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Abstract

The present study has been undertaken to evaluate antihyperlipidemic and antioxidant activities of Terminalia Arjuna (TA) and Commiphoramukul (CM) standardized extracts in combination at their predetermined doses. The hyperlipidemia in animals (rats) was induced by high fat diet by mixing Indian vanaspati ghee and coconut oil in the ratio of 3:1 (v/v). Acute toxicity was performed according to Organization of Economic Cooperation and Development (OECD) -423 and observed for behavioral changes, hematological and biochemical alteration if any or not for 14 days. The result of toxicity studies did not indicate any major changes in the result in comparison with control group of animals. The combination of plant extracts exhibited significant antihyperlipidemic activity in comparison to control group. The level of triglycerides (TGL), cholesterol (CHO), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) got reduced with increased level of high-density lipoprotein (HDL). The result of test drug and standard drug showed similar value with some minor difference. Hematology data of hyperlipidemic rats showed safety level of blood components after treatment with test drug. The test drug also revealed good antioxidant activity by normalization of superoxide dismutase (SOD) and nitric oxide (NO) levels. Thus, further study required to determine the active constituents from plants extracts required for biological activities.



Keywords Terminalia arjuna - Commiphoramukul - Super oxide dismutase - Nitric oxide

# Anti-hyperlipidemic and Antioxidant Activities of a Combination of Terminalia Arjuna and Commiphora Mukul on Experimental Animals

**Jhakeshwar Prasad, Ashish Kumar Netam, Trilochan Satapathy, S. Prakash Rao, and Parag Jain**

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**Keywords** Terminalia arjuna · Commiphoramukul · Super oxide dismutase · Nitric oxide

## 1 Introduction

Higher amount of lipids or fats in the blood is characterized as hyperlipidemia. It is a family disorder in which fatty contents get increased abnormally. However, increased amount of fats increases the risk of coronary heart disease (CHD) and also plays role in body's metabolic processes. Individual's diet also shows impact on

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hyperlipidemia; high cholesterol diet and food containing more saturated fats lead to increased blood cholesterol, and triglycerides levels. Other disorders, such as diabetes mellitus, kidney disease, and hypothyroidism, may promote hypertriglyceridemia (Fronzo and Ferrannini 1991). Most people who have hyperlipidemia also having relevant other complications such as diabetes, high cholesterol and have difficulty in managing all three conditions at a time. Hence combination therapies of more than two or three drugs are prescribed by physicians or clinicians those in turn produce severe adverse effects. Calcium channel blockers (CCBs) are one of the most potentially lethal prescriptions, which may worsen hyperlipidemia if administered excessively (Saeed and Larik 2017).

Frequent administration of CCBs may cause rapid fall in blood pressure, decreased heart rate, and cardiac arrest. However, overdoses of sustained-release formulations result in delayed onset of dysrhythmias, shock, sudden cardiac collapse, and bowel ischemia. Among the anti diabetic agents, Di-Peptidyl Peptidase-IV (DPP-IV) inhibitors are drug of choice for treatment of Type-II diabetes and recent research revealed that, long term administration of DPP-IV inhibitors at their therapeutic doses also causes pancreatic cancers. Herbal drug had been used since ancient times for welfare of the mankind and several research have been done to identify the active compound responsible for therapeutic activity. The active components of plants when taken together may give synergistic effect,when they have co-administered for the treatment of multifactorial disorders such as diabetes associated with hypertension and dyslipidemia. Terminalia Arjuna(TA) is a wild herb containing various chemical constituents. Among these arjunetin and arjunosides acts as a major constituent already been reported for having affinity for Na+ - K+ ATPase Pump. (Urizar and Moore 2003) Commiphoramukul (CM) also reported for having antihyperlipidemic activity. The objective of this present research work is to evaluate the affinity of Terminalia arjuna for Na+ - K+ ATPase Pump blocking effect which in turn may be useful as an antihypertensive agent. When more than two drugs are administered at a time there may be a chance of drug interactions. Hence, toxicity studies need to be carried out for these combination therapies and to achieve better therapeutic response of Terminalia arjuna and Commiphora mukul standardized extract at their predetermined ratio for their synergistic effect in the treatment of high fat diet induced hyperlipidemia using experimental animals in Rats (Dobrian et al. (2000).

