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ORIGINAL ARTICLE

# Antilipidemic Properties of *Calpurnia aurea* Leaf Extract on High-Fat Diet Induced Hyperlipidemia

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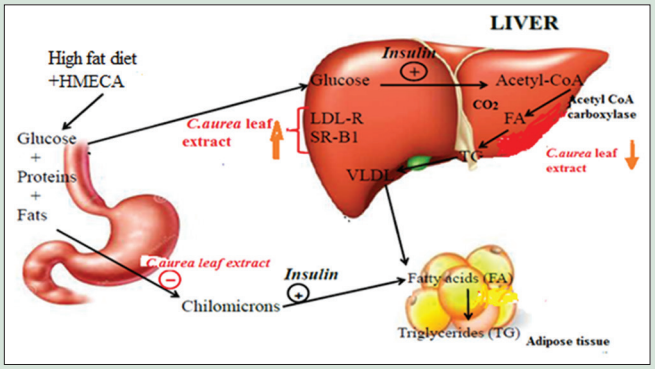
## ABSTRACT

**Background:** Hyperlipidemia is described by raised in the plasma lipids including triglycerides (TG), cholesterols, cholesterol esters, phospholipids as well as plasma lipoproteins, for example, very low-density lipoprotein (LDL), LDL, and reduction in the circling high-density lipoprotein (HDL) levels. **Objective:** To explore the antilipidemic properties of the hydromethanolic extract of *Calpurnia aurea* (HMECA) leafs against high-fat diet-induced hyperlipidemic male albino Wistar rats. **Materials and Methods:** Thirty albino Wistar rats of 60–75 days and weights of 150–200 g were isolated arbitrarily into six groups of five each. Group I fed with normal diet in as typical control, Group II got high-fat-eating routine (48.8% fat w/w) containing fat produced using hamburger fat and blended with hydrogenated vegetable oil, Group III fed with high fat diet plus 3.5 mg/kg/day atorvastatin as standard control, and the remaining Groups IV, V, and VI fed with high fat diet along with different does of HMECA at 200, 300, and 400 mg/kg/day separately for 60 days. Food intake, body weight, body mass index, serum lipid profiles, and liver histopathology were studied. **Results:** The results of this investigation exposed that HMECA has dose-dependent antilipidemic exercises. HMECA treatment of 400 mg/kg caused a noteworthy bringing down of $P < 0.05$ of serum LDL from 28.53 ± 12.2 mg/dl to 9.70 ± 5.77 mg/dL; the serum cholesterol level from 92.00 ± 13.0 mg/dl to 60.33 ± 8.60 mg/dl; the serum TG leve1 from 84.73 ± 19.4 mg/dl to 71.83 ± 13.0 mg/dl mg/dl; and increased the serum HDL-cholesterol levels from 11.66 ± 1.23 mg/dl to 29.66 ± 1.52 mg/dl. At the medium dosage of 300 mg/kg, it was not successful as 400 mg/kg and at the insignificant dosage of 200 mg/kg brought numerical contrast not statistically noteworthy among the serum lipid profile. **Conclusion:** This research discovered that the HMECA possesses a significant antilipidimic activity in dose dependent manner. The molecular mechanism of antilipidemic exercises of this medication should be contemplated.

**Key words:** Antilipidemia, *Calpurnia aurea,* high-fat diet, hyperlipidemia, lipid profile

## SUMMARY

- Hydromethanolic extract of *Calpurnia aurea* had significant amount of alkaloid, saponin, flavonoid, phenolic compound, tannin, and glycosides
- These bioactive components reduced the food intakes, bodyweight, body mass index, serum total cholesterol, triglycerides, low-density lipoprotein levels, and a significant increased high-density lipoprotein cholesterol.




