Objecitve: The objective of this research article is to develop and evaluate polyherbal preparation and comparative studies on diet-induced hyperlipidemia.

Methods: After the extraction, pharmacognostical and phytochemical screening was done. The lipid-lowering activity of polyherbal formulation (T1, T2, T3, T4, and T5) may be attributed to the phytoconstituents present such as alkaloids, carbohydrates, steroids, proteins, tannins, carbohydrates, flavonoids, phenols, glycosides, and triterpenes. In acute oral toxicity study, there were no behavioral changes seen up to 4 h and no mortality was observed up to the end of 24 h even at the maximum tested dose level of 2000 mg/kg per oral. It was considered maximum safe dose. Male and female albino rats weighing 150–200 g were used for the study. Hydroalcoholic extract of all plants was prepared having a dose of 2000 mg/kg. The doses were selected according to the Organisation for Economic Cooperation and Development guideline no. 425. The procedure was divided into two phases: Phase I (observation made on day 1) and Phase II (observed the animals for the next 14 days of drug administration). Animals received a single dose of 2000 mg/kg. After the administration of Healthcare Administration, food was withheld for 3–4 h. In case an animal dies, we have to again perform the test for the determination of LD₅₀. The study was conducted by measuring various parameters, namely, daily feed intake (g), water intake (ml), body weight (g), lipid profile high-density lipoprotein (HDL), low-density lipoprotein (LDL), Cholesterol (CHL) level (mg/dl), and blood glucose level (mg/dl).

Results: Results showed a significant decrease in blood glucose level and serum lipid profile such as total cholesterol, LDL, and increasing serum HDL level, so could be useful in the treatment of hypolipidemia.

Conclusion: Polyherbal formulations (T1, T2, T3, T4, and T5) have hypoglycemic activity and significantly improve lipid profile levels in diet-induced experimental rats.

Keywords: Hyperlipidemia, Atherosclerosis, Allium sativum, Moringa oleifera, Cicer arietinum, Hibiscus rosa-sinensis, Quisqualis indica.

INTRODUCTION

Hyperlpidemia is known as a condition of an increased level of serum total cholesterol (TC), low-density lipoprotein (LDL), very LDL (VLDL), and reduced high-density lipoprotein (HDL) [1].

Cardiovascular diseases (CVDs) are a major risk factor caused by hyperlipidemia. Hyperlipidemia disease has afflicted humankind since antiquity. Hyperlipidemia, or hyperlipoproteinemia, is defined as abnormally elevated levels of one or more of triglycerides, cholesterol, cholesterol esters, and phospholipids and plasma lipoproteins including VLDL and LDL, and reduced HDL levels. These lipoproteins are deposit in the interstitial space of arteries arising from aorta, restricting the blood supply to the heart. This phenomenon is known as atherosclerosis. Due to the privilege deposition of lipoproteins, blood supply to the heart gets blocked, and thus, myocardial infarction occurs, which is usually well known as heart attack.

Among ischemic heart disease and the high mortality rate, there is a strong relation. In addition, high plasma cholesterol levels cause more than 4 million deaths in a year. Secondary hyperlipidemia often mimics familial forms of hyperlipidemia and can have similar effects. Due to secondary hyperlipidemia, risk of premature atherosclerosis increased or, when happen with severe hypertriglyceridemia, may cause pancreatitis and other consequences of the chylomicronemia syndrome [2,3].

Fig. 1: Soxhlet extraction of plant parts [17,18]
From times of yore, several studies have shown that dietary modifications such as high-fiber diets, low-fat diets, and diets rich in flavonoids and phenolic acids can reduce metabolic syndrome risk factors. Statins and synthetic antioxidants such as probucol are modern anti-hyperlipidemic drugs which are widely used to treat atherosclerosis. Regrettably, these drugs are not free of side effects. To provide novel treatments for hyperlipidemia, it has been focused on the natural products that have very few side effects [4-6].