## 2 Materials and Methods

### *2.1 Drug and Chemical Reagents*

Terminalia Arjuna and Commiphoramukuldried extracts were received as a gift sample from SUNPURE Pvt. Ltd New Delhi (India). Carboxy methyl cellulose (0.5–5%) was purchased from LOBA Chemie Pvt. Ltd. Mumbai. Atorvastatin was procured from Sun Pharmaceuticals Pvt. Ltd. Mumbai, Maharashtra,

(India). Halothane was purchased from Korten Pharmaceuical Pvt. Ltd. Shanti-Sthal, Shirgaon-Palghar, Thane-Mumbai (India), and Formaldehyde was purchased from Merck Life Science Pvt. Ltd., Vikroli East, Mumbai, Maharashtra. All other chemicals used was of highest analytical grade-commercially available.

**Experimental Animals**. Healthy adult Male Albino Wistar rats weighing 180–200 gm were obtained from the Animal House Facility of Columbia Institute of Pharmacy, Raipur, Chhattisgarh, (India) having certificate number CIP/IAEC/2017/103 and Regd. No.1321/PO/ReBi/S/10/CPCSEA. The animals were kept and maintained under controlled environmental conditions with temperature (23 ± 2 °C), relative humidity (40–50%), and 12/12 h light/dark cycle. The animals received a standard pellet diet (Hindustan lever limited, India) and water ad libitum. The animals used in the present study were cared as per the principles and guidelines of Institutional Animal Ethics Committee (IAEC), and in accordance with the CPCSEA, New Delhi, India. The animals were acclimatized to laboratory conditions for at least seven days before initiation of the experiment.

**Acute Toxicity**. The acute toxicity was evaluated as per OECD guideline-423. Animals were received dose of Terminalia arjuna along with Commiphora mukul 250 mg/kg body weight orally administered by using an oral feeding needle after short fasting period. The general behavior of the animals was continuously monitored for 30 min, 1, 2, and 3 h after dosing, periodically during the first 24 h (with special attention given during the first 4 h) and then daily observed for 14 days.

**Experimental Study**. The experiment was carried out on animals (albino Wistar rats) to determine therapeutic effectiveness of combination study. In this experiment rats of either gender were randomly divided into four groups. Each group consists of five animals either gender (n = 5). All the animals were administered high fat diet for induction of hyperlipidemia (Table 1). AQ2

**Induction of hyperlipidemia in rats**. Hyperlipidemia was induced by feeding rats on diet rich in fats. It was prepared by mixing India Vanaspati ghee and coconut oil (3:1, v/v). This diet was given per-oral to rats at a dose of 3 ml/kg body weight daily (Munshi et al. 2014).

**Table 1** Allocation of animals into various groups for therapeutic effectiveness study

| Groups | Treatment | Doses |
|---|---|---|
| 1 | Control group | Drinking water (Oral) |
| 2 | Toxic group | High fat diet (3 ml/kg) |
| 3 | Standard drug (Atorvastatin) | 10 mg/kg |
| 4 | Test group [CM+ TA (50:50)] | 500/kg/body weight (Oral) |

**Hematological Study**. The blood was collected with EDTA anticoagulant through retro orbital puncture for biochemical estimation. The evaluated blood parameters were red blood cell count (RBC), blood hemoglobin concentration, basophil, eosinophil and neutrophil granulocytes, lymphocytes, and monocytes, hematocrit value, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC), and platelet counts.

## *2.2 Biochemical Estimation of Antioxidants*

**Superoxide Dismutase (SOD) Assay**. The assay was performed by the production of superoxide from oxygen molecule using reduced b-nicotinamide adenine dinucleotide (NADH) as a reductant and phenazine methosulphate (PMS) as a catalyst. Nitrobluetetrazolium (NBT) was used as an indicator that turned blue when reduced by superoxide. Change in color was monitored spectrophotometrically in the visible range at 560 nm. While adding test drug to the reaction; the antioxidants (superoxide scavengers) competed with NBT to react with superoxide. The percent inhibition of NBT reduction was used to quantify superoxide-scavenging.

