# Investigation of Anti-hyperlipidemic and Antioxidant activity of a polyherbal formulation on Trition X-100 induced animal Model

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## *Abstract*

***Objective**:* Hyperlipidemia is the leading cause of many degenerative disorders, and there is a rise in cholesterol levels in the blood. The current study investigates the effects of PHF on Triton X-100-induced hyperlipidemia in rats and the problems that may arise.

***Methodology*:** The antihyperlipidemic activity of Poly herbal formulation was evaluated for its biochemical characteristics in blood and tissue specimens of Triton X-100-induced hyperlipidemic rats. Twenty-five animals of either sex were put into five groups of five rats in each. Group I animals were under the normal control group, Group II disease control group, which was induced by a Triton X-100 dose of 10mg/kg of b.w., Group III disease standard group, which was treated by an Atorvastatin dose of 10mg/kg of b.w., Group-IV Low Dose Treated group, which was treated by a PHF dose of 100mg/kg of b.w. and Group-V High Dose Treated group, which was treated by a PHF dose of 200mg/kg of b.w. And preserve it for lipid profile analysis.

***Results:*** Mineral examination revealed antioxidant, phenolic content, as well as total flavonoid, as well as the strength of scavenging free radicals using SOD, GSH, and also hydrogen peroxide radicals with dosage dependent activity. When administered with PHF aqueous extract, Triton X-100 produced hyperlipidemic rats demonstrated considerable activity by controlling biochemical components and preserving the lipid profile by reducing TC, LDL-C, and VLDL-C, as well as TG and enhancing HDL-C levels.

***Conclusion***: This content suggests that PHF might be an effective treatment for lowering the risk of hyperlipidemia related consequences.

***Key words.*** *Polyherbal, lipoprotein, Hyperlipoproteinemia*

# 1. INTRODUCTION

Hyperlipidemia is defined as an excess of fatty compounds known as lipids in the blood, mostly cholesterol and triglycerides. Because these fatty compounds circulate in the blood coupled to proteins, it is also known as hyperlipoproteinemia. This is the only method for these fatty compounds to remain dissolved in the bloodstream. Hyperlipoproteinemia, also known as dyslipidemia, is one of the most common lipid metabolism diseases. [1] They are distinguished by alterations changes plasma lipoprotein composition, either in terms of quantity or quality. They can be characterized by one or more of the following changes: either a rise or decrease in triglycerides, LDL cholesterol, or HDL cholesterol (high-density lipoprotein cholesterol). Dyslipidemia is one of numerous risk factors for cardiovascular disease (CVD), along with hypertension, smoking, obesity, and diabetes [2].

It is characterised by extremes of TC, TG, LDL-C, also VLDL-C, and decreasing levels of HDL-C29, and is regarded as more than just a coronary risk factor leading to fatty liver heart disease and atherosclerosis, which is the leading cause of mortality in humans.It has been estimated that by 2030, roughly 23.6 million more people will die from cardiovascular disease related to hyperlipidemia.[3] Furthermore, the frequency of hyperlipidemia is continuously growing and it may worsen as the population ages4. As a result, hyperlipidemia management and prevention are critical.[4]

As a result, the current medicine, which employs allopathy pharmaceuticals, has significant adverse effects. As a result, an essential and alternate therapy is essential. Investigators from several fields are working on herbal supplements and their bioactive components that operate as strong therapeutic drugs.[5] The use of these herbal medications is beneficial, has few or no adverse effects, and is less expensive than allopathic pharmaceuticals. Plants that produce a variety of bioactive molecules with varying activities or Functionality is a critical component in the development of medicines. In the process of developing medications to prevent and treat disease, plants and their natural chemicals play a significant role. in managing various ailments in the pharmaceutical industry. [6]

Antioxidants include the phenolic content of flavonoids, which scavenge oxygen free radicals and decrease enzyme production. Auxiliary metabolites loaded with nutrients are known as phenolic compounds. [7] They have a variety of therapeutic effects involving cardiovascular, antioxidant, antiatherogenic,, anti-inflammatory, and anti-angiogenesis properties. Several animal studies have found the influence of phenolic content, which includes quercetin and rutinosides, on lipid metabolism, resulting in a reduction in blood lipids.[8,9]

Ayurveda has become one of the Indian healthcare treatments that has been practised in India for over 2000 years.

The goal of this research was to evaluate the antioxidant and antihyperlipidemic efficacy of a polyherbal formulation (PHF) in an animal model of Triton X-100-caused hyperlipidemia.

# 2. MATERIALS

## 2.1 Drug and Chemicals

Carboxymethylcellulose, different reagents such as Dragendorff's reagent, also Mayer's reagent and Wagner's reagent, Lead acetate, Magnesium ribbon, Hydrochloric acid(HCL), Ferric chloride, Benzene, Ammonia, Hydrogen peroxide($H_2O_2$), Phosphate buffer, Sodium

dodecyl sulfate, Picric acid, Ethanol, Butenol, Dithiobis-nitro benzoic acid, Pyridin, Sodium phosphate monobasic & dibasic, Salphosalicylic acid Triton X-100, Atorvastatin.

### 2.2 Instrument and apparatus

Digital balance, Gavage (Orchid scientific), Rotatory evaporator, UV Spectrophotometer (UV-3200.)

### 2.3 Collection and Authentication of plant resources

The freshest plant parts were gathered from the surrounding region of Lucknow, (U.P.) India in the month of January-February 2020. Initially identification and authentication were carried out by the NISCAIR (National Institute of Science Communication and Information Resources), New Delhi, India. The herbarium was submitted to the NISCAIR with an authentication no. NISCAIR/RHMD/Consult/2020/3626-27-1 for *Berberis aristata*, authentication no. NISCAIR/RHMD/Consult/2020/3625-27-2 for *Commiphora mukkul* and authentication no. NISCAIR/RHMD/Consult/2020/3626-27-3 for Piper *nigrum*.

## 3. METHODOLOGY

### 3.1 Extraction of plants materials used for Polyherbal formulations

The plant materials, rhizomes of *Berberis Aristata*, fruits of *Piper nigrum*, and entire plant parts of *Commiphora Mukul*, were air dried and placed under shadow at 25 C. The mixture was then crushed using a mechanical grinder and passed through 120 meshes separately.[10]
For extraction, the cold maceration process was applied to *Berberis Aristata* and *Piper nigrum*. The powered sample was stored in an iodine flask with ethanol for 72 hours with frequent agitation. *Commiphora mukul was* extracted by soxhlet in ethyl acetate solvent. The materials were filtered using muslin cloth, and the filtrates were then dried under decreased pressure. The dry extract powder of *Berberis Aristata, Commiphora Mukul,* and *Piper nigrum* was obtained and preserved for further use.[11]

### 3.2 Preparation of Formulation

The extracted materials of *Berberis Aristata, Commiphora Mukul* and *Piper nigrum* in the optimized ratio of 3:2:1 was varied to form the polyherbal formulation.

### 3.3 Preliminary Phytochemical Screening:

The extracted material was tested to find out the fact that the presence of the active chemical moiety such as as-Alkaloids, flavonoids, glycosides, tannins, gum, and saponins by the standard methods. [12]

**Table No. 1 Phytochemical Screening**

| PHYTO CONSTITUENT | IDENTIFICATION TEST | INFERENCE |
|---|---|---|
| **Carbohydrates** | **Molisch's test:** Take 1-2 ml of test, pour 1ml of freshly made α-naphthol compound in alcohol mix, and add conc. $H_2SO_4$ from either the test tube's side wall into tap water. | Violet Circle appear at the connection of both liquids |
| | **Benedict's test:** Warm the test solution with Benedict's reagent on a water bath. | Colour change is seen |
| | **Fehling's test:** Take few ml of extract put in equivalent amount of Fehling's solution A and B heated on hot boiled water bath for approx 4-10 minutes | Dark red colour precipitate is produced. |
| | **Barfoed's test:** Take 1ml of test solution sample add 1ml of Barfoed's reagent and also gently heated on waterbath | Red precipitate was produced. |
| | **Matchstick test:** Immerse the matchstick in the crude extracts. It should be dried.Moisten it with hydrochloric acid and warm near flame. | Wood will turn pink or red in color due to phloroglucinol. |
| **TANNINS** | **Ferric chloride test:** 2ml of 2% solution of ferric chloride was added in to the sample | Dark blue or Greenish black colour |
| | **Lead acetate test:** Lead acetate solution was added to extract drop by drop | White colour precipitate appear at the bottom of test tube |
| **SAPONINS** | **Foam test:** Put a very little portion of extract and mix it with 10 ml of filtered water in a marked cylinder for up to 15 minutes. | 1cm film layer of foam extended |
| | **Hemolytic test:** Put drug sample extract or maybe dry fine particles to singledive of blood, to be found on the side of the glass. | hemolytic region seen. |
| | **Mucilage test:** | look at the precipitate for its |

| | | |
|---|---|---|
| **GUMS** | Take 5 ml of extract and add slowly to 10 ml of absolute alcohol with fixedrousing. Filtered precipitate and dried in air. | bulge properties and for the existence of carbohydrates. |
| | **Hydroly tic test:** Hydrolyze the test sample solution with normal dilute solution of Hcl. Perform Benedict's and Fehling's test. | Dark Red colour is created. |
| **FLAVONOIDS** | **Shinoda test:** Take ethanolic extract sample of powder and add magnesium reasons for change or in foil and add conc. Hcl | Highly dark cherry red colour or may be in red colour is formed. |
| **ALKALOIDS** | **Dragendroff's test:** Sample with little drops of Dragendroff's reagent | Orange red precipitate appearance |
| | **Mayer's reagent test:** Sample with little amount of Mayer's reagent | Cream colour precipitate |
| | **Wagner's test:** Sample with few drops of Wagner's reagent | Reddish brown precipitate formed |

### 3.4 Pharmacological Study

#### 3.4.1 Animals

Albino Wistar rats of body weight 120-200gm (either male or female) were utilize to the study later than the sanction of IAEC ( Institutional Animal Ethics Committee). (IAEC No. HIPER/IAEC/37/20/08)

#### 3.4.2 Source

Albino Wistar have been received from the experimental animal house of Hygia Institute of Pharmaceutical Education and Research, Lucknow U. P, India.

