td>
<td>0.348±0.013</td>
<td>0.238±0.064</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>0.660±0.02</td>
<td>0.470±0.023</td>
<td>0.366±0.021</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>0.790±0.019</td>
<td>0.529±0.019</td>
<td>0.464±0.086</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>600</td>
<td>0.874±0.043</td>
<td>0.774±0.043</td>
<td>0.559±0.032</td>
<td></td>
</tr>
</tbody>
</table>
Figure No 1: standard curve of Quercetin.

![Standard curve of Quercetin](image1)

Figure No 2: Total flavonoid content in methanol extract of *Turbinaria ornata*(mg/g).

![Total flavonoid content in methanol extract of Turbinaria ornata](image2)

Figure No 3: Total flavonoid content in ethyl acetate extract of *Turbinaria ornata*(mg/g).

![Total flavonoid content in ethyl acetate extract of Turbinaria ornata](image3)
Table no. 4: Total flavonoid content in different extracts of *Turbinaria ornata*.

<table>
<thead>
<tr>
<th>S.No</th>
<th><em>Turbinaria ornata</em> extracts</th>
<th>Total flavonoid content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol extract of <em>Turbinaria ornata</em></td>
<td>31.6</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl acetate extract of <em>Turbinaria ornata</em></td>
<td>24.3</td>
</tr>
<tr>
<td>3</td>
<td>Cyclohexane extract of <em>Turbinaria ornata</em></td>
<td>19.7</td>
</tr>
</tbody>
</table>

Table no. 5: Total phenolic content estimation.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (μg/ml)</th>
<th>Absorbance of standard (Gallic acid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>0.115±0.25</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>0.245±0.65</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>0.345±0.05</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>0.476±0.04</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>0.693±0.06</td>
</tr>
</tbody>
</table>

Figure No. 5: Estimation of total phenolic content in Standard
Figure No: 6 Total phenolic content in methanol extract of *Turbinaria ornata*(mg/g).

Figure No. 7: Total phenolic content in ethyl acetate extract of *Turbinaria ornata*(mg/g).

Figure No. 8: Total phenolic content in cyclohexane extract of *Turbinaria ornata*(mg/g).
Table No 6: Total phenolic content of different extracts of *Turbinaria ornata*(mg/g).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Turbinaria ornata extracts</th>
<th>Total phenolic content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol extract of <em>Turbinaria ornata</em></td>
<td>40.3</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl acetate extract of <em>Turbinaria ornata</em></td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>Cyclohexane extract of <em>Turbinaria ornata</em></td>
<td>23.16</td>
</tr>
</tbody>
</table>

**ANTIOBESITY ACTIVITY**

Table No. 7: Effect of different extracts of *Turbinaria ornata* on body weights in high fat diet induced obesity wistar rats.

<table>
<thead>
<tr>
<th>S No</th>
<th>Weeks</th>
<th>Normal Control</th>
<th>POSITIVE CONTROL</th>
<th>Methanol</th>
<th>Ethyl acetate</th>
<th>Cyclohexane</th>
<th>Standard (Atorvastain 3 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>150.28±1.64</td>
<td>170.07±1.90</td>
<td>160.12±1.8**</td>
<td>165.1±1.79*</td>
<td>185.13±1.58*</td>
<td>180.24±1.81**</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>165.10±2.15</td>
<td>180.54±2.93</td>
<td>178.16±2.9**</td>
<td>168.25±2.56*</td>
<td>190.25±3.56*</td>
<td>185.91±2.55**</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>170.55±2.25</td>
<td>190.55±3.38</td>
<td>185.28±3.4**</td>
<td>175.28±2.72*</td>
<td>192.34±2.56*</td>
<td>190.02±3.22**</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>180.40±2.72</td>
<td>195.12±3.70</td>
<td>190.29±3.3**</td>
<td>195.07±2.98*</td>
<td>195.77±3.27**</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>185.31±2.79</td>
<td>235.44±3.75</td>
<td>200.44±3.6**</td>
<td>200.25±3.45*</td>
<td>200.59±2.48*</td>
<td>200.17±3.48**</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>190.85±2.81</td>
<td>262±3.86</td>
<td>195.44±2.9**</td>
<td>210.39±3.51*</td>
<td>215.61±2.72*</td>
<td>198.62±3.72**</td>
</tr>
</tbody>
</table>

All Values were expressed as mean ± SD (n = 5), *p<0.001 when compared to control group = **p<0.001 when compared to standard (One way ANOVA followed by Dunnett’s test).