*Procedure*. 10% w/v tissue homogenate in 0.15 M TrisHCl or, 0.1 M phosphate buffer was prepared and centrifuged at 15,000 rpm for 15 min at 4 °C. The supernatant (0.1 ml) was taken and considered it as sample. Then 0.1 ml sample + 1.2 ml sodium pyrophosphate buffer (pH 8.3, 0.052 M) + 0.1 ml phenazinemethosulphate (186 μM) + 0.3 ml of 300 μM Nitroblutetrazolium + 0.2 ml NADH (750 μM) were mixed and incubated at 30 °C for 90 s followed by addition of 0.1 ml glacial acetic acid. This was then stirred with 4.0 ml n-butanol and allowed to stand for 10 min followed by centrifugation, and butanol layer was separate. The Optical Density (OD) of the rest of the sample was measured at 560 nm by taking butanol as blank (Paoletti et al. 1986; Sapakal et al. 2008).

**Nitric Oxide Estimation**. Nitric oxide is produced due to oxidative stress occurring in the brain. The assay was performed by taking 100 μl of serum sample in a test tube and added 400 μl of carbonate buffer (pH 9.0) followed by addition of copper cadmium alloy fillings (0.15 g). The reaction was stopped by addition of sodium hydroxide (100 μl of 0.35 M) and zinc sulphate solution (400 μl of 120 mM) under vortex mixing. Then the solution was allowed to stand for 10 min and centrifuged at 4000 rpm for 10 min. The clear supernatant solution (500 μl) was transferred to another test tube in which 500 μl of Griess reagent was added. The absorbance was noted spectrophotometrically at 548 nm. A standard curve (1–100 μM) was plotted using sodium nitrite to calculate the concentration of nitrite (Griess Reagent Kit; Sastry et al. 2002).

*Procedure*. Mix the following in a spectrophotometer cuvette (1 cm pathlength) i.e., 100 μL of Griess reagent, 300 μL of the nitrite-containing sample and 2.6 mL of deionized water. Then incubated the mixture for 30 min at room temperature. A

photometric reference sample was prepared by mixing 100 μL of Griess reagent and 2.9 ml of deionized water. Measured the absorbance of the nitrite-containing sample at 548 nm relative to the reference sample. Absorbance readings were converted to nitrite concentrations as described in calibration.

**Histopathological Examination**. The animals were anaesthetized with halothane and blood was collected by retro orbital puncture for biochemical estimation. The animals were again anaesthetized by using excess halothane and sacrificed by cervical dislocation method. The abdominal portions were cut opened and heart was dissected out. The Heart was removed immediately and transferred into 10% formalin solution for routine histopathological examination. The samples were taken from the sections of rat heart tissue with highest macroscopic damage. The heart tissue specimen from each animal was removed and fixed in 10% formalin solution then cut into 5 μm thickness, stained using hematoxylin eosin for the histopathological examination. They were made using a rotary microtome, 5 μm thickness sections were cut from the tissue samples embedded in paraffin and placed on standard glass slides. The paraffin was melted with a period of approx 12 h in an incubator at 58 °C. The samples were then stained with haematoxylene and eosin (H&E) according to the protocol. Qualitative analyses were performed on 400× magnified images.

# 3 Results

## *3.1 The Effect of Terminalia Arjuna Along with Commiphora Mukul on Behavioral Changes*

The results of oral acute toxicity study indicated minor behavioral changes and no mortality observed in animals through the 3-days period following single oral administration at all selected dose levels of the Terminalia arjuna along with Commiphora mukul (Table 2 and Fig. 1).

## *3.2 The Effect of Terminalia Arjuna Along with Commiphora Mukul on Hematological Changes*

See Table 3.