#### 3.4.3 Housing

The rats were residence in clean polypropylene cage. The bedding matter of the cages was changed on alternate day. They were maintained under natural day and night cycle. They were reserved on a normal pellet diet and water *ad labitum.* Seven days of acclimatisation were required before they could be utilised.

#### 3.4.4 Acute toxicity study

According to the regulations, acute toxicity testing was performed with OECD guidelines-423. Rats were kept on during the night, fasting was observed, and water was withheld for 3–4 hours before the delivery of the test drug. The PHF was given orally in increasing doses of

100mg/kg and up to 200mg/kg of body weight. The rats were exposed continuously for up to 4 hours after dosing for indication of body motor activity, tremors, convulsion, spasm, lack of righting reflex, ataxia, drowsiness, hypnosis, salivation, and writing are all symptoms of poisoning. Rats were maintained during this time, and they were housed in cages inspected every 72 hours and finally for 15 days for any mortality.[13]

### 3.4.5 Induction of hyperlipidemia

Rats were given Triton X-100 to produce hyperlipidemia. Triton was i.p. injection (10mg/kg b.w. in combination of normal saline of pH 7.4). [14]

### 3.4.6 Experimental design

Water and a normal pellet meal were available to the rats at will. They be erratically separated in 5 groups, include of five rats in each group. After the introduction of hyperlipidemia by triton, all animal were treated with corresponding drugs/ extraxct for once daily for 14 days. The treatment schedule was as followed:

**Table No. 2 Grouping of animals for triton**

| Groups | | Treatments | Dose/Route |
|---|---|---|---|
| **Group 1** | Positive Control | CMC | 1ml/kg b.w/day |
| **Group 2** | Negative control | Triton + CMC | 10mg/kg b.w |
| **Group 3** | Standard | Triton + Atorvastatin | 10mg/kg b.w./day |
| **Group 4** | Test 1 | Triton + PHF | 100mg/kg b.w./day |
| **Group 5** | Test 2 | Triton + PHF | 200mg/kg b.w./day |

Group-I: Positive Control rats treated with normal saline only.
Group-II: Negative Control Hyperlipidemic rat treated by drug triton.
Group- III: Standard group administered at dose of Atorvastatin (10mg/kg b.w for 14 days).
Group- IV: Test 1 administered with dose of PHF (100mg/kg b.w for 14 days).
Group- V: Test 2 administered with dose of PHF (200mg/kg b.w. for 14 days).

### 3.5 Prediction of serum lipid profile

The lipid profiles of the blood samples were analysed. Triglycerides (TG), Total Cholestrol (TC), and (HDL) High Density Lipoprotein being catalysed enzymatically as assessed by commercial apparatus:

Friedwald formula was used to calculate LDL cholesterol: [15]

$$\boldsymbol{LDL = TC - \left[HDL + \left(\frac{TG}{5}\right)\right]}$$

VLDL cholesterol was calculated by the friedwald formula:

$$\boldsymbol{VLDL = \frac{TG}{5}}$$

### 3.6 Estimation of the coronary risk index (CRI) and the atherogenic index (AI)

The following equation was used to determine the arteriosclerosis index (AI):

$$\mathbf{AI} = \frac{\mathbf{TC-HDL}}{\mathbf{HDL}}$$

Whereas the CRI was find by:

$$\boldsymbol{CRI} = \frac{\boldsymbol{TG}}{\boldsymbol{HDL}} (\boldsymbol{mg/dL})$$

Calculation of LDL HDL ratio

**LDL-c/HDL-c ratio = LDL-c/HDL-c**

**3.7 Measurement of total Body weight**

A weighing scale was utilised every day of the 14-day treatment period to measure the rats' body weights. Calculated by comparing the 21st day's total weight loss to the initial starting weight on day one.

**3.7.1 Measurement of weight of different body organs.**

After withdrawal of blood the rats were sacrificed and then organ (liver, heart, kidney) were isolated.

**3.7.2 Blood collection**

At the 14$^{th}$ day one hours after the last dose of test and standard drug the blood was withdraw from retro-orbital anaesthesia with a little sedative,vein puncture by a thin capillary to an anticoagulant tube and maintained at 37°C for 30 minutes and centrifuged to separate the serum from the plasma to analyize the biochemical Parameter.

**3.7 Estimation of Antoxidant activities [16**

**3.7.1 Brain Homogenate Preparation**

The brains have been cleaned and homogenised 10 times through ice-cold 0.1 M phosphate buffer ( pH 7.4 approx) and allowed to calculate activity of , MDA, SOD and GSH also.

**3.7.2 Lipid peroxidation-**

**Principle** The estimation of the quantity of malondialdehyde (MDA) has determined lipid peroxidation. The procedure is intended to check any MDA in (hydrochloric acid) maybe MDA in conjunction by HAE (methane sulfonic acid) alone. This examine is based on the chromogenic reagent N-methy-2 phenylindole (R1) reagent reaction at 90c by means of MDA and 4 Hydroxyalkenal.

**Procedure-**

0.2 ml of homogeneous tissue was in use in the test tube and also 0.2 ml of 8.1% solution of sodium dodecyl sulphate (SDS), and 1.5 ml solution of TBA (thiobarbituric acid) was mixed. The mixture volume was made with distilled water up to 4ml and after that heated in the hot water bath for 60 min. After this sample should cooled with distilled water to room temperature and volume balanced up to 5ml, Added 5 ml butanol, pyridine (15:1) mixture, and a detailed 2min vortex . Each mixture then centrifuged approx 10 minutes at 300 rpm. Separated from the upper organic layer. Using UV- 1700 Pharma spec shimadzu spectrophotometer against butanol pyridine (15:1) solution as blank, the absorption of organic layer examine at 532 nm.

### 3.7.3 Estimation of Superoxidase Dismutase (SOD)

**Principle:** The elucidation of solution of the riboflavin in the existence of EDTA reduces the Flavin. It then adds oxygen and lowers oxygen to O-2, a fragment of the detector which allows the NBT to react, lowers the NBT to formazan blue. The sample SOD impedes the manufacture of formazan**.**

**Procedure:** Approx a total volume up to 0.2ml of 0.1 M solution of EDTA and 0.1ml of 1.5 M NBT and a phosphate buffer of 2.6 ml, the absorbance of 0.01ml of homogeneous mixed at 560 nm was measured. Both tubes held 15 minutes in the incubator, and blue color absorption has been tested again. The percentage of the reserve had been considered after comparing sample absorbance and control absorbance. The sample quantity needed to check for One enzyme unit activity per milli gramme of protein was evaluated for 50% of the superoxide anion produced.

### 3.7.4 Measurement of glutathione level

**Principle:** A yellow-colored composite of DTNB, which is soluble in acid sulfhydryl. The absorption of the colored compound regarded at 412 nm.

**Procedure-**Its DTNB (Ellman reagent) reaction calculated the amount of GSH to create a light yellow chromophore their absorbance is to be calculated spectrophotometrically. The homogeneous brain tissue was combined at 4°C and centrifuged for 10 minutes at 200 g with 10% trichloroacetic acid (TCA). GSH concentrations were determined using the tissue supernatant. Tissue samples were added to 2 ml phosphate buffer at pH 8.4 and the homogenate mixture was swirled vigorously on the vortex to homogenise the sample. At 412 nm, the absorbance spectra were collected in under 15 minutes.GSH was expressed in the form of protein mol / mg.

### 3.8 Histopathology

The liver was kept for histopathological studies for estimation of serum and biochemical profile.

### 4.1 Statistical analysis

Mean SEM should be used for all experimental outcomes (Standard error of mean). Turkey will conduct a one-way analysis of disagreement (ANOVA) on the data to determine its validity.

