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# EVALUATION OF ANTI-OBESITY POTENTIAL OF *IXORA PAVETTA* AGAINST HIGH FAT DIET INDUCED OBESITY IN WISTAR RATS

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**Abstract:** The aim of the study was to evaluate the anti-obesity effect of butenolic fraction of *Ixora pavetta*in high fat diet induced obesity in wistar rats. Female rats were fed with high fat diet for 8 weeks. Butenolic fraction of *Ixora pavetta* (BIP) was administered at a dose of 250 mg/kg, p.o. and 500 mg/kg for last 3 weeks while high fat diet offering. Atorvastatin 30 mg/kg P.O. was used as a standard. The effect of BIP on following parameters was recorded - body weight, feed consumption and various biochemical parameters like serum glucose, serum lipid profile eg.total cholesterol (TC) and triglyceride (TG), HDL-C, LDL-C, VLDL and atherogenic index. Other biochemical parameters like SGOT, SGPT and total protein were estimated. Finally effect on vital organs like liver, heart and kidney as well as epididymal fat pad was also recorded.There was a significant reduction in body weight and other increased biochemical parameters like serum glucose levels, TG, TC, LDL-C, VLDL, SGOT and SGPT. Decreased HDL-C level in HFD fed animals were significantly improved due to treatment of BIP. Atheroginic index as well as epididymal fat pad weight was found to be reduced due to BIP treatment. The results by test and standard drug were comparable. The result strongly indicates butenolic fraction of *Ixora pavetta*posses anti-obesity action in high fat diet induced obesity in wistar rat's model.

**Keywords:** *Ixorapavetta*, Butenolic fraction, anti-obesity, Atheroginic index.

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## INTRODUCTION

Obesity is a chronic disease of increasing prevalence in most countries, which leads to substantial increase in morbidity, and mortality in association with insulin resistance, diabetes, hyperlipidaemia, hypertension, and other cardiovascular diseases[1]. Among the multiple factors contributing to its etiology, the sedentary life styles, white collar jobs, lack of exercise, psycho-logical factors, and the consumption of energy rich diets are the major ones[2]. In Brief, obesity is an abnormal excessive growth of adipose tissue, results from the combined effects of excess energy intake and reduced energy expenditure[3.]

Although a multitude of pharmaceutical agents are available for the treatment of obesity, the long-term persistence rate with medications is poor probably due to adverse effects, modest efficacy and expense. However, adverse effects of these drugs are not negligible, and must be taken into consideration when it is time to prescribe one of these anti-obesity drugs[4,5.]

While extensive research has been conducted on the development of anti-obesity drugs, the phytoconstituents like flavonoids, saponin, alkaloids derived from plant sources, including caffeine in oolong tea[6], saponin in the roots of broad bellflower[7] and capsiate in sweet pepper[8], for preventing and ameliorating obesity have been investigated.

The plant, *Ixora pavetta* Andr is reported for presence of various phytochemicals like essential oil, flavanoids, saponins, resins, phytosterols, alkaloids, tannins. Due to presence of these phytochemicals, various parts of plants like flowers, root and leaves are claimed to have medicinal benefits in various disorders like such as dysentery, leucorrhoea, dysmenorrhea, haemoptysis bitter tonic, aperients, purgative, urinary disorders and is frequently prescribed in visceral obstructions[9, 10].

However, effect of *Ixora pavetta* or its phytoconstituents have not reported for anti-obesity effect. In the present study, the anti-obesity effects of *Ixora pavetta* was investigated in rats fed with High Fat (HF) diet by measuring changes in body weight, food consumption, serum biochemicalprofile,changes in epididymal fat pad weights

## MATERIALS AND METHODS

### Collection of Plant material

Fresh leaves of Ixora Pavetta was obtained from local area of Kadapa & authentified by Botanist; S.V University, Tirupathi, (A.P). The specimen voucher of same is kept in department of pharmacology; PRRM College of Pharmacy; Kadapa.