**Table 2** Effect of Terminalia arjuna along with Commiphora mukul on lipid profile level in Albino Wistar rats

| Parameters | Duration | Sex | Control | Toxic group | Standard | Test |
|---|---|---|---|---|---|---|
| TGL (mg/dL) | 0 Day | M | 62.5 ± 0.26 | 78.4 ± 0.22 | 63 ± 0.34 | 60.6 ± 0.33 |
| | | F | 63.4 ± 0.36 | 77.3 ± 0.20 | 64 ± 0.32 | 59.4 ± 0.37 |
| | 10th Day | M | 75.5 ± 0.23 | 89 ± 0.21 | 87.9 ± 0.24 | 73 ± 0.25 |
| | | F | 76.6 ± 0.34 | 88 ± 0.19 | 88.7 ± 0.27 | 72 ± 0.28 |
| CHO (mg/dL) | 0 Day | M | 53 ± 0.23 | 74 ± 0.25 | 57.2 ± 0.34 | 51.5 ± 0.36 |
| | | F | 54 ± 0.25 | 73 ± 0.24 | 58.5 ± 0.32 | 50.4 ± 0.34 |
| | 10th Day | M | 56.2 ± 0.34 | 71 ± 0.31 | 70.1 ± 0.33 | 54.5 ± 0.23 |
| | | F | 57.2 ± 0.37 | 70 ± 0.30 | 71.3 ± 0.35 | 53.3 ± 0.25 |
| HDL (mg/dL) | 0 Day | M | 11.4 ± 0.25 | 3 ± 0.27 | 11.5 ± 0.22 | 12.3 ± 0.33 |
| | | F | 12.3 ± 0.34 | 2 ± 0.25 | 12.2 ± 0.20 | 12.7 ± 0.2 |
| | 10th Day | M | 13 ± 0.23 | 4 ± 0.21 | 18.3 ± 0.31 | 17.5 ± 0.27 |
| | | F | 14 ± 0.34 | 3 ± 0.20 | 19.4 ± 0.33 | 18.4 ± 0.25 |
| LDL (mg/dL) | 0 Day | M | 23.6 ± 0.32 | 37 ± 0.30 | 22.5 ± 0.24 | 23.8 ± 0.21 |
| | | F | 24.5 ± 0.31 | 36 ± 0.28 | 23.3 ± 0.25 | 24.3 ± 0.23 |
| | 10th Day | M | 28.9 ± 0.25 | 26.7 ± 0.23 | 34.8 ± 0.32 | 44.8 ± 0.34 |
| | | F | 29.7 ± 0.27 | 25.5 ± 0.21 | 35.5 ± 0.33 | 45.7 ± 0.32 |
| VLDL(mg/dL) | 0 Day | M | 15.2 ± 0.31 | 28 ± 0.29 | 15.7 ± 0.25 | 14.4 ± 0.31 |
| | | F | 14.5 ± 0.33 | 27 ± 0.27 | 16.5 ± 0.23 | 13.2 ± 0.33 |
| | 10th Day | M | 15.8 ± 0.21 | 13.7 ± 0.19 | 17.9 ± 0.33 | 18.5 ± 0.34 |
| | | F | 16.6 ± 0.24 | 12.6 ± 0.17 | 18.7 ± 0.31 | 17.7 ± 0.32 |

Mean ± SEM (n = 5)
Triglycerides (TGL), Cholesterol (CHO), High density lipoprotein (HDL) Low density lipoprotein(LDL), Very-low-density lipoprotein (VLDL)

## 3.3 *Biochemical Parameters Studies*

**Superoxide Dismutase Assay**

See Table 4 and Fig. 2.

**Nitric Oxide (NO) Assay**

See Table 5 and Figs. 3 and 4.

**Histopathological Examination of Heart**. Animal organ (Heart) histopathology report is shown below (Plates 1, 2, 3, 4, 5 and 6).

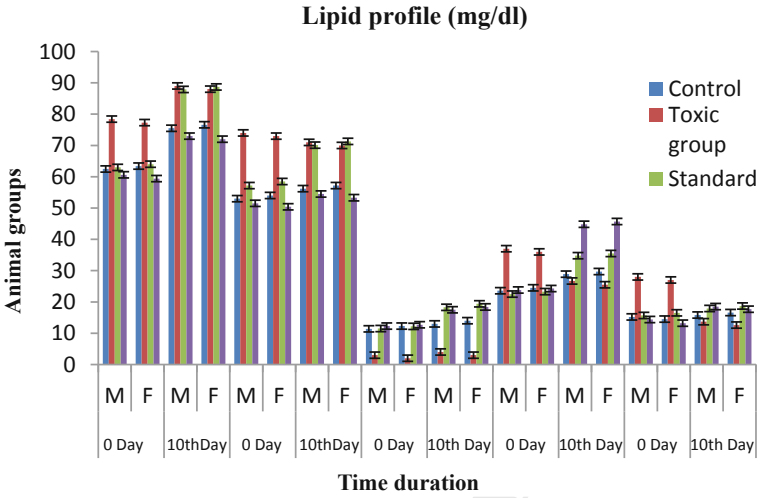



**Fig. 1** Graph showing the effect of various treatments on lipid profile in different group of animals. All values are reported as Mean ± SEM (n = 5)