### 4.2 Compilation of data

## 5 RESULTS

### 5.1 Preliminary phytochemical analysis

The phytochemical screening examinations were carried out to determine the presence of various phytochemical elements in the PHF. Phytoconstituents testing revealed the presence of carbohydrates, alkaloids, glycosides, flavonoids, glycosides, tannins, and a lack of saponins.

### 5.2 Acute toxicity study

Rats were subjected to an acute toxicity test by following the OECD protocol (OECD 2002).In acute toxicity studies, PHF did not show any mortality in the rats upto higher dose i.e. 200mg/kg orally. It does not produce any effect on the behavioral neurological, physical profile, and autonomic profile.

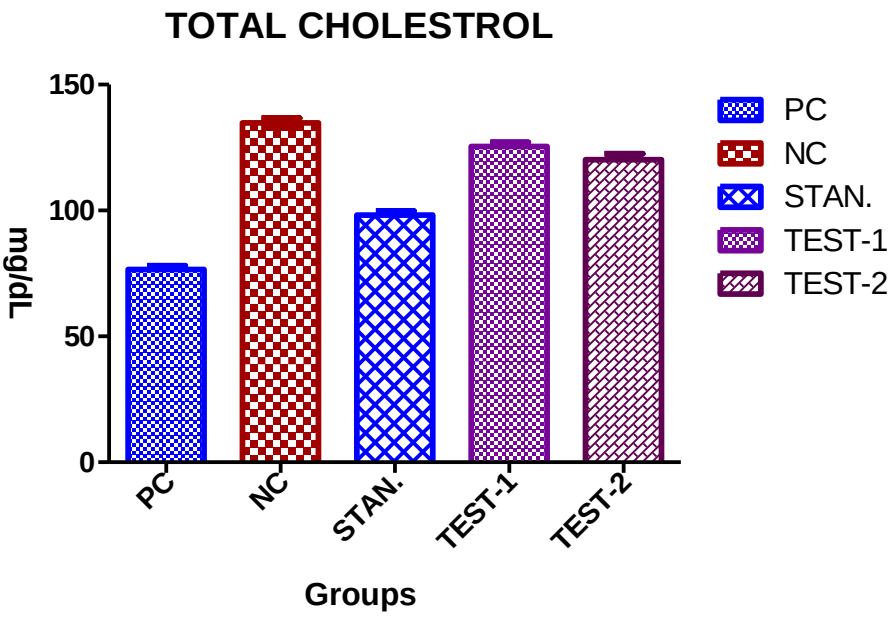
### 5.3 Result of PHF on total cholesterol and triglyceride in rats

The plasma TC and TG levels of PHF (100, 200mg/kg b.w. /day) given groups were shown in table no. 9 and fig. no. 9 and 10. Triton significantly increased plasma TC and TG levels as compared to the usual control group. After treatment with PHF (100, 200mg/kg b.w.) there was a considerable reduction in the levels of TC and TG in dose dependent behavior. TG and TC levels were also significantly reduced by the conventional medication therapy.

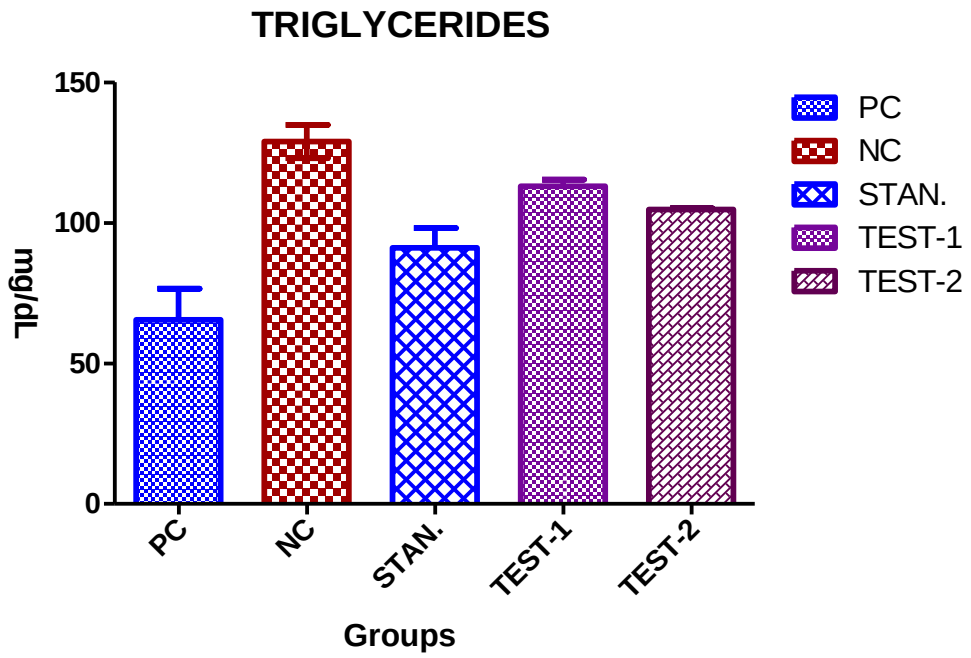
**Table No. 3 Role of PHF on amount of Total Cholesterol and Triglycerides in Triton X-100 (Tyloxapol) induced hyperlipidemic rat**

| S. No. | GROUPS | TOTAL CHOLESTEROL (mg/dL) | TRIGLYCERIDES (mg/dL) |
|---|---|---|---|
| 1. | Positive control | **76.60±1.44** | **54.40±1.12** |
| 2. | Negative control | **134.80±1.85** | **123±1.72** |
| 3. | Standard | **98.20±1.66*** ** | **84±1.48*** ** |
| 4. | Test 1 (100mg/Kg) | **115.40±1.66*** | **110.60±1.57*** ** |
| 5. | Test 2 (200mg/Kg) | **104.20±2.34*** ** | **105.20±0.90*** ** |

All numbers and Mean SEM (n = 5). Significant at ***P0.001, **P0.01, and *P0.1 compared to the negative control group using one-way ANOVA and the post-Tukey test.




Fig; 9 Effect of PHF on Total Cholesterol in Triton X-100(Tyloxapol) induced hyperlipidemic rats




Fig; 10 Effect of PHF on Triglycerides in Triton X-100 (Tyloxapol) induced hyperlipidemic rats

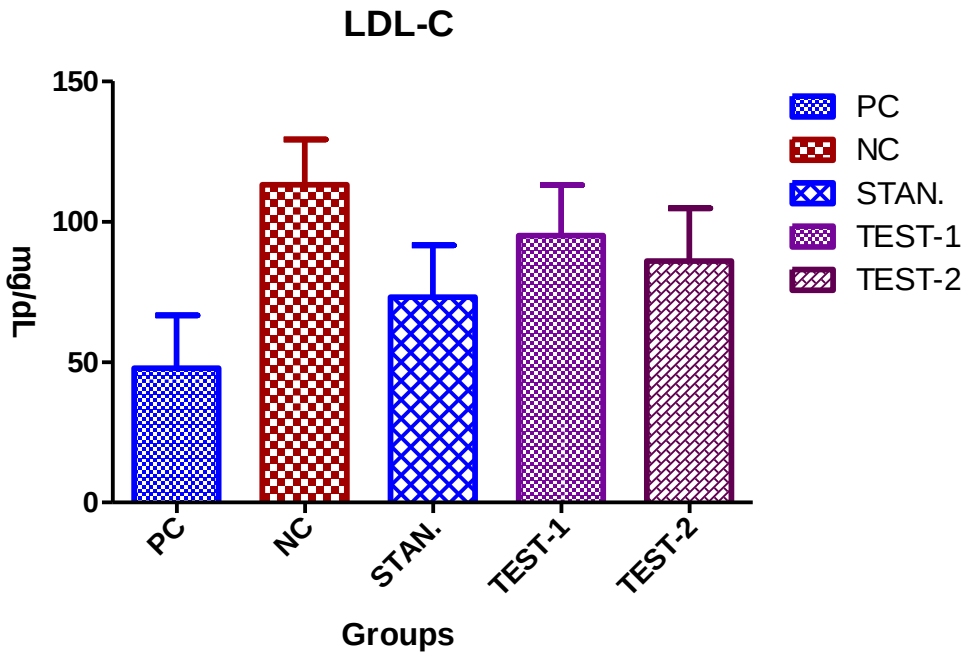
**5.4 Effect of PHF on LDL-C, HDL-C, and VLDL-C Cholesterol in Triton X-100 (Tyloxapol) induced hyperlipidemic rats**

The plasma HDL-c, LDL-c, and VLDL-c levels of PHF (200, 400mg/kg b.w. /day) treated groups are shown in table no.10 and fig. no.11, 12, 13. In comparison with the normal control group, triton caused a marked increase in plasma LDL-c, and VLDL-c levels. After treatment with PHF(100, 200mg/kg b.w.) there was a reduction in the levels of LDL-c, and VLDL-c.The standard drug treatment also showed a significant reduction in the levels of HDL-c, LDL-c, and VLDL-c level.