**Abbreviations Used:** HMECA: Hydromethanolic extract of *Calpurnia aurea*; DPPH: 2,2-diphenyl-1-picrylhydrazyl; HFD: High-fat diet; HED: Human equivalent dose; BMI: Body mass index; TG: Triglycerides; TC: Total cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

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## INTRODUCTION

Hyperlipidemia is described by hoisted in the plasma lipids including triglycerides (TG), cholesterols, cholesterol esters, phospholipids as well as plasma lipoproteins, for example, very low-density lipoprotein (LDL), LDL, and reduction in the circling high-density lipoprotein (HDL) levels.[1] The high fatty substances travels in the circulation attached to proteins make hyperlipoproteinemia.[2] It is also identified that dyslipidemia, to describe the manifestations of atherosclerosis which causes cardiovascular diseases, those records for 33% of total deaths around the world. Furthermore, it is trusted that cardiovascular maladies will end up being the primary driver of death and inability worldwide by the year 2020.[3]

The pervasiveness of hyperlipidemia has drastically expanded worldwide because of current ways of life which realize increment in the utilization of high-fat-eating regimens.[4] Numerous present investigations uncover that there are noteworthy contrasts in blood lipid levels and the commonness of hyperlipidemia between ethnic gatherings, distinctive dietary propensities, the way of life and level of physical activity and in addition their hereditary foundation.[5] Other altering factors in the improvement and movement of hyperlipidemia are age and sexual orientation. It has been demonstrated that cholesterol levels ascend as the individual gets more seasoned.[6] The prevalence of hyperlipidemia is in the range of 39%, 51% and 26% worldwide, developed and developing countries, respectively. Overall, raised cholesterol is evaluated to cause 2.6 million passings and 29.7 million inability-balanced life years.[7]



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*Calpurnia aurea* (Ait.) Benth, a member of the subfamily Papilionoideae belongs to the family of *Fabaceae*. This grows as shrub or a slender tree of up to 15 m tall, widespread along the east coast of Africa. *C. aurea* is known by several local names, chekata in Afaan Oromo and digita in Amharic. *C. aurea* has not been scientifically proven to have antilipidemic potential; it has been found to be medicinally useful in many ailments. Its widespread application for diverse ethnomedicinal uses has made it a subject for pharmacological and phytochemical studies. It is used by the Shinasha people to treat amoebiasis and giardiasis while the Amhara people from the same region use the leaves to treat malaria and the seeds to treat hypertension while a combination of the leaves and seeds is used to treat diarrhea and diabetes. The plant has also been used to induce uterine contractions and to treat coughs, amoebic dysentery, syphilis, leishmaniasis, tapeworm, trachoma, ringworm, scabies, elephantiasis, abscesses, and wounds as well as stomach ache, vomiting, headache, and eye diseases.[8]

Pharmacological studies have shown that methanol extracts of the leaves and stems of *C. aurea* have a good antibacterial and antioxidant properties,[9,10] validating its traditional use for a range of microbial infection. Quinolizidine alkaloids, butin (7,3',4'-trihydroxyflavanone), the flavonoids vicenin-2 (6,8-di-β-D-glucopyranosyl-5,7,4' trihydroxy flavone), and 3'-hydroxydaidzein (isoflavone) were isolated from the seeds of *C. aurea*, in keeping with flavonoids being the other major class of compounds consistently found in the *Fabaceae*. There is a report on the isolation of five isoflavonoids, a pterocarpan, and a quinolizidine alkaloid from stem and bark of *C. aurea* as well as the anticancer activity of the isolated isoflavonoids.[11]

*C. aurea* plant leaves and seeds, both contain flavones and polyphenols though the levels were found more in leaves than in the seeds. The extracts of both leaves and seeds of the plant indicated strong antioxidant activities. Previous study proved that it has antidiarrheal activity through antispasmodic effects, delay intestinal transit, suppress gut motility, stimulate water absorption, or reduce the intraluminal fluid accumulation.[12] *C. aurea* seed extract obsessed hepatoprotective activity against highly active antiretroviral therapy-induced liver injury in rats.[13] Hyperlipidemic factors at present extraordinarily impact our well-being by causing hyperlipidemia and other cardiovascular-related ailments as delighted by many examinations. Hence, *C.* aurea plant which has the board application and assorted ethnotherapeutic uses has no any claim for its hypolipidemic properties. Thus, this examination is expected to discover antilipidemic properties of the hydromethanolic extract of *C. aurea* on high-fat diet (HFD) nourished Wistar rats.

## MATERIALS AND METHODS

### Plant material collection and authentication

The fresh leaves of *Calpurnia aurea* was collected from Debrezeyt district of eastern showa zone which is 45 km from Addis Ababa and authenticated by Taxonomist of Ethiopian National herbarium of Addis Ababa University and 14 voucher specimen Collection number 001/mw/2015.

