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Research Article

# Evaluation of Lipid Profile and Antioxidant Activity of *Opuntia Ficus - Indica* Powder

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ABSTRACT

The obesity incidence has increased at an alarming rate in recent years, becoming a worldwide health problem,with incalculable social costs. A wide variety of natural materials have been explored for their obesity treatment potential. In the present study obese rats were taken and divided in to two groups are to be made of four rats each. The first group of rats will be administered dehydrated nopal and the second group will be administered standard nopal. Blood samples will be collected from each rat by retro-orbital puncture at 0 (predose) & 15, 30, 45, 60 days (after drug administration). Serum lipid levels will be determined by using by UV/Visible spectrophotometer mentioned in the respective kits. The percent reduction at each time was calculated with respect to initial levels. The in-vivo evaluation includes estimation of serum lipid profile which includes estimation of total cholesterol, triglycerides, HDL-Cholesterol, LDL-Cholesterol. The lipid lowering action of nopal was estimated as 40.57% reduction in total cholesterol levels, 50.29% reduction in triglycerides levels, 61.09% reduction in LDL-Cholesterol levels, 50.07% reduction reduction in VLDL levels and 36.125 % increase in HDL-Cholesterol levels were observed.

## 1. Introduction

Obesity is associated with many diseases, particularly diabetes, hypertension, osteoarthritis, and heart disease. The obesity incidence has increased at an alarming rate in recent years, becoming a worldwide health problem, with incalculable social costs[1-3].

Two different obesity-treatment drugs are currently on the market: orlistat, which reduces intestinal fat absorption via inhibiting pancreatic lipase; and sibutramine, an anorectic or appetite suppressant[4]. Both drugs have hazardous side-effects, including increased blood pressure, dry mouth, constipation, headache, and insomnia. For this reason, a wide variety of natural materials have been explored for their obesity treatment potential[5, 6]. These are mainly complex products having several components with different chemical and pharmacological features.

Nopal is used for diabetes, hypercholesterolemia, obesity, alcohol-induced hangover, colitis, diarrhoea, benign prostatic hypertrophy (BPH) and atherosclerosis[7, 8]. It has been demonstrated that nopal leaves are effective for the treatment of diabetes, atherosclerosis, BPH, and alcohol hangover with very almost negligible side-effects. Nopal leaves have a high content of fiber and pectin. It appears that pectin can alter hepatic cholesterol metabolism without affecting cholesterol absorption. Soluble fiber may also help lower the level of cholesterol in the blood[9-12]. The present study is aimed to determine the lipid lowering effect, weight reduction and anti-oxidant effect of nopal in albino wistar rats.

## 2. Materials and Methods

***Preparation of dehydrated nopal:***

*Drying of Nopal pads:* 1kg ($W_1$) of nopal pads were collected, thorns were removed. External cuticle was removed, and the pads were cut into small pieces. These pieces were subjected for air drying at room temperature.

*Pulverisation, sieving:* After complete drying of the sample, the dried pieces were subjected for pulverization to get powder, this powder was passed through sieve no.120 to get a fine powder. The final weight of the

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powder obtained from 1kg of fresh weight of nopal was 100gm ($W_2$). The percentage of drying was calculated using the following formula

$$\text{Moisture content} = \frac{W_1 - W_2}{W_1} \times 100$$

Where, $W_1$ = fresh weight of the sample; $W_2$ = dry weight of the sample

***In-vivo tests:***

*Sources of data:*

This study is planned to generate data by conducting laboratory-based research in animals. It is also planned to use rats in the experiments. Estimation of serum lipid levels at a regular interval after the administration of antihyperlipidaemic agent to obese animals is important criteria for generating required data. This study is also intended to generate some data by using certain pharmacological tools. In addition, it is also planned to take some data from the available literature.

Materials:

Drug : Dehydrated Nopal

Chemicals : Sodium carboxymethyl cellulose

Equipments : UV/Visible spectrophotometer,

Research design: Multiple dose.

Sample size: 8 albino wistar rats, of either sex.