Herbs have a defined kind of potency through which they can stimulate the human body to protect itself against the diseases. These medicinal herbs serve as a great source of remedies in the treatment of human and animal diseases [7].

Here, we are in progress to prepare a polyherbal formulation which consists of the extract of *Allium sativum*, *Moringa oleifera*, *Cicer arietinum*, *Hibiscus rosa-sinensis*, *Quisqualis indica*

### Table 1: Animals were grouped as follows

| Group   | Description                                                                 |
|---------|-----------------------------------------------------------------------------|
| I       | Normal control group (normal diet [ND])                                     |
| II      | Positive control group (ND+HFHS)                                           |
| III     | Standard control group (HFHS+ND+atorvastatin calcium [2.1 mg/kg, p.o. body weight]) |
| IV      | Test control group (HFHS+ND+polyherbal formulation [300 mg/kg, p.o. body weight]) |

HFHS: High-fat high-sugar diet

### Table 2: Morphological characteristics of plants part

| S. No. | Character | Observation |
|--------|-----------|-------------|
| T1     | Color     | Green       |
| T2     | Odor      | Rough       |
| T3     | Taste     | Pungent     |
| T4     | Size      | 5-150 cm    |
| T5     | Color     | Green       |
|        | Odor      | Smooth      |
|        | Taste     | Better to ingest |
|        | Size      | 2-5 cm      |
|        | Color     | Deep green  |
|        | Odor      | Rough       |
|        | Taste     | Sweet       |
|        | Size      | 0.5-3 cm    |
|        | Color     | Green       |
|        | Odor      | Slender     |
|        | Taste     | Edible tangy citrusy |
|        | Size      | 6.0" length |
|        | Color     | Waxy        |
|        | Odor      | Simple      |
|        | Taste     | Simple      |
|        | Size      | 5-10 cm     |

T1: *Allium sativum*, T2: *Moringa oleifera*, T3: *Cicer arietinum*, T4: *Hibiscus rosa-sinensis*, T5: *Quisqualis indica*

### Graph 1: Effect of high-fat high-sugar diet on total feed and water content. Values are expressed as mean±standard deviation; (n=6). All values are mean±standard error of mean, n=6. *p<0.05, **p<0.01 when compared to positive control group, following repeated measures ANOVA parametric methods, using Dunnett’s test.

### Graph 2: Effect of high-fat high-sugar diet, atorvastatin, and *Cordia dichotoma* on body weight. Values are expressed as mean±standard deviation; (n=6). All values are mean±standard error of mean, n=6. *p<0.05, **p<0.01 when compared to positive control group. Following repeated measures ANOVA parametric methods, using Dunnett’s test. NC: Normal control group, PC: Positive control group or high-fat high-sugar diet group, standard: ND+high-fat high sugar (HFHS)+atorvastatin (2.1 mg/kg/day), test control: ND+HFHS+polyherbal formulation (300 mg/kg/day)
Garlic (Allium sativum L., family: Alliaceae) has played significant medicinal and dietary roles throughout the ages. A. sativum (Garlic) preparations are commercially available in the form of garlic oil and garlic powder; and pills are widely used for certain therapeutic purposes, including improving lipid profile and lowering blood pressure. Extract of A. sativum is alleged to possess valuable effects for the prevention of CVDs. Garlic contains active hypcholesterolemic and hypoglycemic components, known as diallyl disulfide and dipropyl disulfide; it was proven by several studies [8-10].

Leaves of M. oleifera Lam., Moringaceae, are claimed to possess several pharmacological activities such as cholesterol-reducing effect and are used to treat patients with heart disease and antiobesity potential that protects the body against adverse effects of high-fat diet-induced obesity. The presence of β-sitosterol in crude extracts of M. oleifera possesses potential hypolipidemic properties [11,12].