### Preparation of plant extract

The plant leaves were cut into little sections, air-dried under shade range at room temperature, and afterward ground to fine powder utilizing clean mortar and pistil. The powder was gone through strainer number 30 and put away in a glass holder. The coarse powder of *C. aurea* leaves was 627 g, and it was macerated in 80% methanol (V/V) for three back to back days (72 h) with mechanical shaking three times each day. Then the extract was filtered by Whatman No.1 filter paper and then the filtrate was evaporated to be dried by rotary evaporator. Then, the filtrate was taken to thermostatic broiler at 40°C and kept overnight to vanish the rest of the methanol. From that point onward, the last concentrate which is free of methanol was taken to profound cooler less to have a strong consistency. This solidified and set concentrate was lyophilized over and again utilizing solidify dryer (lyophilizer) until the point that the water was totally expelled. At that point, the last concentrate was pressed in a glass which was not straightforward to light.

Percentage of yield = weight of the dried concentrate/weight of coarse powder × 100.

### Preliminary phytochemical screening

The phytochemical examination of the *C. aurea* leaves extract was performed by the standard procedures endorsed beforehand by Mulata *et al*.[13]

### Determination of *in vitro* antioxidant activity hydromethanolic extract of *Calpurnia aurea*

The *in vitro* antioxidant activity dot-blot by 2,2-diphenyl-1-picrylhydrazyl (DPPH) staining as indicated by Soler-Rivas *et al*. and determination of $IC_{50}$ (photometric test) as indicated by Gyamfi *et al*. using DPPH.[14,15]

### Acute oral toxicity test

In the acute oral toxicity study of hydromethanolic extract of *Calpurnia aurea* (HMECA), a limit dose of each 2000 mg/kg body weight of the creature was directed on solitary rats orally by gavages. Then Animals were individually observed for changes in skin, general behavioral pattern, tremors, convulsions, salivation, diarrhea, sleep, coma and mortality for a period of 14 days and the results indicated that there were no toxicity was observed.

### Experimental animals

Six females and three male Albino Wistar rats were obtained from my colleagues and allowed to breed them and wait for 3 months. After 3-month stay, male sex rats of weight 150–190 g with age of 60–75 days in this study were used. All the male rats were derived from single cross isolation in one cage. The animals acclimatized to laboratory condition for 1 week before the experiments. They were kept in plastic cages at 22°C ± 2°C, in relative humidity of 55% and on a 12-h light dark cycle with free access to pellet food and tap water *ad libitum* at the same animal house. Then, rats were randomly divided into six groups of each five in number.

### High-fat diet preparation

The HFD was prepared using melted Ox fat with standard pellet mixtures (36%/64% w/w) [Figure 1]. Standard rats' food pellets contained 20% fat, 60% carbohydrates, and 20% proteins. Therefore, the term "high-fat diet" in this study refers to a diet containing 58% fat by weight.

### Extrapolation of atorvastatin dose

The human's doses of atorvastatin drug were extrapolated to animal's dose by the formula: human equivalent dose mg/kg = Animals dose in (mg/kg) × (Animal km ÷ Human km), where Km is a correction factor reflecting the relationship between body weight and body surface area (Natural Health Research Institute, 2008).

### Experimental protocol

The animals were randomly selected, weighed then marked for individual identification. The thirty rats were randomly assigned into six groups of five rats per cage [Table 1].

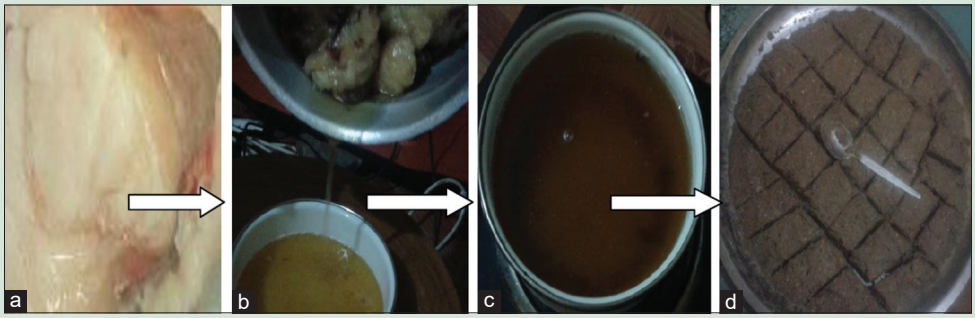


**Figure 1:** Preparation high-fat diet. The Ox fat (a), liquefied by heating in a pan on a stove (b), then nonfat material including connective tissue and meat were removed and the liquefied fat was cooled to allow it to solidify into lard which was supplemented with few hydrogenated vegetable oil (c) and finally placed in powdered pellet while mixing properly and baking (d)