Figure No. 9: Effect of different extracts of *Turbinaria ornata* on body weights in high fat diet induced obesity wistar rats All Values were expressed as mean ± SD (n = 5), *p<0.001 when compared to control group = **p<0.001 when compared to standard (One way ANOVA followed by Dunnett’s test).
Table No. 8: Effect of different extracts on biochemical parameters and in high fat diet induced wistar rats.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Groups</th>
<th>TC</th>
<th>TG</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NORMAL</td>
<td>83.46±1.47</td>
<td>80.86±2.12</td>
<td>36.51±5</td>
<td>39.6 ±4</td>
<td>25.91±3.9</td>
</tr>
<tr>
<td>2</td>
<td>POSITIVECONTROL</td>
<td>195±8.2</td>
<td>125.97±8*</td>
<td>78.24±3.8*</td>
<td>85.25±5.7**</td>
<td>61.43±3.9**</td>
</tr>
<tr>
<td>3</td>
<td>METHANOL</td>
<td>125±3.9</td>
<td>85.21±8.2</td>
<td>22.9±6.3**</td>
<td>59.55±4.8*</td>
<td>26.2±6.1</td>
</tr>
<tr>
<td>4</td>
<td>ETHYLACETATE</td>
<td>165.18±8.7</td>
<td>94.4±6.4</td>
<td>29.44±3.9</td>
<td>67.78±6.1</td>
<td>35.8 ±4.9</td>
</tr>
<tr>
<td>5</td>
<td>CYCLOHEXANE</td>
<td>170.32±5.9</td>
<td>100±5.6</td>
<td>34.89±4.2**</td>
<td>71.46±3.9*</td>
<td>41.68±3.5**</td>
</tr>
<tr>
<td>6</td>
<td>ATROVASTATIN</td>
<td>90.18±3.8</td>
<td>81.4±4.9*</td>
<td>32.43±2.7**</td>
<td>41.34±3.8**</td>
<td>22.8±4**</td>
</tr>
</tbody>
</table>

All Values were expressed as mean ± SD (n = 5), *p<0.001 when compared to control group = **p<0.001 when compared to standard (One way ANOVA followed by Dunnett’s test).

**GROUPS**

Figure No. 10: Histogram representing effect of different extracts of *Turbinaria ornaata* on serum lipid levels against high fat diet induced obesity.

**MICROSCOPIC EVALUATION BY HISTOPATHOLOGY ANTIOBESITY ACTIVITY**

Histopathological Changes In The Adipocytes
Figure No 11: (Control group of *Turbinaria ornata*), the adipocytes of the normal shows which indicates obese adipocytes.

Figure No 12: (Standard group of *Turbinaria ornata*), the adipocytes shows the reduction in size, shrinkage as well as arrow shows destruction of adipocytes.

![Figure 11](image1)

![Figure 12](image2)

Figure No 13 (Positive control group of *Turbinaria ornata*), the arrow indicates the reduction of size It also shows that the shrinkage as well as the destruction of the adipocytes. obese adipocytes after receiving the high fat diet.

Figure No 14 (Methanol extract of *turbinaria ornata*), after the administration of the methanolic extracts arrow shows the destruction in cell wall which affects the integrity of cell and show shrinkage of adipocytes as well as destruction of cell wall.

![Figure 13](image3)

![Figure 14](image4)

Figure No 15 (Cyclohexane extract of *turbinaria ornata*), After administration of the cyclohexane extracts it indicates the shrinkage of cell as well as the arrow shows destruction of cell wall, this destruction can leads to the leakage of the cell contents which cause apoptosis of the adipocytes.

![Figure 15](image5)

![Figure 16](image6)
Figure No. 16: (ethyl acetate extract of turbinaria ornata) After administration of ethyl acetate the arrow indicates. severe shrinkage of adipocytes as well as severe destruction of cell wall

RESULT AND DISCUSSION

 Obesity is defined as a chronic metabolic disorder that is characterized by increased lipid concentration and enlarged fat mass. It is a result of imbalance between energy expended and energy taken in. At the cellular level, it is characterized by an increase in size and number of adipocytes differentiated from fibroblastic preadipocytes in adipose tissues. Further more, obesity has led to reduced life expectancy and health problems such as type 2 diabetes, and cardiovascular diseases and obstructive sleepapnea etc among others.