C. arientum reported a rich source of vitamins, minerals, and phytoestrogens. Seeds of C. arientum were used as stimulant, aphrodisiac, tonic, anthelmintic, appetizer, relieving burning sensation in stomach, and in the treatment of obesity and in patients who consume excess oily and heavy foods. Cholesterol-lowering effects of C. arientum in different types of hyperlipidemias such as induced by diet are demonstrated in several research studies [13].

H. rosa-sinensis Linn. flowers exhibited a significant reduction in serum lipid parameters such as triglycerides, TC, LDL, VLDL, and increase in HDL [14].

Cholesterol diet and passive smoking raise the lipid and cholesterol levels with reducing the HDL level which causes hypercholesterolemia and hyperlipidemia existing heart disease such as heart attack and heart stroke in the future. Q. indica Linn. raised the HDL level which is good cholesterol and produced a significant reduction in harmful lipids. Q. indica extracts contain flavonoids and phenolic compounds helpful in CVD [15,16].

**METHODS**

**Collection of plant**

*A. sativum, M. oleifera, C. arientum, Q. indica, H. rosa-sinensis,* and Q. indica were collected from various places from BHEL area, Govindpura, Bhopal, Madhya Pradesh, during the month of May 2018.

**Authentication of plant material**
The plant has been identified and authenticated by Janatta PG College, A.P.S. University, Rewa, Madhya Pradesh, voucher specimen no. is Number/JJ/BOT/H-352 to H-356.

**Preparation of extract**

Extraction of *A. sativum, M. oleifera, C. arientum, Q. indica, H. rosa-sinensis,* and *Q. indica* was done by Soxhlet extraction method (Fig. 1).

- Soxhlet extraction: Soxhlet apparatus was used for the extraction and hydroalcoholic solvent (1:1) was selected as a solvent for extraction and calculated percentage yield of the extract.

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### Table 3: Consistency and color of all plant extracts

| Extract | Solvent uses | Color | Consistency |
|---------|--------------|-------|-------------|
| T1      | Hydroalcoholic | Dark green | Semi-solid |
| T2      | Hydroalcoholic | Black   | Semi-solid |
| T3      | Hydroalcoholic | Black   | Semi-solid |
| T4      | Hydroalcoholic | Dark green | Semi-solid |
| T5      | Hydroalcoholic | Dark green | Semi-solid |

1. Allium sativum, T2: Moringa oleifera, T3: Cicer arientum, T4: Hibiscus rosa-sinensis, T5: Quisqualis indica

### Table 4: Percentage yield of all plant extracts

| S. No. | Extracts | Yield (g) | Percentage yield (%) |
|--------|----------|-----------|-----------------------|
| 1.     | T1       | 12.801    | 14.94                 |
| 2.     | T2       | 11.502    | 12.50                 |
| 3.     | T3       | 13.200    | 15.20                 |
| 4.     | T4       | 15.020    | 16.25                 |
| 5.     | T5       | 14.250    | 13.65                 |

T1: Allium sativum, T2: Moringa oleifera, T3: Cicer arientum, T4: Hibiscus rosa-sinensis, T5: Quisqualis indica

### Table 5: Physiochemical analysis of powder of all plant leaves

| S. No. | Parameters | Observation (%) |
|--------|------------|-----------------|
| 1.     | Loss on drying | 1.58 2.05 1.35 1.55 1.75 |
| 2.     | Total ash value  | 4.50 3.80 3.59 4.89 3.74 |
| 3.     | Acid-insoluble ash value | 0.85 0.95 0.95 1.00 1.10 |
| 4.     | Water-soluble ash value | 0.89 0.93 0.085 0.97 1.0 |
| 5.     | Roaming index (cm)  | 1.00 1.25 0.62 0.5 0.45 |

T1: Allium sativum, T2: Moringa oleifera, T3: Cicer arientum, T4: Hibiscus rosa-sinensis, T5: Quisqualis indica