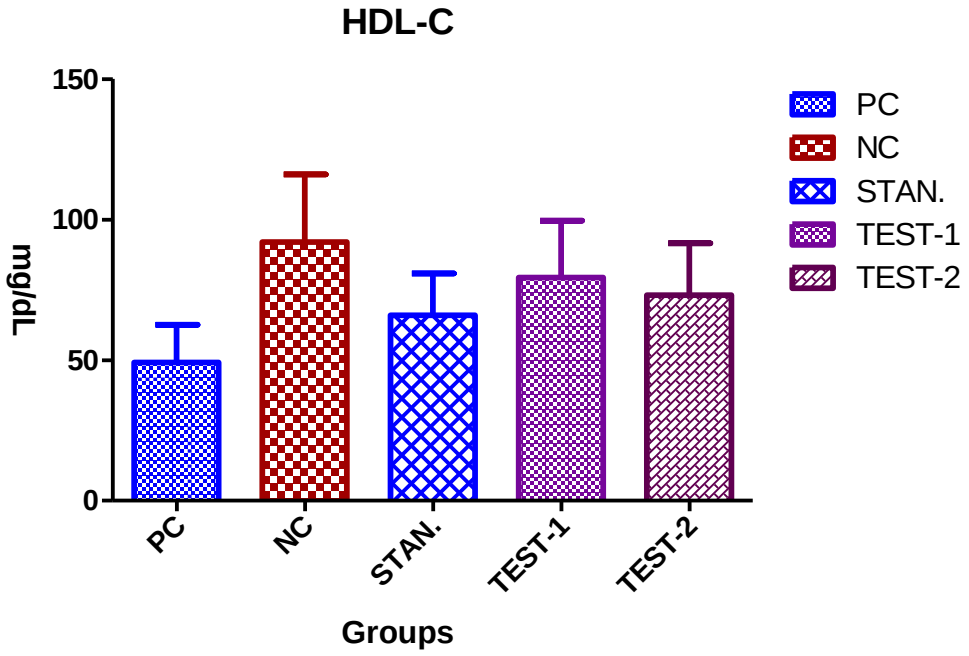
**Table No. 4 Effect of PHF on HDL-C, LDL-C, and VLDL-C Cholesterol in Triton X-100 (Tyloxapol) induced hyperlipidemic rats**

| S.NO. | GROUPS | LDL-C (mg/dL) | HDL-C (mg/dL) | VLDL-C (mg/dL) |
|---|---|---|---|---|
| 1. | Positive control | 12.32±2.61 | 53.40±1.30 | 28.11 ± 0.57 |
| 2. | Negative control | 81.60±2.45 | 28.60±0.88 | 100.25 ± 1.96 |
| 3. | Standard | 37.20±3.31*** | 44.20±1.60*** | 59.33 ± 1.16*** |
| 4. | Test 1 (100mg/Kg) | 59.08±2.46*** | 32.40±1.27** | 99.93 ± 1.86** |
| 5. | Test 2 (200mg/Kg) | 48.56±2.43*** | 34.60±0.98*** | 81.65 ± 2.34*** |

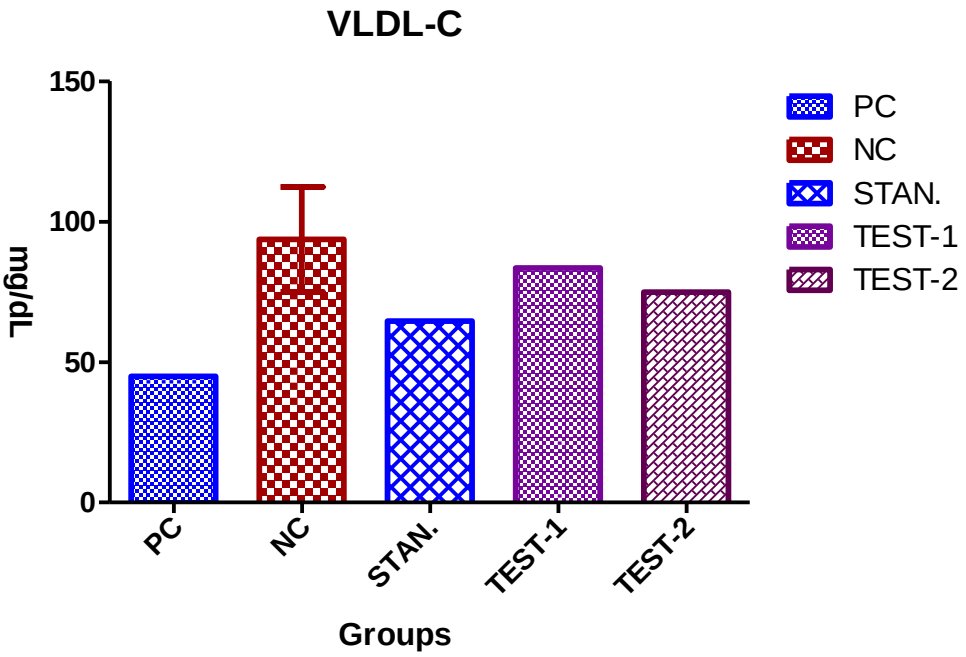
All numbers and Mean SEM (n = 5). Significant at ***P0.001, **P0.01, and *P0.1 compared to the negative control group using one-way ANOVA and the post-Tukey test.




Fig; 11 Effect of PHF on LDL-C in Triton X-100(Tyloxapol) induced hyperlipidemic rats




Fig; 12 Effect of PHF on HDL-C in Triton X-100(Tyloxapol) induced hyperlipidemic rats




Fig; 13 Effect of PHF on VLDL in Triton X-100 induced hyperlipidemic rats

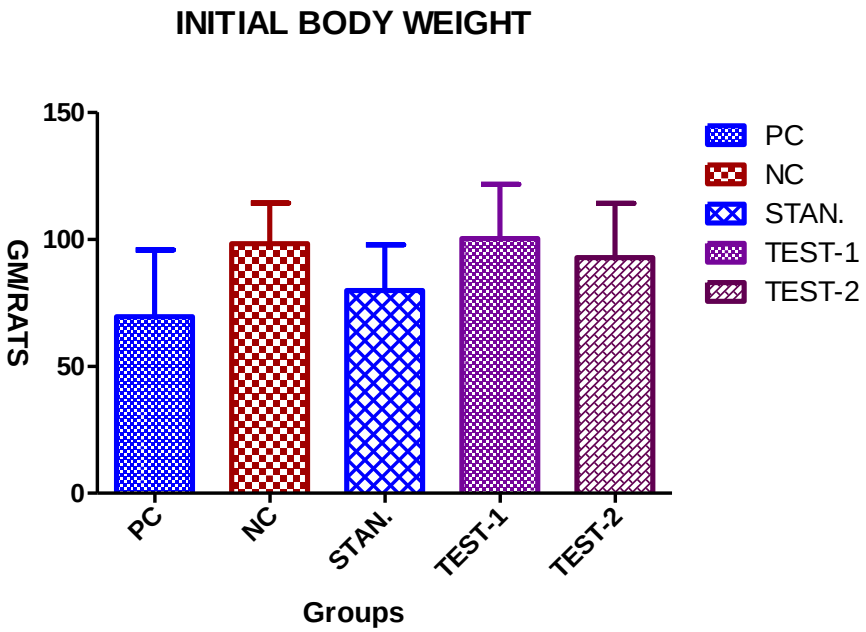
**5.5 Effect of PHF on Body weight changes of rats after 14 days of experimental period**

The body weight of animals are changes with on the basis of there dosing and induction agent the initial and final body weight of rat will be mentioned in table no. 11 and shows in graph no. 14 and 15.

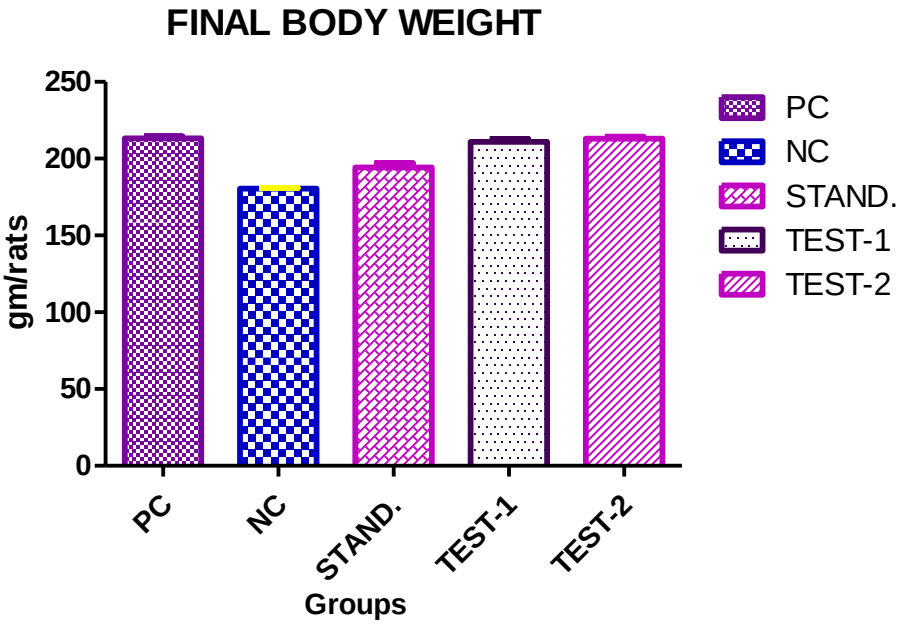
**Table no. 5 Body weight changes of rats after 14 days of experimental period**

| S.NO | GROUPS | INITIAL BODY WEIGHT (g/rats) | FINAL BODY WEIGHT (g/rats) | BW=Final B.W-Initial B.W/Initial B.W |
|---|---|---|---|---|
| 1. | Positive control | 192.40± 1.95 | 213.2± 1.33 | 10.8% |
| 2. | Negative control | 121.67 ± 0.22 | 180.61± 0.26 | 48.7% |
| 3. | Standard | 156.20 ± 3.08*** | 194.20 ± 2.73*** | 24.35% |
| 4. | Test 1 (100mg/Kg) | 184.20 ± 1.39*** | 211.00 ± 1.81** | 14.67% |
| 5. | Test 2 (200mg/Kg) | 182.40 ± 1.95*** | 213.00 ± 1.33** | 17% |