## The food intake measurement

The daily food intake of the rats was measured in the morning using a weighing balance. Food intake was calculated by subtracting the amount of food left over in each cage from the measured amount of food provided at the previous day (g/day/cage). The mean of food intake was represented in g/day/cage.

## Anthropometrical determinations

The body weight of the rats were measured weekly and recorded. The body length of the rats were measured at the end of the experimental day after anesthetized. The body weight and body length were used to determine the body mass index (BMI) as described by Novelli *et al.*[16]

## Blood collection and serum preparation from rats

At the end of the study, the rats were fasted overnight and anesthetized with diethyl ether. The blood was collected by cardiac puncture using a 10-cc syringe. Blood collected (3–4 ml) were placed in serum separating test tube, left for 30 min at room temperature to clot, and centrifuged at 4500 rpm for 5 min and serum was separated and analyzed the lipid profiles.

## Tissue histopathological studies

To see the effect of rats treated with HFD and counter effect of *C. aurea* leaves extract-fed rats, histopathological examination of liver was performed; small pieces of liver tissue were taken from each rat carefully performed; small pieces of liver tissue were taken from each rat carefully and fixed 10 % of formalin tissue can which contain 10% of formalin. Then the tissue was dehydrating using different percentage of ethanol for one hour. Tissue section was treated with xylene solution to remove ethanol from the tissue and replace this ethanol with fluid that is readily miscible with paraffin wax which enhances the tissue to embed easily with the paraffin wax to form tissue blocks and prepared section then stained with hematoxylin and eosin and examined for histopathological changes[17] under the microscope (MoticAE21, Germany). The microphotographs were taken using Moticam 1000 camera at the original magnification of 100 × s.

## Statistical analysis

Collected quantitative data were coded, entered to computer, processed, edited, and analyzed using Microsoft Excel and expressed to SPSS (SPSS, Chicago, IL) version 20 statistical software for analysis. All results are expressed as mean ± standard deviation. The difference among the group was evaluated by independent sample *t*-test and one-way analysis of variance and the *P* values < 0.05 consider as significant difference.

**Table 1:** Animal grouping and dose of extract

| Group | Category | Treatment dose |
|---|---|---|
| I | Normal control | Regular pellet + distil water |
| II | Positive control | HFD + distil water |
| III | Atorvastatin as control | HFD + 3.5 mg/kg |
| IV | Experimental | 200 mg/kg HMECA + HFD |
| V | Experimental | 300 mg/kg HMECA + HFD |
| VI | Experimental | 400 mg/kg HMECA + HFD |

HMECA: Hydromethanolic extract of *Calpurnia aurea*; HFD: High-fat diet

# RESULTS

## Percentage yield of hydromethanolic extract of *Calpurnia aurea*

The various unrefined extract which was acquired from 627 g coarse powder of *C. aurea* leaf was 68.4 g. Along these lines, the rate yield of this concentrates by utilizing hydromethanol (80/20 v/v) was and given as:

Percent yield = Actual yield/theoretical yield × 100

= (68.4/627) × 100 = 15.9% (w/w).

## Phytochemicals present in hydromethanolic extract of *Calpurnia aurea*

The preparatory phytochemical examination of hydromethanolic leaves' extraction of *C. aurea* uncovered the nearness of phytochemical constituents, for example, alkaloid, saponin, flavonoid, phenolic compound, tannin, and heart glycosides [Table 2].