### Table 6: Phytochemical screening of hydroalcoholic extract

| S. No. | Identification test | Test name | T1 | T2 | T3 | T4 | T5 |
|--------|---------------------|-----------|----|----|----|----|----|
| 1.     | Alkaloids           | Mayer's test | +  | +  | −  | +  | +  |
| 2.     | Glycosides          | Dragendorff’s test | +  | +  | +  | +  | +  |
| 3.     | Carbohydrates       | Wagner’s test | +  | −  | +  | +  | +  |
| 4.     | Tannins and phenols | Fehling test | +  | −  | +  | −  | −  |
| 5.     | Flavonoids          | Gelatin test | +  | +  | +  | +  | +  |
| 6.     | Steroids            | Ferric chloride test | +  | +  | +  | +  | +  |
| 7.     | Saponins            | Shinozaki test | +  | +  | +  | +  | +  |
| 8.     | Protein             | Alkaline reagent test | −  | −  | +  | +  | +  |
| 9.     | Gums and mucilage   | Liebermann-Burchard test | −  | −  | +  | +  | +  |

1. Allium sativum, T2: Moringa oleifera, T3: Cicer arientum, T4: Hibiscus rosa-sinensis, T5: Quisqualis indica. +: Present, −: Absent
Graph 3: Effect of high-fat high-sugar diet, atorvastatin, and *Cordia dichotoma* on HDL level. Values are expressed as mean±standard deviation; (n=6). All values are mean±standard error of mean, n=6. *p<0.05, **p<0.01 when compared to positive control group. Following repeated measures ANOVA parametric methods, using Dunnett’s test. NC: Normal control group, PC: Positive control group or high-fat high-sugar diet group, standard: ND+high-fat high sugar (HFHS)+atorvastatin (2.1 mg/kg/day), test control: ND+HFHS+polyherbal formulation (300 mg/kg/day)

Graph 4: Effect of high-fat high-sugar diet, atorvastatin, and *Cordia dichotoma* on LDL level. Values are expressed as mean±standard deviation; (n=6). All values are mean±standard error of mean, n=6. *p<0.05, **p<0.01 when compared to positive control group. Following repeated measures ANOVA parametric methods, using Dunnett’s test. NC: Normal control group, PC: Positive control group or high-fat high-sugar diet group, standard: ND+high-fat high sugar (HFHS)+atorvastatin (2.1 mg/kg/day), test control: ND+HFHS+polyherbal formulation (300 mg/kg/day)

Graph 5: Effect of high-fat high-sugar diet, atorvastatin, and *Cordia dichotoma* on CHL level. Values are expressed as mean±standard deviation; (n=6). All values are mean±standard error of mean, n=6. *p<0.05, **p<0.01 when compared to positive control group. Following repeated measures ANOVA parametric methods, using Dunnett’s test. NC: Normal control group, PC: Positive control group or high-fat high-sugar diet group, standard: ND+high-fat high sugar (HFHS)+atorvastatin (2.1 mg/kg/day), test control: ND+HFHS+polyherbal formulation (300 mg/kg/day)
Preparation of polyherbal formulation

The hydroalcoholic extract of *A. sativum* (50 mg), *M. oleifera* (50 mg), *C. arietinum* (50 mg), *F. rosa-sinensis* (50 mg), and *Q. indica* (50 mg) was dissolved in suspending agent (1% carboxymethylcellulose [CMC] aqueous) before orally administered to the rats. Standard drug was dissolved in suspending agent (1% CMC) before orally administered to the rats [19].

Preparation of high-fat high-sugar diet (HFHSD)

Bread (30 g) + Biscuits (30 g) + Vanaspati ghee (3 ml) + Coconut oil (1 ml) 25% fructose was added in drinking water bottle.

These diets were fed along with normal diet for a total period of 6 weeks to rats.

Experimental protocols

In the HFHSD model, the animals were divided into five groups and each group composed of six animals (Table 1).