Study period: Three months

Methodology: The entire study was categorized into following phases

Pharmacodynamic study in rats: Multiple dose Pharmacodynamic study in Obese Rats: Eight rats (210 to 280 g) are to be taken. Two groups are to be made of four rats each. The first group of rats will be administered dehydrated nopal and the second group will be administered standard nopal. Multiple dose: Blood samples will be collected from each rat by retro-orbital puncture at 0 (predose) & 15, 30, 45, 60 days (after drug administration). Serum lipid levels will be determined by using various methods mentioned in the respective kits. The percent reduction at each time was calculated with respect to initial levels. The serum lipid levels will be estimated from these samples by UV/Visible spectrophotometer.

Methods of Collection of Data (Including Sampling Procedures, If Any)

Data on drug will be collected through survey from physicochemical database, handbooks. Experimental design assisted replicated experiment would be conducted to generate other data pertinent to formulation under investigation.

Animals: Animals were procured from TINA Bio-labs, Hyderabad and Registration number 177599 CPCSEA.

Data analysis method: The results obtained will be subjected to statistical analysis.

Estimation of lipid profile: The *in-vivo* evaluation includes estimation of serum lipid profile which includes estimation of total cholesterol, triglycerides, HDL-Cholesterol, LDL-Cholesterol.

Total cholesterol estimation: The total cholesterol content was estimated by using total cholesterol kit which works on the principle COD/PAP method. The contents within the kit are ready to use. Contents include enzyme reagent 1, enzyme reagent 2 and cholesterol standard.

Reagent preparation: Pour contents of enzyme reagent 2 into the contents of enzyme reagent 1.

| | Blank (ml) | Standard(ml) | Test(ml) |
|---|---|---|---|
| **Working reagent** | 1 | 1 | 1 |
| **Distilled water** | 0.01 | - | - |
| **Cholesterol STD** | - | 0.01 | - |
| **Sample** | - | - | 0.01 |

Mix and incubate at 370C for 5 min and measure at 505 nm.

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

*HDL-Cholesterol estimation:*

The HDL-Cholesterol content was estimated by using HDL-Cholesterol kit which works on the principle method. The contents within the kit are ready to use. Contents include HDL-precipitating agent and cholesterol standard. *Procedure:* the procedure includes two steps – HDL separation and manual assay. *HDL separation:* pipette serum (0.5ml) and HDL- precipitating agent (0.5ml). mix well and centrifuge at 4000rpm for 10 min to get clear supernatant. *Manual assay:* assay the supernatant for HDL-Cholesterol within 2hrs using working solution of autozyme cholesterol reagent.

Incubate for 10 min at 37 $^0$C and measure at 510 nm.

| | Blank | Standard | Test |
|---|---|---|---|
| **Supernatant** | 0.05 ml | - | 0.05 ml |
| **Cholesterol working reagent** | 1 ml | 1 ml | 1 ml |

$$\text{HDL-Cholesterol in mg\%} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

Triglyceride estimation:

The triglyceride content was estimated by using triglyceride kit which works on the principle GPO/PAP method. The contents within the kit are ready to use. Contents include enzyme reagent and standard.

| | Blank (ml) | Standard(ml) | Test(ml) |
|---|---|---|---|
| **Enzyme reagent** | 1 | 1 | 1 |
| **Standard** | - | 0.01 | - |
| **Serum** | - | - | 0.01 |

Mix well and incubate for 10 min at 37$^0$C and read the absorbance at 546 nm.

$$\text{Triglycerides in mg\%} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

*LDL-Cholesterol estimation:*

The LDL-Cholesterol content was estimated by using LDL-Cholesterol kit which works on the principle method. The contents within the kit are ready to use. Contents include LDL-C Direct $R_1$, LDL-C Direct $R_2$ and LDL-C Direct Calibrator. Preparation: The reagent 1 and reagent 2 are ready. Calibrator: Reconstitute with 3 ml of distilled water and let it stand for 2hrs at room temperature. Dissolve the contents of the vial by swirling gently to avoid the formation of foam. Stability: Reconstituted calibrator is stable only for 7 days at 2-8$^0$C.