All values are proceeded as Mean ± SEM. (n=5).




Fig; 14 Effect of PHF on body weight (Initial)




Fig; 15 Effect of PHF on body weight (final)

**5.6 Accurate measurement of AI, CRI and LDL/HDL ratio.**

The AI index, LDL-HDL ratio was significantly declined to normal group value with atorvastatin. The AI index, LDL-HDL ratio was significantly declined to normal group value with PHF (100mg/kg, 200 mg/kg). The CRI was decrease to normal group value with atorvastatin and a similar effect show with the PHF (100mg/kg, 200 mg/kg).

**Table No. 06 Accurate measurement of AI, CRI and LDL/HDL ratio.**

| S. No. | Groups | AI | CRI | LDL HDL ratio |
|---|---|---|---|---|
| 1. | Positive control | 0.47± 0.07 | 1.47± 0.07 | 0.28± 0.06 |
| 2. | Negative control | 3.87 ± 0.19 | 4.86± 0.18 | 3 ± 0.16 |
| 3. | Standard (10 mg/kg) | 1.35 ± 0.13*** | 2.35 ± 0.13*** | 0.95 ± 0.11*** |
| 4. | Test 1 (100mg/Kg) | 2.72 ± 0.15*** | 3.72 ± 0.15*** | 1.94 ± 0.12*** |
| 5. | Test 2 (200mg/Kg) | 2.08 ± 0.12*** | 3.08 ± 0.12*** | 1.44 ± 0.10*** |

All numbers and Mean SEM (n = 5). Significant at ***P0.001, **P0.01, and *P0.1 compared to the negative control group using one-way ANOVA and the post-Tukey test.

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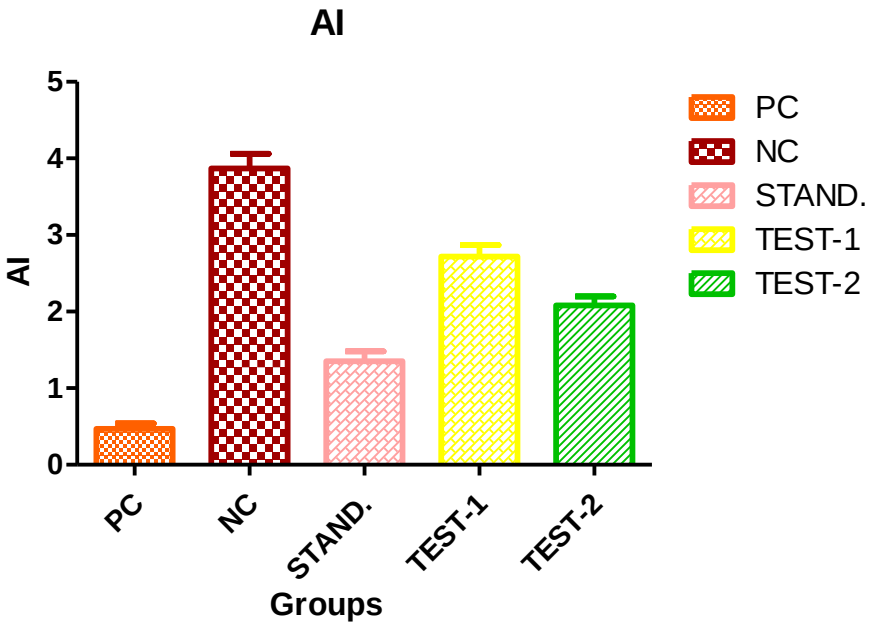
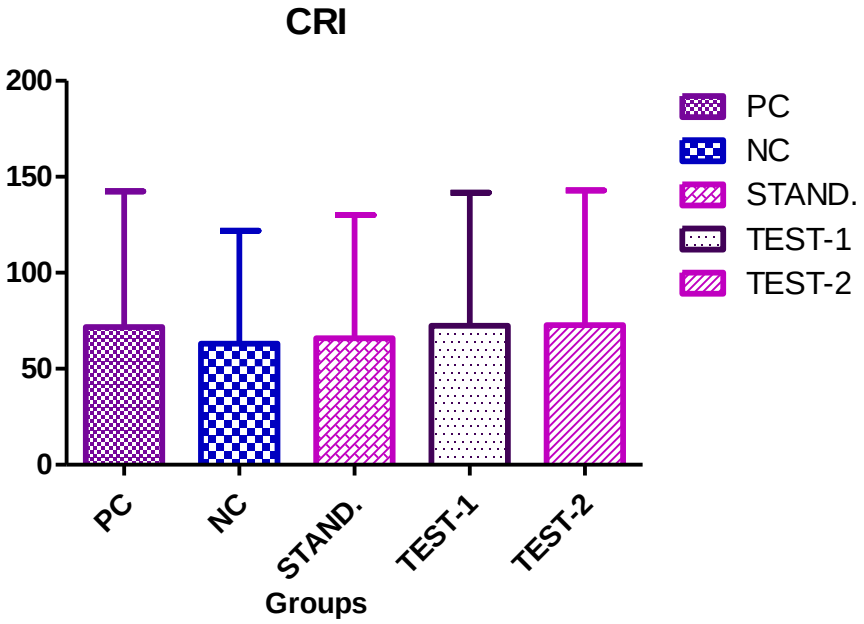
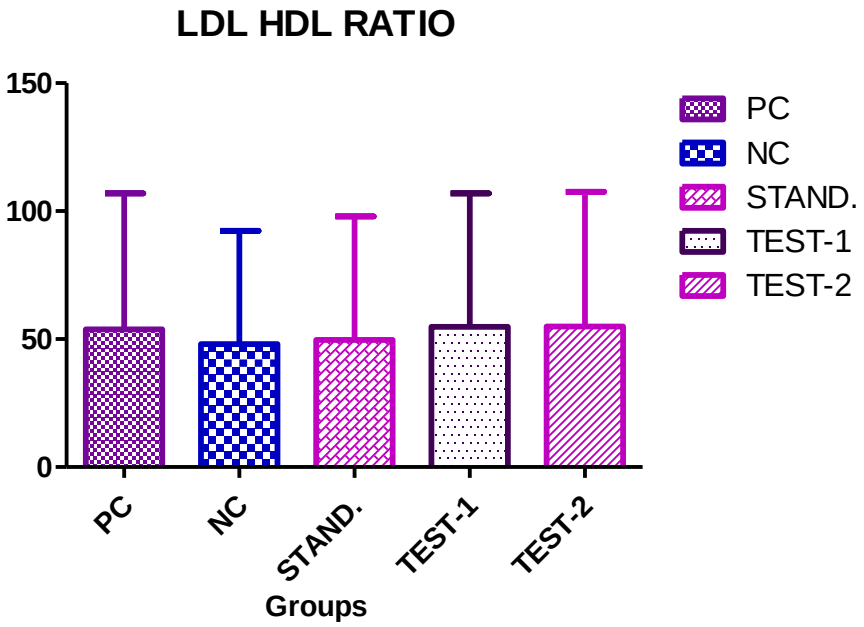



Fig; 16 Determination of atherogenic index (AI)




Fig; 17 Determination of coronary risk index (CRI)




Fig;18 Determination of LDL-HDL ratio.