All the treatments were carried out for 42 days. Before and after the treatment, the animals were fasted for 2 h to improve the absorption rate. Parameters studied for this test were body weights, blood glucose, and total high-density lipoprotein cholesterol levels.

RESULTS

- Morphology (Table 2)
- Consistency and color (Table 3)
- Practical and percentage yield (Table 4)
- Screening of powder (Table 5)
- Phytochemical screening: There is the presence of different phytochemicals in hydroalcoholic extract T1, T2, T3, T4, and T5 (Table 6).

Table 7: Results of acute oral toxicity study of HACD

| Group name | Animal mark | Dose mg/kg | Body weight (g) | Observation | Mortality (if any) |
|------------|-------------|------------|-----------------|-------------|------------------|
| Control    | H           | Normal saline (0.91%) | 153 | 148 | 146 | No sign of toxicity and no mortality occurs |
| Test       | HT          | 2000 mg/kg polyherbal formulation (once dosing at start of acute oral toxicity study) | 205 | 208 | 202 | all animals survived |

Table 8: Total feed (g) and water (ml) intake

| Group | Normal pellet diet (g) | HFHSD (g) | Water intake (ml) |
|-------|------------------------|-----------|-------------------|
| NC    | 89.38±27.53            | 152       | 154.33±1.86*      |
| PC    | 53.09±4.70*            | 49.34±2.34| 47.83±2.14        |
| STD   | 57.08±21.13*           | 170.66±4.58| 50.83±2.48**     |
| Test  | 72.40±12.78            | 50.5±1.51 | 49.83±1.84       |

All values are mean standard error of mean, n=6, *p<0.05, **p<0.01 when compared to positive control group. (Following repeated measures ANOVA (parametric methods, using Dunnett’s test). HFHSD: High-fat high-sugar diet (Graph 1)

Table 9: Body weight (g)

| Group | 0th day | 7th day | 14th day | 21st day |
|-------|---------|---------|----------|---------|
| NC    | 133.17±3.25 | 137.5±3.20 | 138.00±1.78 | 142.66±1.36** |
| PC    | 128.5±2.42 | 130.8±2.63 | 133.00±2.36 | 136.33±1.67   |
| STD   | 158.5±2.88 | 158.0±2.34 | 157.33±3.07  | 156.16±2.49   |
| Test  | 166.00±5.03* | 164.8±4.53* | 161.83±4.07* | 160.83±4.35   |

All values are mean standard error of mean, n=6, *p<0.05, **p<0.01 when compared to positive control group. (Following repeated measures ANOVA (parametric methods, using Dunnett’s test) (Graph 2)

Table 10: HDL level (mg/dl)

| Group | 0th day | 7th day | 14th day | 21st day |
|-------|---------|---------|----------|---------|
| NC    | 46.67±1.64 | 48.17±1.17 | 48.66±2.25 | 50.00±1.90 |
| PC    | 48.67±2.74 | 49.34±2.34 | 49.83±1.84 | 50.83±1.48 |
| STD   | 46.16±2.48* | 49.16±2.13 | 50.83±2.48** | 50.5±1.51 |
| Test  | 50.5±2.48 | 57.00±3.35 | 55.83±3.12 | 54.55±2.58 |

All values are mean standard error of mean, n=6, *p<0.05, **p<0.01 when compared to positive control group. (Following repeated measures ANOVA (parametric methods, using Dunnett’s test). HDL: High-density lipoprotein (Graph 3)

Table 11: LDL level (mg/dl)

| Group | 0th day | 7th day | 14th day | 21st day |
|-------|---------|---------|----------|---------|
| NC    | 73.00±2.28 | 71.16±1.72 | 68.16±2.04 | 69.5±1.76* |
| PC    | 49.5±3.08 | 56.66±2.94 | 66.16±2.92 | 75.16±3.48 |
| STD   | 43.16±2.31 | 48.67±2.58 | 53.53±1.76** | 54.34±2.59* |
| Test  | 43.33±1.87 | 47.83±2.14 | 51.00±3.03** | 53.34±2.43** |