### Extraction and fractionation of Plant material

The collected plant material of*Ixora Pavetta* was washed thoroughly in water, and air dried for two weeks. The 500 gm of air dried and coarsely powdered material of plants were extracted

with 95% of ethanol by cold maceration method for 72 hrs. Then the extract was filtered with muslin cloth and filtrate was evaporated under reduced pressure and vacuum dried. The yielded a greenish residue was 20- 25% W/W extract with reference to dry starting material. Further this alcoholic extract was fractioned with non polar solvents to polar solvents system (Pet ether – Chloroform - Ethyl acetate – n Butenol) and all fractions were tested for preliminary phytochemical tests. Butenolic fraction of the plant showed presence of maximum phytochemicals and hence this fraction is labeled as Butenolic Fraction of *Ixora Pavetta* (BIP)

**Experimental Animals**

Healthy adult female albino rats were procured from Raghavendra enterprises, Bangalore weighing between 100-150 g was used. They were housed under standard laboratory conditions and food and water were provided *ad libitum*. The temperature was kept at 22 ± 2º c. The animals were maintained under a 12 h light / 12 h darkness cycle. All animal procedures were approved by the Institutional Animal Ethical Committee of P. Rami Reddy Memorial college of pharmacy, Kadapa(Ref No: 1423/PO/a/11/CPCSEA/001).

**Chemicals:**

Most of Ingredients for High fat diet obtained from local market Kadapa. Atorvastatin and all other chemicals were procured from SD fine chemicals Ltd. India andwere of analytical grade.

**Composition of High fat diet and Induction of obesity:**

The composition of high fat diet contains 23% whole wheat, 23% yellow corn, 11% barley, 17% milk powder, 1% bone meal, 1 % calcium chloride, 1% sodium chloride, 11% coconut oil, 11% butter and one multivitamin capsule. The diet was prepared daily morning freshly and offered in form of ball to animals[11]. The rats were rendered obese by a HF diet, while the control rats had access to a normal (N) diet for the total period of 8 weeks. After 5 weeks of HF diet, the rats received daily treatment with test and standard drugs for another 3 weeks[1].

**Experimental design**

The animals offered with High fat diet (HFD) for 5 weeks were divided into four groups (n=6). All groups were treated with test and standard drugs for further 3 weeks as fallows. Normal control group offered with normal diet was maintained separately and received vehicle for treatment period.

Butenolic Fraction of Ixora pavetta (BIP)and thestandard drug Atorvastatin were freshly suspended in 1% CMC daily and administered to animals by oral feeding needle immediately.

| SN | Group | Treatment schedule (3 weeks): |
|---|---|---|
| I | Normal | Normal feed + 1 % CMC (p.o.) |
| II | Control | High fat diet + 1 % CMC (p.o) |
| III | Standard | High fat diet + Atorvastatin (30 mg/kg ,p.o) |
| IV | Low dose 250 mg/kg | High fat diet + BIP (250 mg/kg, p.o.) |
| V | High dose 500mg/kg | High fat diet + BIP (500 mg/kg, p.o.) |

**Measurements and sample collection**

Body weights and food intake were recorded twice a week throughout experimental period. At the end of experiment, the blood samples were collected from the retro orbital venous plexus of rats without any coagulant for the separation of serum. After collecting the blood into micro centrifuge tubes it is kept aside for 30 min at room temperature and then serum was separated by centrifugation at 2000 rpm for 15 min and stored at -80º C until analyzed for various biochemical parameters.

After blood collection, animals were sacrificed by deep anesthesia and vital organs like liver, kidney, heart as well as epididymal fat pads were isolated and weighted immediately.