## 4 Discussion

Hyperlipidemia is a multifactorial disorder involving interactions among environmental, vascular, neuroendocrine, and genetic factors. The prevalence of hyperlipidemia is increasing in India as well as all over the world. Apart from these, the other cause include is more complex i.e., association of type-2 diabetes mellitus as well as obesity. Those are polygenic factors. This complexity makes it difficult to diagnose the disorder properly that make the researchers to look major contributions toward the developments of new drug/new entity for effective treatment. As the drugs available in the market for the treatment of hyperlipidemia associated with diabetes are limited, many patients need the combination therapy of anti-lipidemics that in turn causes various side effects. Hence the herbal therapy has come into existence.

Terminalia arjuna has traditionally been used for the treatment of various heart disorders for more than centuries. It improves cardiac muscle function subsequently improving pumping activity of the heart. Among the active constituent present in the Terminalia arjuna the saponin glycoside thought to be responsible for the ionotropic effect while flavonoids and oligomeric proanthocyanidins (OPCs) provide free radicals antioxidant activity. In other way Commiphora mukul an oleo gum-resin has been used as medications since Vedic period for the effective treatment of number of vascular disorders such as atherosclerosis, hypercholesterolemia, obesity, etc., but the scientific evidence for the combination of these two (Terminalia arjuna and Commiphora mukul) has not been established till yet. So this present study has been undertaken to evaluate the safety and effectiveness of both the drug at their

**Table 3** Effect of combination therapy on hematological data of various groups of animal

| S. no. | Particulars | Sex | Control Group | Toxic Group | Standard group | Test Group |
|---|---|---|---|---|---|---|
| 1 | Heamoglobin (gm%) | M | 16.22 ± 0.08 | 5.21 ± 0.04 | 15.3 ± 0.07* | 11.28 ± 0.10* |
| | | F | 15.26 ± 0.107 | 4.20 ± 0.03 | 14.46 ± 0.120* | 10.4 ± 0.141 |
| 2 | Total WBC Count (cmm) | M | 4280 ± 37.41 | 1120 ± 31.21 | 4030 ± 50.99 | 5060 ± 143* |
| | | F | 4100 ± 70.710 | 1090 ± 29.19 | 3880 ± 135.64 | 5010 ± 86.023* |
| 3 | Neutrophils (%) | M | 61.4 ± 0.50 | 24 ± 0.39 | 58.8 ± 0.8 | 57.2 ± 0.8 |
| | | F | 59.4 ± 0.748 | 23 ± 0.37 | 57.4 ± 0.927 | 56.2 ± 0.8 |
| 4 | Lymphocytes (%) | M | 33.8 ± 0.37 | 15 ± 0.35 | 31.6 ± 0.50 | 30 ± 0.70* |
| | | F | 32 ± 0.707 | 14 ± 0.32 | 30.6 ± 0.927 | 28.2 ± 0.860 |
| 5 | Eosinophils (%) | M | 6.2 ± 0.37 | 2 ± 0.31 | 4.4 ± 0.50 | 3.4 ± 0.87 |
| | | F | 4.6 ± 0.509 | 1 ± 0.30 | 3 ± 0.707 | 3.1 ± 0.860 |
| 6 | Monocytes (%) | M | 03 ± 00 | 0.3 ± 0.25 | 02 ± 0.70 | 02. ± 0.45 |
| | | F | 02 ± 00 | 0.2 ± 023 | 01 ± 0.583 | 0.1 ± 0.43 |
| 7 | Basophiles (%) | M | 00 ± 00 | 00 ± 00 | 00 ± 00 | 00 ± 00 |
| | | F | 00 ± 00 | 00 ± 00 | 00 ± 00 | 00 ± 00 |
| 8 | RBC Count (%) | M | 8.302 ± 0.00 | 1.3 ± 0.27 | 6.766 ± 0.00 | 5.694 ± 0.03 |
| | | F | 7.286 ± 0.012 | 1.1 ± 0.24 | 5.73 ± 0.010 | 4.75 ± 0.014 |
| 9 | Platelet Count (%) | M | 3.728 ± 0.00 | 0.2 ± 0.00 | 2.354 ± 0.00 | 2.174 ± 0.01 |
| | | F | 2.726 ± 0.009 | 0.1 ± 0.01 | 1.33 ± 0.010 | 1.34 ± 0.018 |
| 10 | Mean Platelet Value (Million/cmm) | M | 10.28 ± 0.09 | 1.65 ± 0.07 | 8.722 ± 0.15 | 8.502 ± 0.14 |
| | | F | 9.38 ± 0.106 | 1.35 ± 0.05 | 7.56 ± 0.107 | 7.46 ± 0.145 |
| 11 | Packed Cell Volume (Million/cmm) | M | 41.74 ± 0.05 | 21 ± 0.03 | 39.24 ± 0.09* | 37.36 ± 0.10 |
| | | F | 40.42 ± 0.106 | 20 ± 0.01 | 38.5 ± 0.141* | 36.6 ± 0.114 |
| 12 | Mean Corpuscular Volume (Cu micron) | M | 50.548 ± 0.00 | 14 ± 0.04 | 48.57 ± 0.00 | 57.584 ± 0.00 |
| | | F | 47.76 ± 1.788 | 13 ± 0.02 | 47.5 ± 0.141 | 56.55 ± 0.014 |
| 13 | Mean Corpuscular Hemoglobin (Pictograms) | M | 19.28 ± 0.06 | 2.65 ± 0.07 | 17.602 ± 0.00* | 16.742 ± 0.00* |
| | | F | 18.42 ± 0.106 | 1.58 ± 0.05 | 16.54 ± 0.012 | 15.74 ± 0.014 |
| 14 | Mean Corpuscular Hemoglobin Con. (mg/dl) | M | 38.174 ± 0.00 | 14 ± 0.09 | 36.4 ± 0.50 | 35.29 ± 0.00* |
| | | F | 37.18 ± 0.012 | 13 ± 0.07 | 35.2 ± 0.860 | 34.75 ± 0.018 |
| 15 | Red Cell Distribution Width (%) | M | 15.62 ± 0.05 | 5 ± 0.04 | 13.64 ± 0.05 | 11.36 ± 0.10* |
| | | F | 14.36 ± 0.107 | 4 ± 0.02 | 12.5 ± 0.141* | 10.5 ± 0.141 |