| | Blank (µl) | Calibrator (µl) | Sample (µl) |
|---|---|---|---|
| **Reagent 1** | 450 | 450 | 450 |
| **Calibrator** | - | 5 | - |
| **Sample** | - | - | 5 |
| Mix and incubate for 5 min at 37$^0$C. | | | |
| **Reagent 2** | 150 | 150 | 150 |

Mix and incubate for 5 min at 37$^0$C and measure the absorbance of calibrator and sample against reagent blank at 546 nm.

$$\text{LDL-C Conc in mg/dl} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{calibrator conc.}$$

*Weight reduction:* Weight of the animals was observed before dosing and on 2$^{nd}$, 4$^{th}$, 6$^{th}$ & 8$^{th}$ week of the experiment after giving dose to the animals. The reduction in weight of the animals was calculated and the percentage weight was calculated.

***Evaluation of Antioxidant Activity by DPPH method***

*Methanolic extract of nopal pads:* 100gm of coarsely grinded powder was subjected for soxhalation and the extraction was run for 10 cycles.

After the extraction process, the extract obtained was found to 1.9gm. This extract was used for the evaluation of antioxidant activity.

*Methanolic extract of nopal fruits:* 2gm of fruit powder was subjected for maceration using 25ml of methanol for 7 days. The obtained extract was found to be 0.50gm. This extract was used for the evaluation of antioxidant activity.

Extract of *Opuntia* pads

Extract of *Opuntia* fruit

**Fig No. 1.** Methanolic extracts of pads and fruits

*Antioxidant:* An agent that prevents or inhibits oxidation. Antioxidants are substances that may protect cells from the damaging effects of oxygen radicals highly reactive chemicals that play a part in atherosclerosis, some forms of cancer and reperfusion injuries.

*Mechanism of Antioxidants:* Hydrogen donation to free radicals by antioxidants. Formation of a complex between the lipid radical and the antioxidant radical (free radical acceptor).

*Preparation of standard solution:* Butylated hydroxyl toluene (BHT) was used as standard for antioxidant activity. The weight equivalent to concentration of 20, 40, 60, 80 and 100 µg/ml was weighed and dissolved in methanol.

*Preparation of test solution:* Stock solutions of samples were prepared by dissolving 10 mg of test sample in 10 ml of methanol to give concentration of 1000µg/ml.

From the above stock solutions the concentrations of 20, 40, 60, 80 and 100 µg/ml were prepared by dissolving equivalent quantity in methanol.

*Method:* The method of Liyana-Pathiana and Shahidi (2005) was used for the determination of scavenging activity of DPPH free radical. To 1 mL of 0.135 mM DPPH prepared in methanol was mixed with1.0 ml of test compounds ranging from 20-100 µg/ml. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 min.

The absorbance was measured spectrophotometrically at 517 nm. The scavenging ability of the test compounds was calculated using the standard equation. The % inhibition and $IC_{50}$ values were given in table.

The amount of DPPH radical was calculated following this equation:

$$\% \text{ inhibition of DPPH} = [A_0 - A_s]/A_0 \times 100$$

Where $A_0$ is the absorbance of control and $A_s$ is the absorbance of sample. Standard drug is Butylated hydroxyl toluene (BHT). Each experiment was carried out in triplicate and results were expressed as mean % antiradical activity ± SD. The antioxidant activity was compared between the standard (BHT), fruit(I), pad extract(II) by using DPPH method (**Table 3** & **Graph 1-3**).

According to Friedewald's formula,

$$\text{VLDL-Cholesterol} = \frac{\text{TG (Triglycerides)}}{5}$$

LDL-Cholesterol = Total cholesterol – (VLDL + HDL-Cholesterol)

## 3. Results and Discussion

*Preliminary evaluation of dehydrated nopal powder:*

Dehydrated *opuntia* powder was subjected for preliminary organoleptic and phytochemical investigations. The powder was standardized, and it was flowable, spherical shaped with light green colour and was slightly bitter in taste. Results of organoleptic observations were presented in **Table 1**.