**Estimation of Biochemical Parameter:**

Various biochemical parameter like serum glucose, total cholesterol (TC) , HDL, Triglycerides (TG), SGOT, SGPT and total protein were estimated by using Erba kit and semi auto analyzer (Maxlyzer, Avecon model no: NB-201). Other parameters like LDL, VLDL and atherogenic index were calculated by using equitation-

- VLDL= TG/5
- LDL = {TC – (HDL + VLDL)}
- Atherogenic index = $\frac{\text{LDL + VLDL}}{\text{HDL}}$

**Statistical analysis**

All the data was expressed as Mean ± S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Dunnet's test using computer based fitting program (Prism graph pad.). Statistical significance was taken as $P< 0.01$.

## RESULTS

### Body weight and Food consumption

The body weight of each group was recorded twice a week throughout the study period. The results obtained where shown as Mean ±SEMin Fig No 1. Animals Offered with high fat deit showed gradual body weight gain with time as compaired to animals maintainted on normal deit.

After 5 week offering of High fat deit (HFD), Animal treated with standered and test group for next 3 weeks showed significant reduction of body weight gain. Butenol fraction of Ixora pavetta (BIP) showed dose dependant reduction in body weight from Day 48 ($p<0.01$) to Day 57 ($p<0.0001$) when compaired to HFD control group. Animals treated with standered drug i.e. Atorvastatin 30 mg/ kg P.O also showed statistically significant ($p<0.0001$) reduction in body weight gain

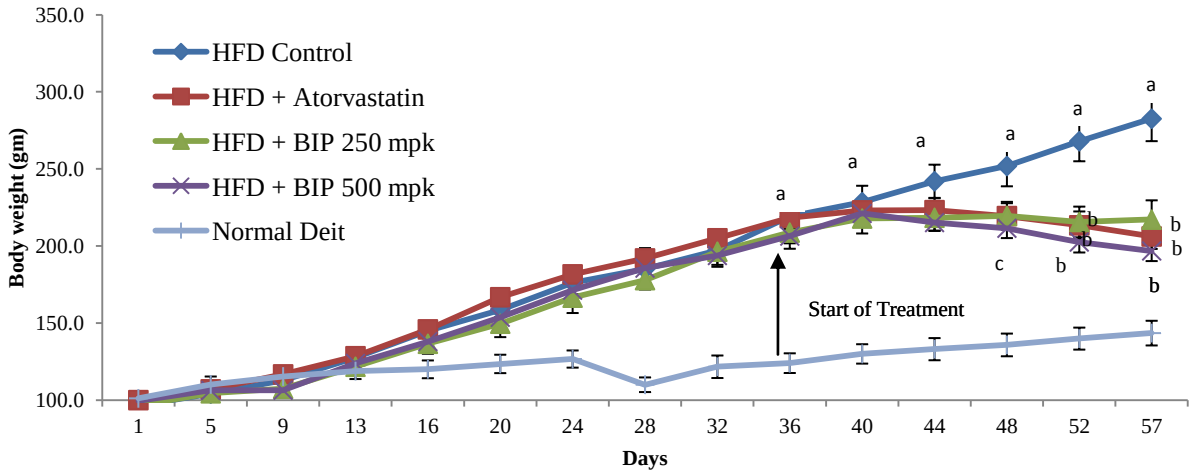


**Figure 1:- Effect of BIP on body weight gain**

Day wise body weight for experimental group. Values are represented as Mean ± SEM. Statistical analysis performed using one way ANOVA followed by dunnett's test for each individual day. a- P<0.0001vs Normal Diet group; b- P<0.0001 vs HFD control group; c- P<0.01 vs HFD control group (should be written as caption for Fig 1) (Start New paragraph) Feed consumption was calculated from feed offered and feed left to next day (including weastage of feed in cage). Feed consumption of all the groups was recored every day throughout experimental period. The results were expressed as feed consumed per animal per day in gram. Feed consumption pattern for all the treated and control group was found simillar as shown in Fig No 2.