High fat diet-induced obesity has been considered as the most accepted model among researchers due to its high similarity of mimicking the usual route of obesity episodes in human and so why it is considered as a reliable tool for studying obesity as they will readily gain weight when feed high-fat diets.

The extracts of Turbinaria ornata like methanol, cyclohexane and ethyl acetate were screened for the presence of the phytoconstituents both qualitatively and quantitatively. Phytochemical screening showed the presence of saponins, glycosides, phenols, flavonoids, alkaloids and tannins, carbohydrates, steroids. All the extracts were successively cold macerated for 72 hours. The flavonoid content in the methanolic extract was calculated to be 31.6 mg/g Quercetin equivalent, ethyl acetate contained 24.3 mg/g Quercetin equivalent while the cyclohexane extract contained 19.7 mg/g Quercetin equivalent. The phenolic content was estimated to be 40.3mg/g gallic acid equivalent in methanolic extract of, ethyl acetate extract of Turbinaria ornata contained 30 mg/g gallic acid equivalent while the cyclohexane extract of Turbinaria ornata contained 23.16 mg/g gallic acid equivalent phenolic content. On acute oral toxicity the extract was found to be safe up to 4000 mg/kg and thus the 1/10th of the dose 400mg/kg was selected for the studies.

Effects of High-Fat Diet and Turbinaria ornata extracts on Body Weight: In the present study, the effect of Turbinaria ornate was observed on the rats fed with HFD (High fat diet). The study was conducted for about 6 weeks. High Fat Diet substantially increased body weights of rats when compared to normal control group. On VIth week, when compared to
HFD control, HFD+ atorvastatin mg/kg, *Turbinaria ornata* low and high dose showed significant activity in decrease in the body weight. *Turbinaria ornata* high dose showed similar activity when compared to standard drug atorvastatin (Table no-7). The result also suggests that *Turbinaria ornata* extracts at 400 mg/kg are capable of preventing body weight gain.

**Serum Lipid Profiles:** Biochemical parameter’s such as HDL, Triglycerides and Total cholesterol were measured using biochemical kits. LDL and VLDL were calculated with the formulas respectively. Rats fed with HFD showed increased levels of serum Triglycerides, LDL, VLDL and total cholesterol and decreased HDL levels. However oral administration of *Turbinaria ornata* extract significantly suppressed the rise of Lipid profile and rise in the HDL levels were observed. When compared to HFD+ atorvastatin 3 mg/kg, *Turbinaria ornata* high dose showed significant difference in reducing the serum Lipid profile (Table No.-8) Effects of *Turbinaria ornata* extracts on lowering the TG, TC and LDL. There might be two probable mechanisms behind the observed anti-obesity and hypolipidemic effect of the extract, i.e., reduce in dietary cholesterol absorption in the intestinal tract or interference in the synthesis of cholesterol. The inhibition in the absorption of dietary fats usually limits the excess energy required for the storage of fats in adipose tissue which was seen with the significant suppression of visceral fats in the treated rats.

As shown in microscopic evaluation by histopathology dietary obesity, therefore, leads to an increase in number of adipocytes (hyperplasia) and their size (hypertrophy) (figure no-11 to 16). After the administration of the different extracts of *Turbinaria ornata* it shows the destruction in cell wall which affects the integrity of cell and show shrinkage of adipocytes as well as destruction of cell wall.

All the three extracts were found to significant at p<0.001 and inhibit the antiobesity activity. The present findings emphasize that the methanolic extract shows the highest antiobesity activity followed by the ethyl acetate and cyclohexane extracts. Atrovastatin (standard drug) showed the highest antiobesity activity.

**CONCLUSION**
The anti-obesity activity of *Turbinaria ornata* the methanolic extract shows the highest antiobesity activity followed by the ethyl acetate and cyclohexane extracts against the
atrovastatin as the standard which showed the highest antiobesity activity and extract of *Turbinaria ornata* was confirmed by the following measures in high fat diet.

Reduced the body weight
Reduced LDL levels
Reduced Triglycerides
Reduced cholesterol levels
Reduced fad pad weights
Increased HDL levels

From the above, it may be concluded that the *Turbinaria ornata* shows anti-obesity property. When taken along with diet, the plant is shown to reduce obesity and the *Turbinaria ornata* extracts may be further explored for its potential in treatment of obesity.

**REFERENCES**


6. M. Ouimet, “Autophagy in obesity and atherosclerosis: interrelationships between cholesterol homeostasis, lipoprotein metabolismand autophagy inmacrophages and other systems,”