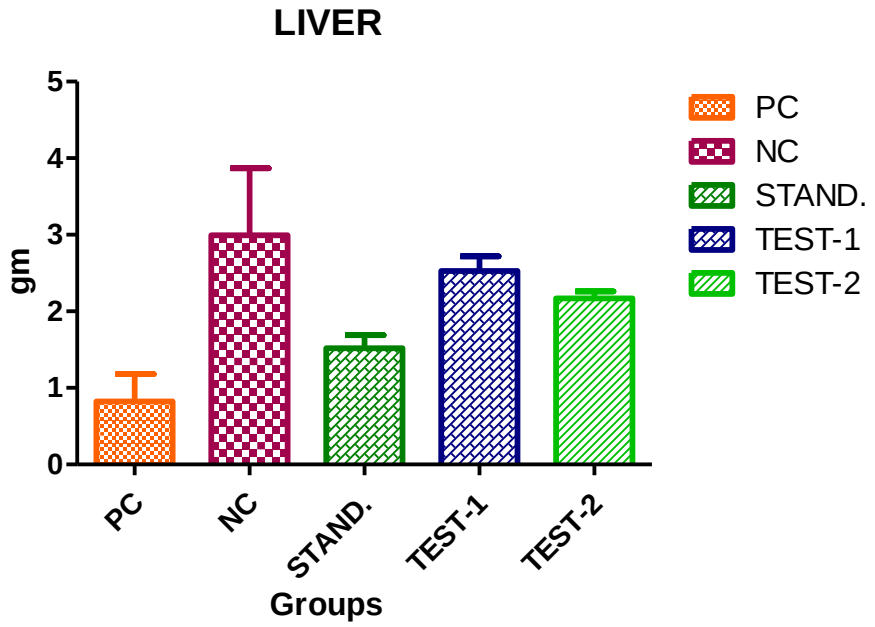
**5.7 Effect of PHF administration for 14 days on organ weight**

Organ's weights were declined to normal group value with atorvastatin. In the case of the liver, weight was decreased significantly on the administration of a lower dose (100 mg/kg) of PHF in comparison to the negative control group. Weight of Heart was also decreased in the administration of dose of PHF. The weight of the kidney was declined significantly relating to management of both doses (100 and 200 mg/kg) of PHF.

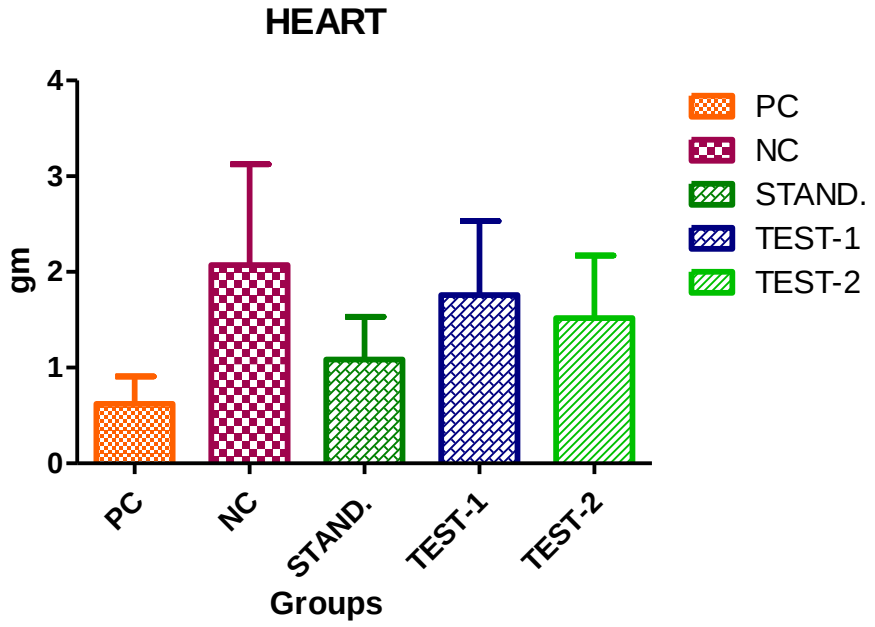
**Table No. 7. Effect of PHF administration for 14 days on organ weight**

| S. No. | Groups | Liver | Heart | Kidney |
|---|---|---|---|---|
| 1. | Positive control | 1.18 ± 0.01 | 0.21 ± 0.01 | 0.51 ± 0.01 |
| 2. | Negative control | 2.12 ± 0.03 | 0.23 ± 0.01 | 0.38 ± 0.01 |
| 3. | Standard (10 mg/kg) | 1.69 ± 0.05*** | 0.21 ± 0.01*** | 0.60 ± 0.01*** |
| 4. | Test 1 (100 mg/kg) | 2.33 ± 0.03** | 0.23 ± 0.01*** | 0.45 ± 0.01*** |
| 5. | Test 2 (200 mg/kg | 2.26 ± 0.05*** | 0.22 ± 0.01*** | 0.53 ± 0.01*** |

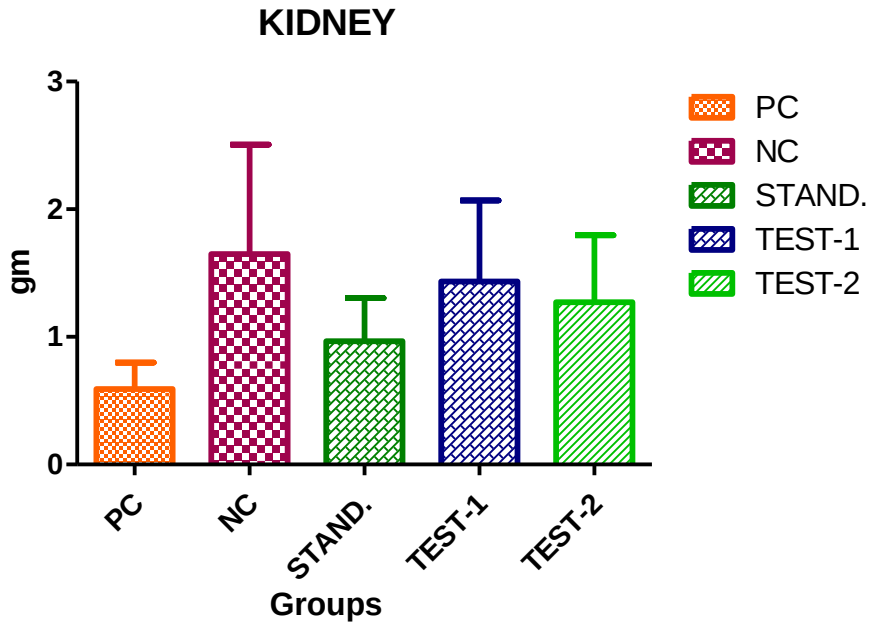
All numbers and Mean SEM (n = 5). Significant at ***P0.001, **P0.01, and *P0.1 compared to the negative control group using one-way ANOVA and the post-Tukey test.




Fig; 19 Effect of PHF administrations for 14 days on organ weight (liver)




Fig; 20 Effect of PHF administrations for 14 days on organ weight (heart)




Fig;21 Effect of PHF administrations for 14 days on organ weight (kidney)

**5.7 BIOCHEMICAL PARAMETER**

**5.7.1 LPO Activity**

Lipid peroxidation is an autocatalytic cycle that occurs as a result of cell passage. This cycle has the potential to injure peroxidative tissue in irritation, disease and harmfulness of xenobiotics and maturing. MDA is individual final results in the lipid per-oxidation measure. High lipid peroxidation esteems show exorbitant free l. revolutionary prompted peroxidation

and estimation of lipid peroxidation is marker of hepatocellular harm. Treatment with PHF essentially turned around these changes (P<0.001) contrasted with control rodents. Adequacy of PHF was equivalent with standard Atorvastatin. Consequently it could be conceivable that the system of antihyperlipidemic impact of PHF is because of its cancer prevention agent impact.

**5.7.2 SOD activity**

In this examination, SOD assumes a significant part in the end of ROS (Reactive oxygen species) obtained as a result of the peroxidative action of xenobiotics in liver tissues 30. The noticed increment of SOD movement proposes by the Vedic gatekeeper treatment has a proficient defensive system (P<0.001) because of ROS. And furthermore, these discoveries demonstrate that Vedic gatekeeper might be related with diminished oxidative pressure and free radical mediated tissue harm. High portion of Vedic watchman 180mg/kg b. w., p. o. was similar with reference Silymarin 25mg/kg (Table 14).

**5.7.3 Glutathione action (GSH)**

GSH is generally dispersed in cells of sample. GSH is an little intra-cell reducess and assumes significant part in catalysis, digestion and also its transport. It ensures cells adjacent to free extremists, peroxides and other harmful mixtures. Undoubtedly, GSH decrease expands the affectability of all the cells to different animosities and furthermore has a little metabolic impacts, for instance, a reduction in the pace of gluconeogenesis or an expansion in process of glycogenolysis 29. Treatment with PHF has viably recuperated the decreased glutathione level, (P<0.001) contrasted with control rodents. High portion of PHF was practically identical with standard Atorvastatin (Table 7).