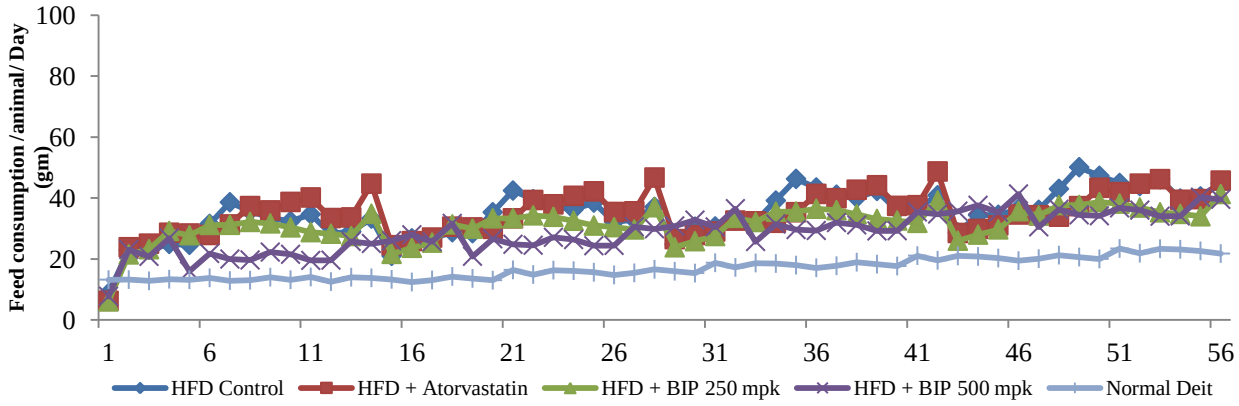


**Figure 2:- Feed consumption**

Feed consumption data for each group. Values are represented as feed consumed per animal per day in gram.

**Serum Parameters**

**Effect on Biochemical Parameters**

Serum biochemical parameters were estimated by using Erba kit. The results obtained where shown as Mean± SEM in Fig No 3, 4 and Table 1. The animals fed with high fat diet showed significant increase ($P<0.0001$) in glucose and lipid parameters like Cholesterol (TC), Triglycerides (TG), VLDL and LDL as well as other biochemical parameters like SGOT and SGPT level when compared to the normal group animals. The treatment with BIP showed a dose dependent decrease in all elevated biochemical parameter level in high fat feed rats. BIP 250 mg/kg b.w. p.o and 500 mg/kg b.w. p.o. showed significant decrease ($P<0.001$, $P<0.0001$ respectively) in glucose and lipid parameter level when compared with HFD control group. In case of SGOT and SGPT, the high dose of BIP i.e. 500 mg/kg treatment was able to produce significant reduction ($P<0.001$), However treatment with low dose of BIP (250mg/kg) was unable to produce any significant achievement. Similar kind of results was obtained in case of HDL-C. HDL-C level of high fat diet control animals were found be significantly decreased ($P<0.0001$) as compared to the normal group animals.BIP 500 mg/kg treatment was able to normalized HDL level and showed significant increased level ($P<0.001$) when compared with HFD control group, However BIP 250 mg/kg treatment was unable to produce significant improvement in HDL levels. Standard drug treatment i.e. Atorvastatin showed significant result in all parameters which were comparable to results shown by high dose treatment of BIP