Mean ± SEM (n = 5), P = < 0.005 (*)

**Table 4** Superoxide dismutase levels in different groups of animal

| S. no. | Groups | SOD level |
|---|---|---|
| 1 | Control | 42.00 ± 0.690 |
| 2 | Toxic group | 11.25 ± 1.24 |
| 3 | Standard | 39.41 ± 0.597 |
| 4 | Test group | 27.47 ± 1.194 |

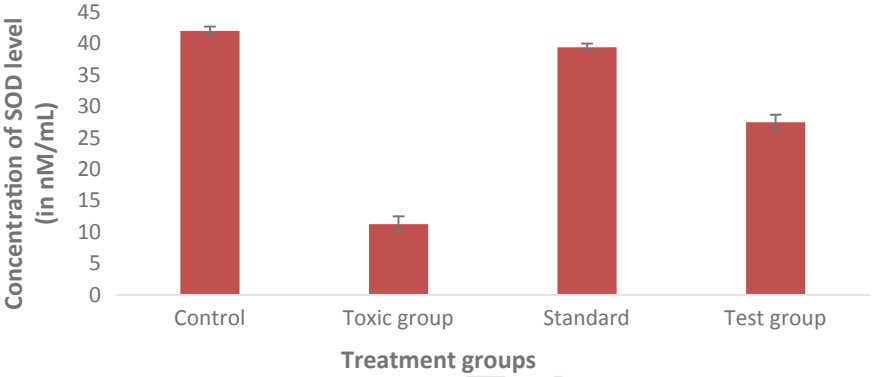



**Fig. 2** Graph showing the SOD levels in homogenized heart tissue of different groups of animal

**Table 5** Nitric oxide levels in different groups of animal

| S. no. | Groups | NO level |
|---|---|---|
| 1 | Control | 13.42 ± 0.144 |
| 2 | Toxic group | 2.12 ± 0.683 |
| 3 | Standard | 11.67 ± 0.382 |
| 4 | Test group | 10.17 ± 0.289 |