**Table 1.** Organoleptic properties of dehydrated nopal powder.

| Parameters | Observation |
|---|---|
| Physical tests | |
| Shape | Spherical shape |
| Colour | Light green |
| Odour | Characteristic |
| Taste | Slight bitter |
| Loss on drying | 89% |
| Ash Values | |
| Total ash | 24.64% |
| Acid insoluble ash | 6.66% |

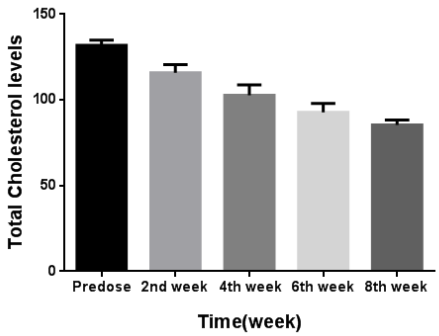
In order to identify chemical constituents of *Opuntia* powder preliminary chemical test were performed. These results were summarized in **Table 2**. It was observed that *Opuntia* powder showed presence of carbohydrates, amino acids, flavonoids proteins and alkaloids.

**Table:2.** Preliminary phytochemical investigations

| Test Performed | Results |
|---|---|
| Test for alkaloids | +ve |
| Test for proteins | +ve |
| Test for carbohydrates | +ve |
| Test for flavonoids | +ve |
| Test for glycosides | - ve |
| Test for aminoacids | +ve |

*Toxicity studies*

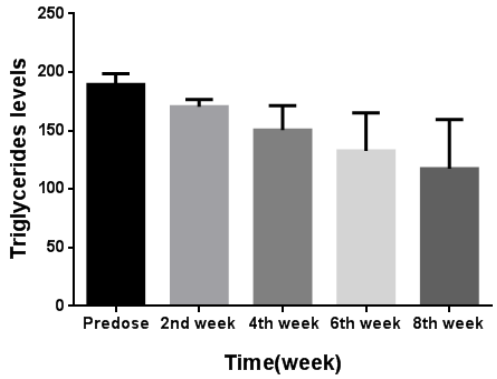
Acute toxicity studies were carried out and the effective dose was found to be 3000 mg/kg.. Toxicity studies were conducted on albino wistar rats. Acute toxicity study was carried out according to OECD guidelines. Starting dose was selected to be 2000 mg/kg body weight up to 3000 mg/kg body weight (as specified for natural products). At 3000mg/kg body weight, no mortality was observed which is considered as the end point So 1/10th of this dose was taken as experimental dose.