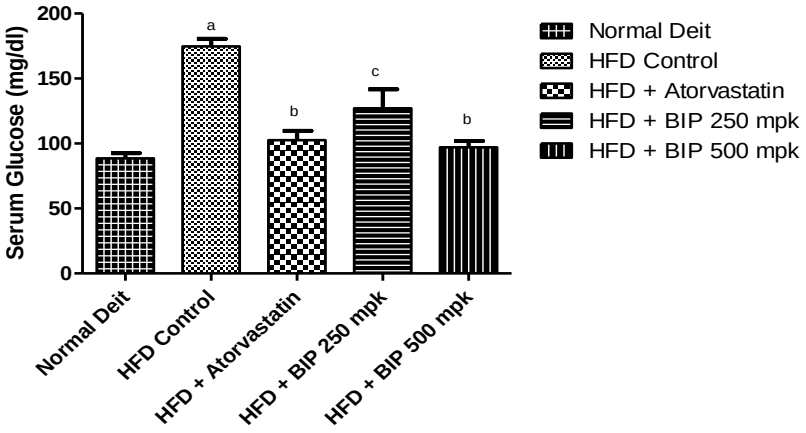


**Figure 3:- Effect on serum glucose level**

Values are represented as Mean ± SEM .Statistical analysis performed using one way ANOVA followed by dunnett's test for each individual day. a- P<0.0001vs Normal Diet group; b- P<0.0001 vs HFD control group; c- P<0.001 vs HFD control group.

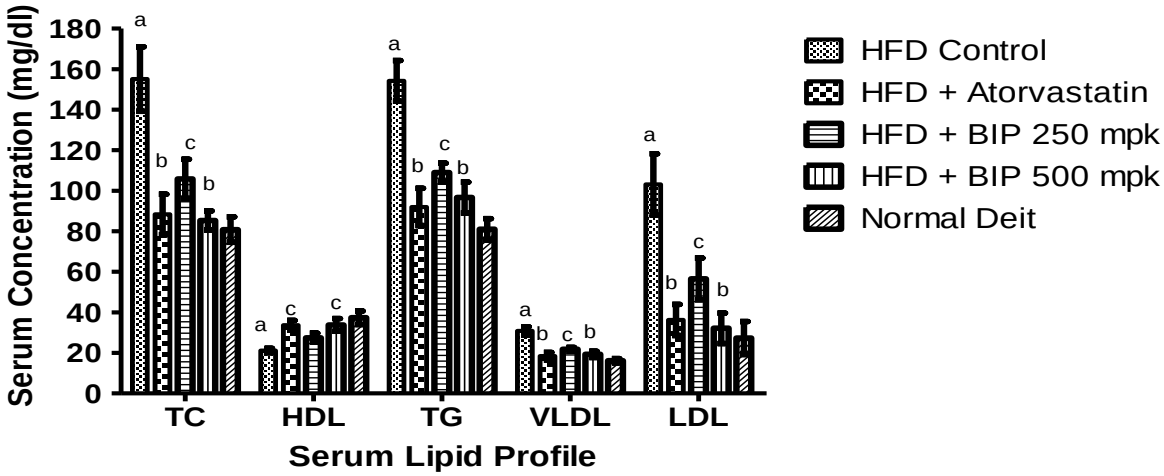


**Figure 4:- Effect on serum Lipid Profile**

Values are represented as Mean ± SEM .Statistical analysis performed using one way ANOVA followed by dunnett's test for each individual day. a- P<0.0001vs Normal Diet group; b- P<0.0001 vs HFD control group; c- P<0.001 vs. HFD control group.

**Table 1:- Effect on Serum SGOT, SGPT and Total Protein**

| Effect on other biochemical parameters | Groups Values (Mean ± SEM) | | | | |
|---|---|---|---|---|---|
| | Normal Diet | HFD Control | HFD + Atorvastatin | HFD + BIP 250 mpk | HFD + BIP 500 mpk |
| SGOT (mg/dl) | 81.0 ± 6.09 | 144.13 ± 5.54[a] | 92.83 ± 5.02[b] | 122.33 ± 14.05 | 101.50 ± 9.24[c] |
| SGPT (mg/dl) | 18.83 ± 1.86 | 42.96 ± 5.23[a] | 20.83 ± 2.4[b] | 32.0 ± 2.43 | 24.0 ± 2.32[c] |
| Total Protein (g/dl) | 5.9 ± 0.42 | 6.66 ± 0.39 | 6.33 ± 0.28 | 6.5 ± 0.40 | 6.66 ± 0.39 |