All values are mean standard error of mean, n=6, *p<0.05, **p<0.01 when compared to positive control group. (Following repeated measures ANOVA (parametric methods, using Dunnett’s test). LDL: Low-density lipoprotein (Graph 4)

Table 12: CHL level (mg/dl)

| Group | 0th day | 7th day | 14th day | 21st day |
|-------|---------|---------|----------|---------|
| NC    | 140.34±3.67 | 146.17±3.31 | 150.3±3.45 | 153.5±2.95** |
| PC    | 146.00±1.42 | 153.5±2.58 | 170.66±4.58 | 192.5±2.74 |
| STD   | 148.00±3.46 | 154.33±1.86* | 170.34±3.07 | 180.2±4.60 |
| Test  | 156.00±2.36 | 169.66±3.15 | 174.67±2.43 | 183.5±2.50 |

All values are mean standard error of mean, n=6, *p<0.05, **p<0.01 when compared to positive control group. (Following repeated measures ANOVA (parametric methods, using Dunnett’s test). CHL: Cholesterol (Graph 5)

- Antihyperlipidemic activity of polyherbal formulation from diet-induced model on experimental rats Acute toxicity studies (LD₅₀): In both Phase I and Phase II procedures, none of the animal mortal or any signs of behavioral changes or show any toxicity on the single administration of Healthcare Administration (2000 mg/kg p.o.). Thus, 300 mg/kg dose was selected for the present study (Table 7).

Evaluation parameters

- Effect on feed (g) and water (ml) intake (Table 8)
- Effect on body weight (g) (Table 9)
- Effect on HDL level (mg/dl) (Table 10)
- Effect on LDL level (mg/dl) (Table 11)
- Effect on CHL level (mg/dl) (Table 12)
- Effect on blood glucose level (mg/dl) (Table 13)

Graphical representation

- Effect on total feed and water intake
- Effect on body weight
- Effect on HDL level

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DISCUSSION

The study was carried out to evaluate antihyperlipidemic activity of polyherbal formulation (T1, T2, T3, T4, and T5) in HFHSD-induced model of rats.

The coarse powder of the shed dried part of the plant was subjected to extraction using Soxhlet apparatus. The plant material was extracted with hydroalcoholic solvent system (1:1). The obtained practical yield of extract sequentially (T1, T2, T3, T4, T5) was 12.801 g, 11.502 g, 13.200 g, 15.020 g, and 14.250 g or percentage yield of extract was 14.94%, 12.50%, 15.20%, 16.25%, and 13.65%.

After the extraction, pharmacognostical and phytochemical screening was done. The lipid-lowering activity of polyherbal formulation (T1, T2, T3, T4, and T5) may be attributed to the phytoconstituents present such as alkaloids, carbohydrates, steroids, proteins, tannins, carbohydrates, flavonoids, phenols, glycosides, and triterpenes.

In acute oral toxicity study, there were no behavioral changes seen up to 4 h and no mortality was observed up to the end of 24 h even at the maximum tested dose level of 2000 mg/kg per oral. It was considered maximum safe dose.

The study was conducted by measuring various parameters, namely, daily feed intake (g), water intake (ml), body weight (g), lipid profile HDL, LDL, CHL level (mg/dl), and blood glucose level (mg/dl).

CONCLUSION

We can say that polyherbal formulations (T1, T2, T3, T4, and T5) have hypoglycemic activity and significantly improve lipid profile levels in diet-induced experimental rats. Results showed significant decrease in blood glucose level and serum lipid profile such as TC, LDL, and increasing serum HDL level, so could be useful in the treatment of hyperlipidemia as mentioned in traditional medicine. However, further studies required to isolate the phytochemicals those responsible for hypolipidemic activity.

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AUTHORS’ CONTRIBUTIONS

All the authors have equally contributed.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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