## *In vitro* antioxidant activity of hydromethanolic extract of *Calpurnia aurea*

### *Rapid screening of antioxidant by dot-blot and 2,2-diphenyl-1-picrylhydrazyl staining*

Figure 2 demonstrates the rapid screening of antioxidant by dot-blot, DPPH staining of the leaves' extracts of *C. aurea*. The outcomes showed that the restraint of oxidation was specifically relative to the amount of the plant extract. The width of lessened zone expanded in guide extent to the dosage of plant extract. The width of oxidation inhibition is directly proportional to the amount of plant extract, 200 mg showing less width and 400 mg showing the highest width of oxidation inhibition.

## Determination of $IC_{50}$ value of hydromethanolic extract of *Calpurnia aurea*

As shown in Figure 3, the $IC_{50}$ of the HMECA is 230 μg/ml (0.23 mg/ml) whereas that of ascorbic acid is 160 μg/ml (0.16 mg/ml). This result

shows that the HMECA *has* a closer antioxidant activity as compared with ascorbic acid. The absorbance of 0.008% DPPH was 0.298 which was used as a control, and the absorbance of methanol was 0.007 which was taken as a blank at 517 nm.

## Effect of hydromethanolic extract of *Calpurnia aurea* on food intake

The food intake of HFD-fed control (Group II) rats did not show any significant difference as compared to the normal control up to 8 weeks. The food intake significantly reduced in Group III rats (HFD + atorvastatin) from the 4th week to 8 weeks (($P < 0.01$). These results were lined with previous study of Seneca *et al.*[18] On the other hand, the food intake of rats fed on HFD supplemented with *C. aurea* leaves extract at 200 mg/kg, 300 mg/kg, and 400 mg/kg dose did not bring any significant alteration up to the 5th week. However sixth and seventh week at the highest dose of 400mg/kg of HMECA significantly decreased ($P < 0.05$) the food intake and the same results maintained upto 8th week. Further analysis at a moderate dose of 300 mg/kg extracts showed a significant decrease ($P < 0.05$) on food intake on the 7th and 8th week. The lowest dose of 200 mg/kg extract showed a significant decrease ($P < 0.05$) on food intake in the 8th week as compared to the HFD fed alone albino rats. Therefore, the result of the present studies indicates that HMECA leaves coadministered with HFD was effective in reduction of food intake in albino rats in a dose-dependent manner when compared with atorvastatin [Table 3].

## Effect of hydromethanolic extract of *Calpurnia aurea* on body weight

Adult rats were given access to HFD (Group II), and a standard laboratory diet (Group I) displayed no significant difference in body weight gain. Atorvastatin treatment (Group III) brings numerical difference only up to the 6th week and significantly reduced body weight from 6th to the 8th week. The high (Group V) and moderate (Group V) doses-treated group rats significantly reduced the body weight gain but the low dose was not effective when compared with high and moderate doses [Table 4].

**Table 2:** Phytochemicals present in the hydroethanolic leaf extract of *Calpurnia aurea*

| Phytochemical constituent | Status |
|---|---|
| Flavonoid | ++ |
| Saponins | ++ |
| Phenolic compounds | ++ |
| Glycosides | + |
| Alkaloid | + |
| Tannins | + |
| Anthraquinones | – |

## Effect of hydromethanolic extract of *Calpurnia aurea* on body mass index

The BMI of the rats fed with HDF (Group II) was notably increased as compared to the normal control. The atorvastatin-treated rats (Group II) drastically decreased BMI from 0.77 to 0.67. However HMECA treated groups significantly reduced the BMI in a dosedependent manner [Table 5].

## Effect of hydromethanolic extract of *Calpurnia aurea* on serum lipid profile of rats which were fed high-fat diet

The animal which was fed with HFD significantly increased ($P < 0.05$) the serum TC levels from 58.35 ± 6.15 mg/dl to 92.0 ± 13.0 mg/dl, the LDL level from 7.7 ± 2.40 mg/dl to 28.53 ± 12.2, the serum TG level increased significantly ($P < 0.05$) from 61.00 ± 2.82 mg/dl to 171.83, and serum HDL level decreased significantly ($P < 0.05$) from 33.50 ± 2.12 mg/dl to 11.66 ± 1.23 mg/dl when compared with rats which were fed a normal diet. The HDF-altered lipid profiles were restored by *C. aurea* leaf extract in a dose-dependent manner. The low dose (200 mg/kg) was not as effective as either the moderate dose or as the higher dose. The moderate dose (300mg) significantly lower the serum TC, LDLC, serum TG level but no alteration in HDL-cholesterol (HDLC) [Figure 4]. However, high does (400mg) effects was similar to the atorvastatin treatment.