Values are represented as Mean ± SEM .Statistical analysis performed using one way ANOVA followed by dunnett's test for each individual day. a- P<0.0001vs Normal Diet group; b- P<0.0001 vs HFD control group; c- P<0.001 vs. HFD control group

**Atherogenic Index**

Atherogenic index (AI) was calculated for each animal and the results obtained where shown as Mean ± SEM inFigure no 5. Mean AI for animals fed with normal diet was found to be 1.25 ± 0.27. Animals offered with High fat diet showed significant increase (P<0.0001) in AI (6.48 ± 0.97). Mean AI for Atorvastatin 30 mg/kg, p.o., BIP 250 mg/kg p.o and 500 mg/kg p.o. treatment groups were found to be 1.64 ± 0.18, 3.0 ± 0.55 and 1.70 ± 0.99 respectively. The reduction of AI by all treatments were significant (P<0.0001) as compared to HFD control group.

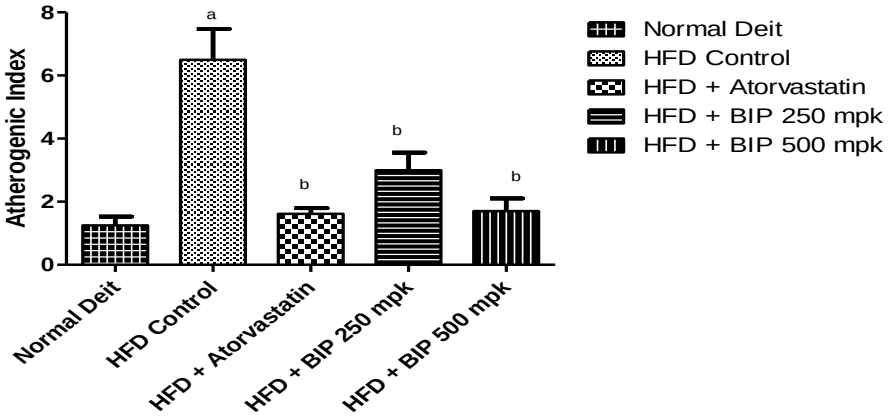


**Figure 5:- Atherogenic Index**

Values are represented as Mean ± SEM .Statistical analysis performed using one way ANOVA followed by dunnett's test for each individual day. a- P<0.0001vs Normal Diet group; b- P<0.0001 vs HFD control group

**Organ Weight and Epididymal Fat pad weight**

End of the experiment, all animals were humanely sacrificed and vital organs like Liver, Kidney and Heart as well as epididymal fat pads were isolated and weighted immediately. The relative organ weights (gm/ Kg b.w) for each animal were calculated as per following formulae and results were represented in Mean ± SEM in Table 2.

Relative Organ weight (g/kg b. wt) = (Absolute organ weight in grams/body weight in grams of final day) X 1000

The animals fed with high fat diet showed significant increase in Liver (P<0.0001), Heart (P<0.001) and Epididymal fat pad (P<0.0001) weight (relative) when compared to the normal group animals. However Kidney weights were found unaltered due to High fat diet. Standard drug treatment i.e. Atorvastatin showed significant decrease in Liver weight (P<0.01) and Epididymal fat pad weight (P<0.0001) when compared to control group. The treatment with BIP showed a dose dependent decrease in increased eipididymal fat pad weights in high fat feed rats. BIP 250 mg/kg b.w. p.o and 500 mg/kg b.w. p.o. showed significant decrease (P<0.001) in eipididymal fat pad weights when compared with HFD control group. Moreover high dose of BIP was found to effective in decreasing increased liver weight.