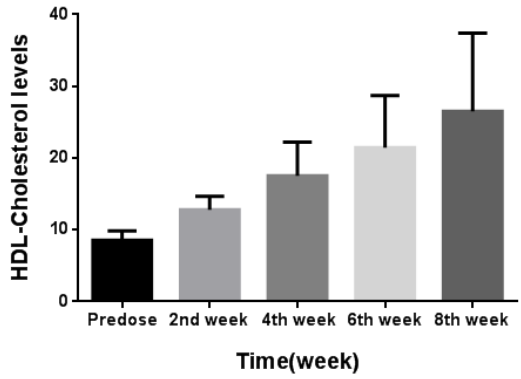

**Graph 1:** Total Cholesterol levels from predose to 8th week of dosing

**Table No.3 . Serum Lipid Levels & Weight reduction Estimation from pre-dose to 8th week of dosing**

| | Predose | 2nd week | 4th week | 6th week | 8th week | % Reduction |
|---|---|---|---|---|---|---|
| **Total cholesterol levels:** | | | | | | |
| **Control** | 129.97 ± 9.13 | 127.65 ± 8.08 | 118.59 ± 5.54 | 111.94 ±13.70 | 94.98 ± 9.80 | **26.93** |
| **Test** | 135.47 ± 10.02 | 119.48 ± 7.91 | 103.20 ± 5.82 | 98.44 ± 8.43 | 80.51 ± 2.70 | **40.57** |
| **Standard** | 130.20 ± 13.2 | 110.40 ± 2.83 | 96.52 ± 4.07 | 88.04 ± 1.47 | 82.74 ± 3.08 | **36.46** |
| **Triglyceride levels:** | | | | | | |
| **Control** | 179.49 ± 13.89 | 176.94 ± 14.91 | 174.32 ± 14.14 | 170.15 ± 16.83 | 165.90 ± 20.34 | **7.58** |
| **Test** | 192.13 ± 14.21 | 169.57 ± 12.87 | 141.19 ± 12.9 | 115.13 ± 3.98 | 96.64 ± 10.94 | **50.29** |
| **Standard** | 197.22 ± 9.90 | 164.48 ± 16.89 | 136.09 ± 16.01 | 112.80 ± 13.73 | 108.03 ± 6.81 | **45.13** |
| **HDL-Cholesterol levels:** | | | | | | |
| **Control** | 9.80 ± 3.77 | 10.67 ±3.71 | 12.07 ± 4.10 | 12.98 ± 4.18 | 14.00 ± 4.13 | **4.85** |
| **Test** | 7.25 ± 1.67 | 13.58 ± 2.80 | 19.97 ± 3.06 | 25.36 ± 2.12 | 34.24 ± 8.78 | **36.12** |
| **Standard** | 8.62 ± 4.66 | 14.13 ± 5.98 | 20.46 ± 6.91 | 25.92 ± 7.41 | 31.19 ± 7.71 | **26.04** |
| **LDL-Cholesterol levels:** | | | | | | |
| **Control** | 63.31 ± 10.91 | 61.83 ± 14.62 | 61.60 ± 12.93 | 60.98 ± 14.78 | 60.80 ± 10.99 | **55.17** |
| **Test** | 89.79 ± 6.79 | 74.74 ± 4.05 | 54.82 ± 3.77 | 44.54 ± 3.75 | 34.94 ± 8.85 | **61.09** |
| **Standard** | 82.14 ± 15.01 | 63.37 ± 6.03 | 48.84 ± 7.35 | 39.55 ± 5.17 | 38.73 ± 7.95 | **52.85** |
| **VLDL-Cholesterol levels:** | | | | | | |
| **Control** | 35.89 ± 2.77 | 35.38 ± 2.98 | 34.86 ± 2.82 | 34.02 ± 3.36 | 33.17 ± 4.06 | **7.58** |
| **Test** | 39.48 ± 3.71 | 33.91 ± 4.76 | 28.23 ± 4.99 | 23.02 ± 0.79 | 19.32 ± 2.18 | **50.07** |
| **Standard** | 39.44 ± 1.98 | 32.89 ± 3.37 | 30.46 ± 3.57 | 27.55 ± 3.70 | 22.08 ± 3.36 | **44.02** |
| **Weight reduction** | | | | | | |
| **Control** | 220.25 ± 0.12 | 220.00 ± 0.65 | 219.63 ± 0.63 | 219.40 ± 0.33 | 218.00 ± 0.76 | **1.22** |
| **Test** | 250.75 ± 0.89 | 231.25 ± 0.12 | 218.75 ± 0.54 | 191.25 ± 0.67 | 166.25 ± 0.32 | **33.7** |
| **Standard** | 236.25 ± 0.76 | 222.25 ± 0.23 | 212.50 ± 0.43 | 197.50 ± 0.78 | 171.25 ± 0.32 | **27.52** |




**Graph 2:** Triglycerides levels from predose to 8th week of dosing




**Graph 3:** HDL-Cholesterol levels from predose to 8th week of dosing

## 4. Conclusion

The lipid lowering action of nopal was estimated as **40.57%** reduction in total cholesterol levels, **50.29%** reduction in triglycerides levels, **61.09%** reduction in LDL-Cholesterol levels, **50.07%** reduction reduction in VLDL levels and **36.125%** increase in HDL-Cholesterol levels were observed. The weight reduced before dosing (predose) and during the complete duration of the experiment (2nd, 4th, 6th & 8th week) was found to be **33.7%**. The test sample showed better results compared to the standard powder. Hence, administration of nopal would significantly reduce the weight and cholesterol levels in obese patients with almost less or no side effects. Hence, it could be more preferred than the commercially available drugs in the market. The antioxidant activity was found to be more for methanolic extract of fruit than methanolic extract of pads. Hence, the fruit has more antioxidant activity than the pads.