## Effect of hydromethanolic extract of *Calpurnia aurea* histopathological changes of the albino rat's liver

Light microscopic examination of liver histology of Wistar albino rats which were fed a HFD showed normal liver histology with no lipid deposition in the hepatocytes of the liver, and no fatty liver was seen in the 8 weeks' feeding period [Figure 5].

# DISCUSSION

The target of the present examination was to screen the antihyperlipidemic action of HMECA. It has been entrenched that nourishment assumes an imperative part in etiology of

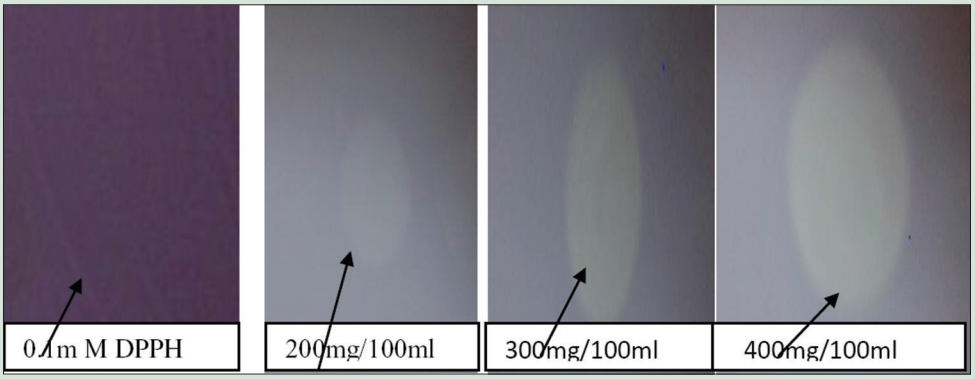


**Figure 2:** *In-vitro* antioxidant properties of various concentration of 80% hydromethanolic extract of *Calpurnia aurea* on thin-layer chromatography plate which is immersed in 0.1 mM 2,2-diphenyl-1-picrylhydrazyl

hyperlipidemia. In the present examination, HFD model demonstrates a critical increment in body weight, BMI, serum TC, TG, LDL levels, and a significant decrease in HDL-C in rats bolstered with the high-fat-eating routine group when contrasted with the typical control group ($P < 0.001$). This is correlated with the work done by Novelli *et al.*[16] But both groups did not showing any statistical difference in food intakes. However, HMECA-treated Group IV (300 mg/kg) and Group V (400 mg/kg) significantly reduced the food intake, body weight gain, and BMI. These reductions may be due to its ability to suppress the animals' appetite by the bioactive components such as saponins and phenolics. This was like the results reported by Chidrawar *et al.*[19] The high does (400mg) restored the HFD affected parameters near to control. This effect seams to the effect of atorvastatin. Chávez-Santoscoy *et al.* reported that some of the chemical constituents of plants, such as flavonoids and saponins, lower cholesterol absorption by the inhibition of cholesterol micellar solubility.[20] The plant secondary metabolites saponins have an *in vivo* antiobesity activity which led to significant decrease in weight and lipid profile such as TG, TC, LDLs, and very LDLs[21] and similar results was confirmed by Jin Son *et al.*[22] Therefore, plants, flavonoids, saponins, phenolics, and other bioactive compounds found in our hydromethanolic extract of *C. aurea* could be instrumental to its hypolipidemic effect in a dose-dependent manner.[23,24] Light microscopic examination of liver histopathology of albino Wistar rats which were fed a HFD showed normal liver histology with no lipid deposition in the hepatocytes of the liver and no fatty liver was seen. This might be short duration of the study.