**Table 2:- Effect on organ weights and fat pad weight**

| Groups | Normal diet | HFD control | HFD + Atorvastatin | HFD + BIP 250 mpk | HFD + BIP 500 mpk |
|---|---|---|---|---|---|
| **Relative Liver Weight (gm/kg b.wt.)** | 41.39 ± 4.19 | 53.72 ± 2.34[a] | 40.26 ± 3.22[c] | 52.52 ± 1.58 | 41.20 ± 4.16[c] |
| **Relative Kidney Weight (gm/kg b.wt.)** | 9.40 ± 0.55 | 9.51 ± 0.29 | 9.54 ± 0.25 | 9.73 ± 0.38 | 9.92 ± 0.30 |
| **Relative Heart Weight (gm/kg b.wt.)** | 3.63 ± 0.27 | 5.60 ± 0.48[b] | 5.66 ± 0.37 | 5.16 ± 0.30 | 5.47 ± 0.47 |
| **Relative Epididymal Fat Pad Weight (gm/kg b.wt.)** | 10.20 ± 0.22 | 16.50 ± 0.99[a] | 11.51 ± 1.05[e] | 12.16 ± 0.62[d] | 12.05 ± 0.82[d] |

Values are calculated as relative weight and represented as Mean ± SEM .Statistical analysis performed using one way ANOVA followed by dunnett's test for each individual day. a- P<0.0001vs Normal Diet group; b- P<0.001 vs Normal Diet group; c- P<0.01 vs. HFD control group; d- P<0.001 vs. HFD control group ; e- P<0.0001 vs. HFD control group.

**DISCUSSION**

The present study demonstrates the beneficial effect of butenolic fraction of *Ixora pavetta* in high fat diet induced obesity model. The body weights, feed consumption, serum biochemical parameters and vital organ weight as well as epididymal fat pad weight were investigated in rats feed with normal diet and High fat diet. Effects of standard and test drugs on all this parameters were explored. The outcome of the study clearly indicates the offering of High fat diet for period of 8 week to the animals resulted into drastic body weight gain as well as alteration of various biochemical parameters. These caused alterations were normalized by 3 week treatment of butenolic fraction of *Ixora pavetta* at 250 mg/kg p.o. and 500 mg/kg p.o. treatment. These doses were selected from earlier published literature where *Ioxa pavetta* was found safe even at 4000mg/kg [12]. The results were compared to standard treatment with hypolipedemic drug Atorvastatin 30 mg/kg p.o. However cumulative food intake of all groups throughout experimental period was similar.

It is well established that elevated cholesterol, Triglycerides and LDL-cholesterol levels in serum as well as a low level of HDL-C is associated with high risk of obesity and associated coronary artery disease [13,]. Reduction in Total cholesterol, LDL- cholesterol and increase in HDL-concentration are significantly related to lipid-lowering therapy. 3 week treatment with BIP 250mg/kg p.o. as well as 500 mg/kg p.o. to high fat diet offered animals resulted into reduction of total cholesterol, Triglycerides, LDL – Cholesterol as well as increase in HDL – cholesterol indicates its potential in lipid lowering therapy.

Generally, it is difficult to detect the CVD risk factor based on individual lipoproteins (TC, LDL-C, HDL-C) and TG levels. Consequently, the Atherogenic index (TC to HDL-C ratio) was used for this purpose[14]. Treatment of BIP at both doses to HFD feed rats resulted into reduction of atherogenic index

The increase in epididymal fat pad weight, Liver weight and Heart weight in HFD fed animals suggested tissue deposition of the fats. Results obtained were in accordance with the changes noticed in body weight pattern. Parallel to increased Body weight reduction, all the treatments were found to be effective to reduce regional epididymal fat pad weight.

**CONCLUSION**

Thus the present study indicates that the butenolic fraction of Ixora pavetta posses anti-obesity effect. The presence of various phytochemicals like essential oil, flavanoids, saponins and

alkaloids in BIP may responsible for its effect. Further studies to understand actual underlying mechanism as well as studies on isolation and structural determination of active principles are in progress.

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