**Antioxidant Activity**

The antioxidant values of standard (BHT), fruit(I), pads(II) at different concentration by using DPPH method are as follows (**Table 4**).

**Table No. 4.** % inhibition of compounds by DPPH method

| Concentration (µg/ml) | Standard | Fruit | Pads |
|---|---|---|---|
| 20 | 69.84±0.20 | 40.39±0.87 | 36.36±0.54 |
| 40 | 73.02±0.43 | 44.77±1.21 | 43.76±1.23 |
| 60 | 76.19±0.12 | 46.46±0.31 | 45.95±0.98 |
| 80 | 80.95±0.34 | 51.51±0.21 | 47.97±0.64 |
| 100 | 47.44±0.33 | 58.58±1.20 | 54.54±0.32 |

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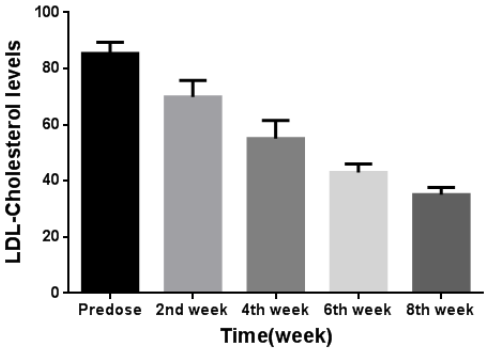
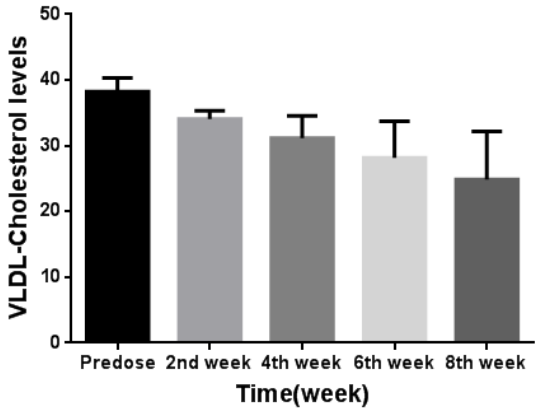
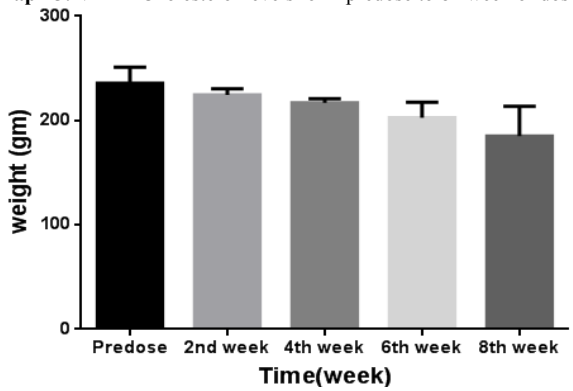


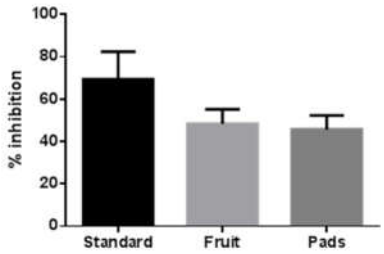

**Graph 4:** LDL-Cholesterol levels form predose to 8th week of dosing




**Graph 5:** VLDL-Cholesterol levels form predose to 8th week of dosing




**Graph 6 :** Weight reduced from predose to 8th week of dosing




**Graph 7.** % inhibition of compounds by DPPH method

## Conflict of Interest

The author(s) confirm that this article content has no conflict of interest.

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