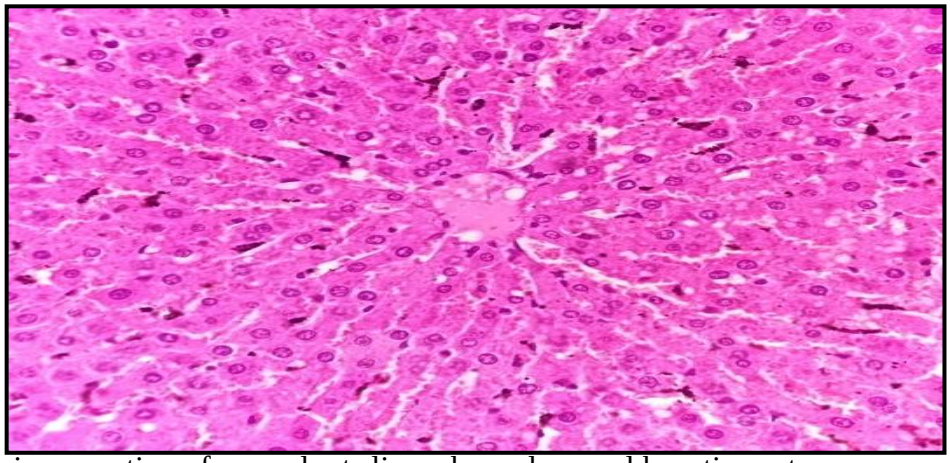
**Table No. 8 Effect PHF on MDA, GSH, SOD in Triton induced animal model liver homogenate.**

| Treatment groups | MDA | GSH | SOD |
|---|---|---|---|
| **Positive Control** | 77.43 ± 10.09 | 4.7 ± 0.15 | 37.53 ± 11.61 |
| **Negative Control** | 94.18± 1.13** | 2.08 ± 0.04** | 39.83 ± 10.53 |
| **Standard** | 41.65 ± 0.99*** | 3.62 ± 0.14*** | 22.74 ± 4.23 |
| **Test 1** | 73.3± 0.42*** | 2.925 ± 0.05*** | 62.73 ± 20.18 |
| **Test 2** | 65.6 ± 2.05** | 3.37 ± 2.04** | 42.63 ± 13.18 |

All values as well as Mean ± SEM. (n=5). Significant at ***P<0.001, ** P<0.01, *P<0.1 vs negative control group by one way ANOVA proceeded by the post Tukey test..

**5.7.4 Effect of PHF on the histopathology of the liver.**

Treatment with the dose of 300 mg/kg of Triton X-100 The central hepatic vein and sinusoidal blockage were caused by the extensive fatty destruction of hepatocytes relative to the normal hepatic architecture. (Figure 22). These histological changes were the the greatest substantial benefit brought about by 100mg/kg of PHF (Figure 25) and 200mg/kg of PHF (Figure 26), roughly in line with what was seen in atorvastatin treated rats (Figure 24).



Fig; 22 A specimen section of normal rats liver showed normal hepatic anatomy.

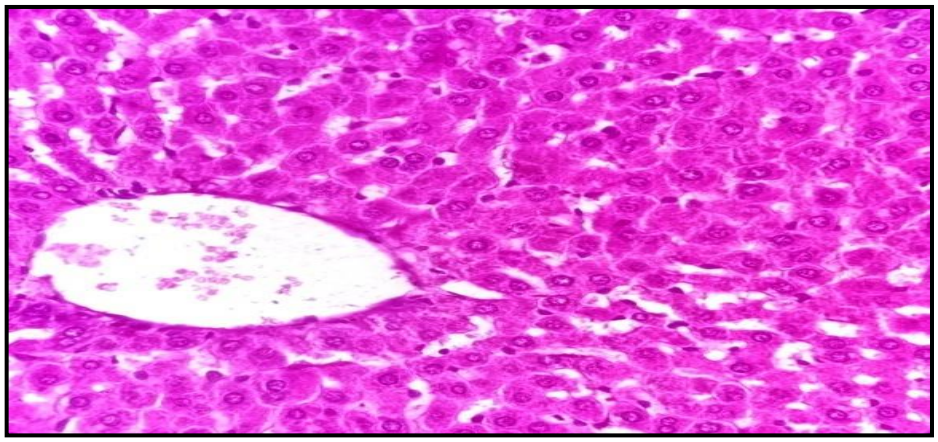
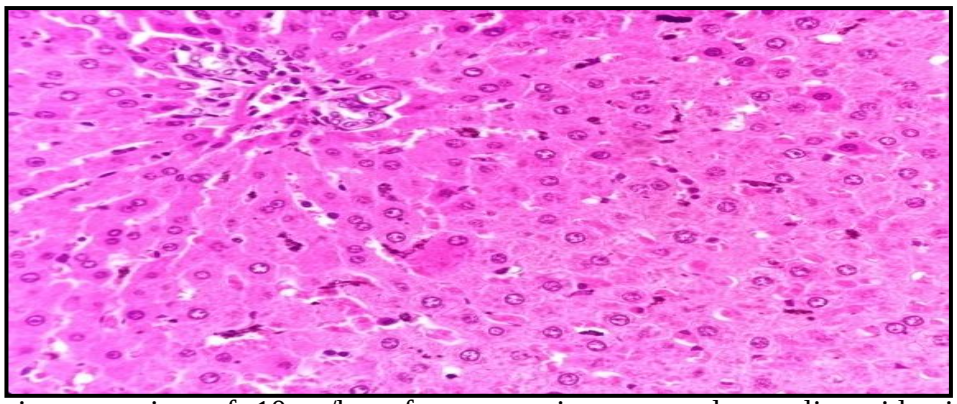
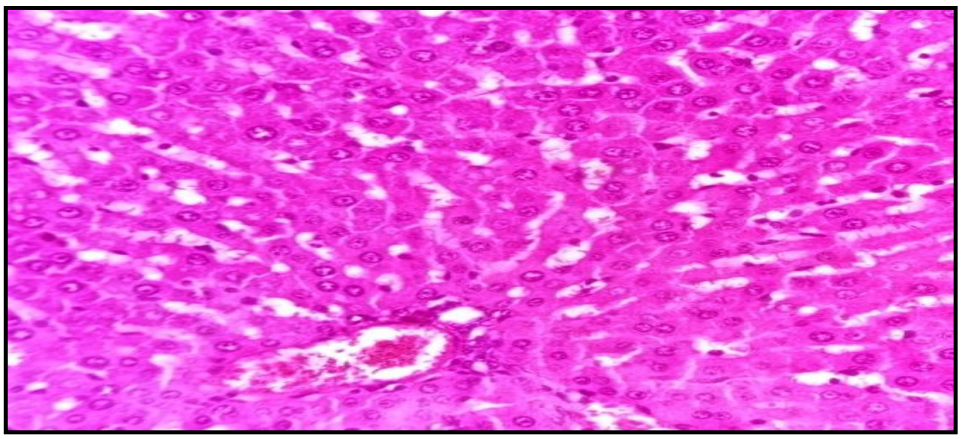


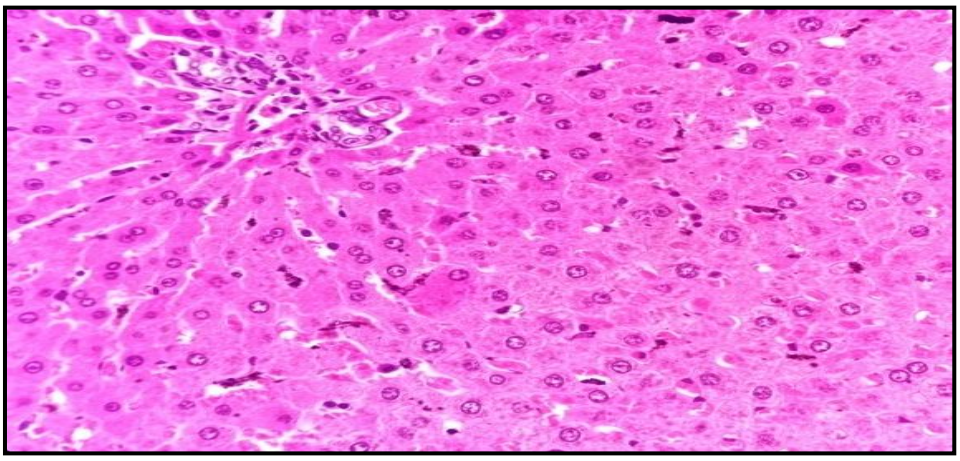
Figure 23 A specimen section of Triton X-100 treated rats liver identified obstructed central hepatic vein and sinusoids as well as extensive hepatic fatty deterioration.



Fig; 24 A specimen section of 10mg/kg of atorvastatin-pretreated rats liver identified obstructed hepatic portal triad, mild sinusoidal obstruction and also normal hepatocytes



Fig; 25 A representative section of 100mg/kg of PHF treatedrats liver showed mildly congested central hepatic vein, portal triad, sinusoidal congestion, and normal hepatocytes.



Fig; 26 A specimen section of 200mg/kg of PHF treated rats liver identified mildly obstructed central hepatic vein, portal triad, sinusoidal obstruction and also normal hepatocytes.