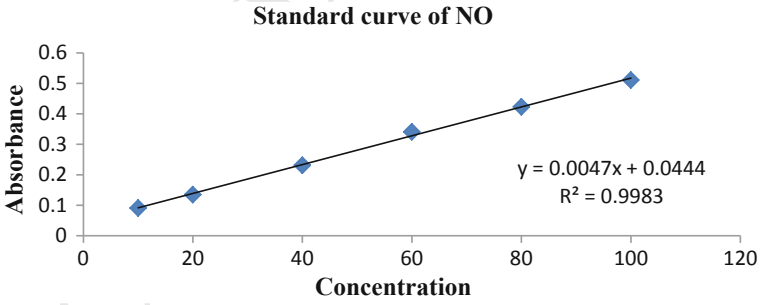



**Fig. 3** Graphical representation of standard curve of NO (Nitric Oxide)

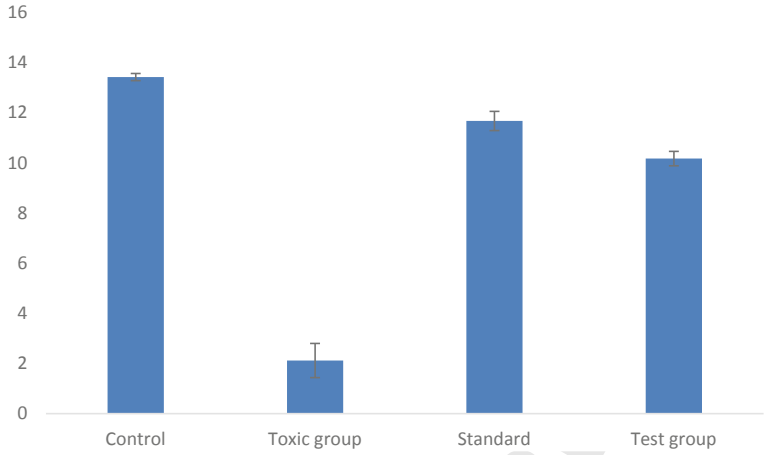
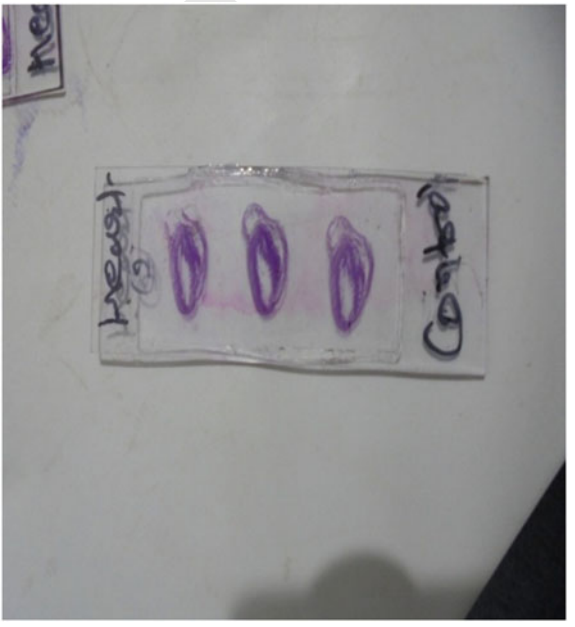



**Fig. 4** Graph showing the levels of NO homogenized heart tissue of different groups of animal

**Plate no. 1** Sample preparation of heart control group



predetermined dose level ratio by using high cholesterol diet hyperlipidemia in rat model.

The oral acute toxicity study for combination of both drugs in rats was carried out. The results for the acute toxicity study indicated that, there were no morbidity and mortality in animals of all the groups. The combination of drugs exhibited decreased level of TGL, CHO, LDL, and VLDL but increased level of HDL. Thus, representing

**Plate no. 2** Effect of vehicle on histopathological changes of heart tissue in control group

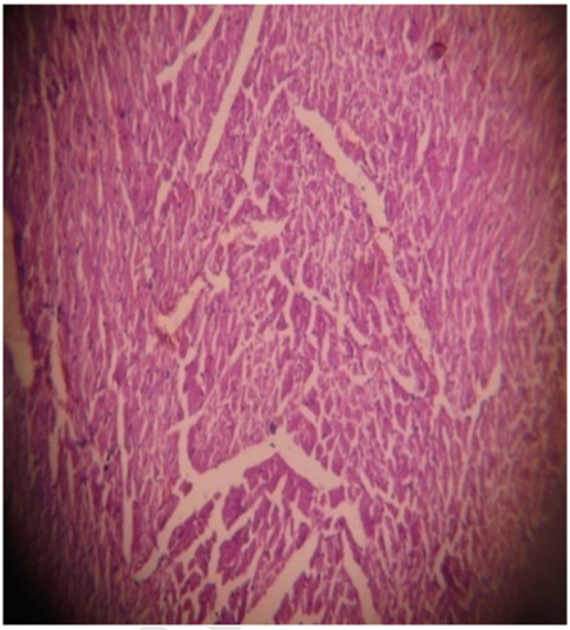


**Plate no. 3** Sample preparation of heart test group




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**Plate no. 4** Effect of Terminalia arjuna along with Commiphora mukul histopathology report of heart test group

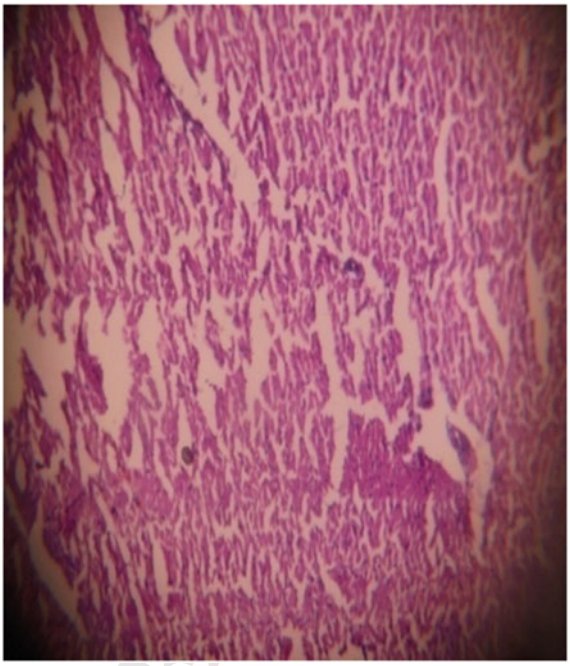
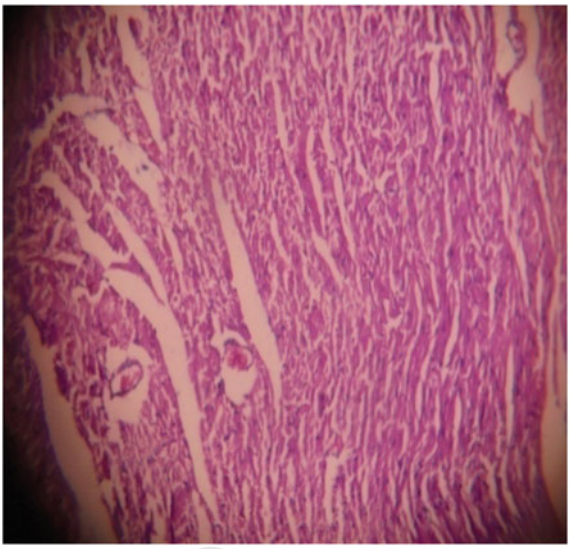


**Plate no. 5** Sample preparation of heart standard Group

**Plate no. 6** Effect of Atorvastatin histopathology report of heart standard group



antihyperlipidemic effect in comparison to control group. The results of antioxidant activity (SOD and NO level) revealed that the combination therapy showed good antioxidant activity on 10th day. Further, exhaustive study is required to determine active constituents and establish the exact mechanism responsible for biological activities.

## 5 Conclusions

In this present study, various parameters were evaluated for establishment of safety and effectiveness of combination therapy containing Terminalia arjuna and Commiphora mukul. Both the drugs in combination with their predetermined ratios exhibited significant antihyperlipidemic and antioxidant properties. The result of oral acute toxicity study did not show any behavioral changes and mortality.

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