## 6. Discussion

Hyperlipidemia is defined as an increase in fatty acids and cholesterol in the blood; therefore, the system in preventing or treating hyperlipidemia is most possibly utilising drugs that contain a collection of compounds that can delay fat incorporation and accumulation through the gastrointestinal tract by suppressing pancreatic lipase and pancreatic cholesterol esterase activities.[17]

The motivation behind this the examination was to assess the hypolipidemic activity of PHF on triton X-100 instigated hyperlipidemia in rodents. On 14 days of dosing of PHF had the option to forestall the increment of TC, TG, LDL-c, VLDL-C, blood glucose levels, body weight however it couldn't forestall the reduction on HDL-C levels incited by the triton X-100 organization in this trial convention.

As a class of HMG-CoA reductase, atorvastatin functions by determining the rate of protein synthesis in cholesterol biosynthesis. This may be due to the increased availability of acetyl CoA, which invigorates the cholesterogenesis rate. LDL receptors may be down-regulated as

a result of cholesterol and unsaturated fats in the diet, which may also explain the rise in serum LDL-C levels.

The nonionic cleanser, Triton X-100, has been utilized broadly to obstruct the take-up of triacylglycerol-rich amount of lipoproteins from blood plasma by fringe tissues by deliver intense hyperlipidemia in creature models, which are frequently utilized for various destinations, specifically for observation common or synthetic hypolipidemic drugs. Test proof backings the idea that Triton X-100 actually changes exceptionally low thickness lipoproteins (VLDL), delivering them refractive to the activity of lipolytic catalysts of both blood and tissue [19]. This forestalls or postpones their expulsion from blood and optionally animates the cholesterol biosynthesis in hepatic region, upgrading the hyperlipidemia. There was stamped expansion in the degree of serum complete cholesterol, fatty substances, LDL-c, VLDL-c and lessening in the degree of cholesterol transporter HDL in the rodents treated with Triton X-100.[20]

Triton WR-1339 goes about as a surfactant and stifles to the activity of enzymes lipases to obstruct and also take-up of lipoproteins as of dissemination by additional hepatic tissues, bringing about expanded blood lipid fixation .[21]

The lipid bringing down impact of berberine might be because of expanding insulin emission or adjustment of the hepatic LDL receptor (LDLR) by animated extracellular sign directed kinase-subordinate pathway.

Berberine. The primary component of berberine shows the inhibitory impact on hypoglycemic adjusting lipids metabolic impacts and it's capacity to rummage free revolutionaries (23). The hindrance of intestinal glucose retention or incitement of fringe glucose take-up additionally could be the another system of hypoglycemic impact of berberine. Expanded AMP-enacted protein kinase (AMPK) movement in 3T3-L1 adipocytes and L6 myotubes and diminished the lipid amassing in T3-L1 adipocytes came about of berberine treatment (24). Then again, restraint of mitochodrial work (repressed oxygen utilization, increment AMP/ATP proportion and AMPK enactment) by berberine can prompt up-guideline of glucose and lipid digestion.

Black pepper has dynamic constituent piperine, showed commented decline in the amount of cholesterol (both free and also ester cholesterol divisions), free unsaturated fats, phospholipids and fatty substances. Besides, supplementation of the great fat took care of rodents with dark pepper raised the convergence of high thickness lipoprotein-cholesterol (HDL-c) and decreased the centralizations of low thickness lipoprotein-cholesterol (LDL-c) and also very low thickness lipoprotein-cholesterol (VLDL-c) in the blood plasma.

Guggulsterones are thought to repress the arrangement of lipoproteins and lower the pace of intestinal retention of fat and cholesterol prompting an expanded pace of fecal discharge of bile acids and cholesterol. Guggulsterone was proposed to improve the pace of take-up of LDL by incitement of LDL receptor-restricting action in the cell films of hepatocytes. The lipid-bringing down action is likewise a consequence of direct incitement of the thyroid organ.

Flavonoids were researched diminished the degree of TG, TC, leptin and insulin with a specific reach and improved insulin obstruction liver capacity, HDL-c levels. The component behind the flavonoids antihyperlipidemic job is digestion of liver discharged bi items.

Lipophilic substances are difficult to dissolve in blood, and cholesterol is no exception. Lipoproteins including phospholipids and apolipoproteins are where it is found. Cholesterol esters and fats are included in the lipoprotein's lipid core, whereas phospholipid, apolipoprotein, and free cholesterol are found on the lipophilic outer film. For example, this permits the lipid particles to be distributed to cells throughout the body through blood. Proteins in the blood known as lipoproteins have a variety of activities. Lipoproteins are classified as high-, mid-, low-, or very low-thickness, with HDL being the thickest, IDL being the middle, and LDL being the thinnest (VLDL). At least 66% of the circulating cholesterol is believed to reside in LDL particles, which then transport it to the body's peripheral tissues. On the other hand, HDL atoms have been hypothesised to do the reverse. They remove a large amount of cholesterol from the body and send it to the liver, where it is excreted. Because high LDL and low HDL raise a patient's chance of developing atherosclerotic disorders, these two lipoproteins are of clinical importance.[22]

HDL is a little molecule made out of phospholipid and apolipoproteins and delivered in hepatic and intestinal cells. The frequency of coronary illness occasions in an ordinary populace is conversely identified with the serum HDL-cholesterol focus - low levels convey an expanded coronary danger. HDL is believed to be against atherogenic and high HDL levels shows a good cardioprotective action. This impact might be intervened by switch to a cholesterol transport, a cycle whereby abundant cholesterol in cells and in atherosclerotic plaques is eliminated and moved back to the liver. The danger for myocardial localized necrosis increments by around 25% for each 5 mg/dL decrement in serum HDL cholesterol underneath middle qualities for people. Low HDL-cholesterol is a segment of the metabolic condition that is described by corpulence, insulin obstruction, dyslipidemia, and hypertension.

LDL (low-density lipoprotein) is the plasma metabolite of low-density lipoprotein (VLDL). The breakdown of the fatty material (TG) in VLDL particles is catalysed by circulating lipoprotein lipase or hepatic lipase. Consequently, then under activity of cholesterol ester transport proteins (CETPs), the cholesterol ester (CE) of HDL is transported to VLDL, while phospholipids, apolipoprotein C (ApoC), and cholesterol are migrated to the exterior of VLDL. This conversation is continuing. VLDL has a continuous decrease in TG, an increase in CE, smaller particles, and a gradual increase in thickness. First, half-thickness lipoprotein (IDL) is formed, and then LDL is formed.

The GSH is essential in all cells, as it provides an antioxidant defenses and protects tissues against ROS production. Also, it serves in the elimination of ROS and actes both as a non-enzymatic oxygen radical scavenger and as a substrate for various enzymes such as GPx [23]

Lipid peroxidation as an indicator of LPO makes an indirect measurement of antioxidative deficit. This reduction in LPO is associated with a decline in the atherosclerosis caused by hyperlipidemia. LPO is a free radical that prompts oxidation of protein, disrupts cell membrane structure and function and produces

MDA showed that LPO cause many changes in the membrane structure and function (increased permeability to ions like Ca2+), causing cellular abnormalities such as cell mutation and death. [24]

Atherogenic dyslipidemia because of modification of lipid profile for the duration of the postmenopausal era is a typical marvel and careful as a piece of few metabolic disorder. It is

described by an increment of fatty oil (TG) and low thickness lipoprotein (LDL-C) and abatement of high thickness of lipoprotein (HDL-C) in blood stream.7 This lipid ternion anomaly expands atherogenicity among postmenopausal ladies contrasted and in its premenopausal partner and well thought-out as a solid danger factor of atherosclerotic heart diseases.8, 9 This atherogenic dyslipidemia has arisen as a significant symbol of upcoming CVD occasion, and henceforth, unique exploration attempted to deal with this matter by utilizing different files of lipid profiles. [25]

The PHF shows huge expansions in HDL levels and diminishes in LDL, VLDL levels. The expansion in LDL, VLDL levels increment the danger of cardiovascular illness. The expansion in HDL has a cardioprotective impact and is demonstrated by different examinations.

Examination on natural meds prompts the improvement of elective medications and systems. The as of now utilized hyperlipidemic drugs behind the ideal properties like adequacy and security on long haul use, cost and straightforwardness of organization. These elements satisfy the conditions for patient confusion. Spices are the mine of restorative specialists needs for analysts are felt to discover efficacious, modest, and safe hyperlipidemic specialists from among characteristic items.

The rhizome of *Berberis,* the fruit of *Pepper Nigrum,* gum of *guggul* have large amounts of flavonoids and saponins. The preliminary phytochemical screening of PHF has exposed the presence of flavonoids and saponins. In the earlier studies, alkaloids, carbohydrates, tannins, glycosides, flavonoids, gums, and saponins have been shown to have many types of biochemical and pharmacological activities, including hypolipidemic effects.

## 7. CONCLUSION

The data obtained from the study it may be concluded that the PHF drugs possessed an antihyperlipidemic activity in triton model. The hyperlipidemic activity of PHF probably due to its antioxidant property, presence of flavonoids, saponins, and tannins, etc. The implemented additional research into alternative bioactivity undertake fractionation of these extracts to separate and define putative bioactive compounds.

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