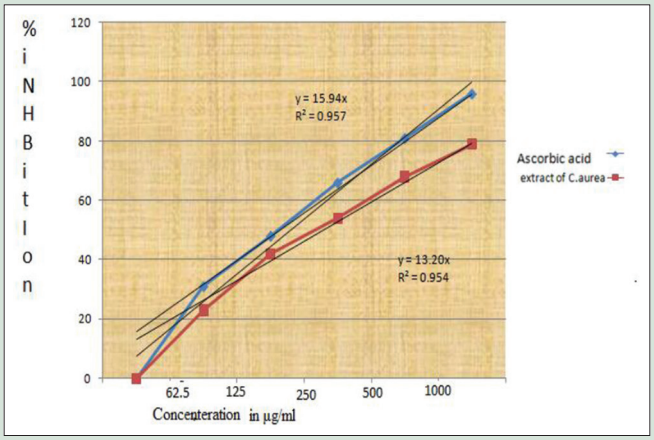


**Figure 3:** Antioxidant activities of HMECA versus % inhibition of 2,2-diphenyl-1-picrylhydrazyl

## CONCLUSION

The results of this study revealed that the HMECA decreased food intake, body weight, and BMI and altered the serum lipid profiles in albino Wistar rats. This was interpreted to indicate that the plant bioactive molecules were involved in decreasing appetite, serum TC, TG, LDL-C and increasing serum HDL-C level. The extract has good antioxidant property and could be due to the presence of flavonoids, saponins and phenolics compounds. Therefore, results of this study show that HMECA might be a good candidate for lowering hyperlipidemia and an easily accessible source of natural antioxidants against free radical that was produced by different mechanism of action [Figure 6].

**Table 3:** Effect of different doses of *Calpurnia aurea* leaf extract on food intakes

| Group | 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week | 7th week | 8th week |
|---|---|---|---|---|---|---|---|---|
| Normal control | 35.0±1.90 | 39.50±2.25 | 45.14±2.03 | 53.14±3.33 | 63.85±2.79 | 72.14±3.13 | 80.57±2.76 | 86.71±4.85 |
| HFD control | 34.14±1.49 | 39.00±1.41 | 48.00±1.84 | 56.00±2.00 | 67.00±3.82 | 76.85±1.88 | 85.15±1.86 | 91.0±7.83 |
| Atorvastatin 3.5 mg/kg + HFD | 33.42±2.00 | 38.07±1.48 | 45.14±1.86 | 53.85±3.28 | 62.57±3.8[a] | 70.42±4.7[a] | 74.60±6.2[b] | 71.28±5.7[b] |
| HFD + 200 mg/kg extract | 34.85±1.37 | 41.42±3.49 | 48.21±1.99 | 57.71±1.49 | 66.14±1.21 | 74.4±2.07 | 80.01±1.0 | 82.28±3.4[a] |
| HFD + 300 mg/kg extract | 34.78±1.82 | 41.43±2.90 | 48.00±4.12 | 54.00±4.50 | 65.00±2.51 | 73.6±2.41 | 78.00±2.6[a] | 77.3±2.50[b] |
| HFD + 400 mg/kg extract | 33.71±1.49 | 38.59±2.50 | 46.70±2.63 | 54.65±3.31 | 64.73±3.23 | 71.0±3.43[a] | 76.04±4.5[a] | 74.25±8.0[b] |

[a]significant at $P < 0.05$, [b]significant at $P < 0.01$. Values are mean±SD ($n$=6). Values are statistically significant at $P<0.05$ and statistically strong significant at $P<0.01$ using one-way ANOVA followed by *post hoc* Tukey test. HFD: High-fat diet; SD: Standard deviation

**Table 4:** Average body weights measurement in gram during the 8 weeks of male albino rates during high-fat diet treatment with different dose *Calpurnia aurea* extract

| Group | Initial weight | 1st week weight | 2nd week weight | 3rd week weight | 4th week weight | 5th week weight | 6th week weight | 7th week weight | 8th week weight |
|---|---|---|---|---|---|---|---|---|---|
| Normal control | 160.6±6.65 | 172.6±7.90 | 186.7±8.64 | 201.2±10.8 | 216.4±12.28 | 230.1±14.17 | 247.4±14.4 | 263.8±14.28 | 275.6±16.3 |
| HFD control | 160.8±7.72 | 172.4±10.43 | 189.2±13 | 207.90±13.6 | 223.2±15.0 | 246.2±15.7 | 264.8±16.8 | 279.2±17.12 | 291.6±19.0 |
| Atorvastatin 3.5 mg/kg + HFD | 161.6±5.85 | 176.0±7.3 | 190.4±8.41 | 205.4±9.31 | 219.0±10.0 | 230.4±102 | 236.2±10.2[a] | 243.4±11.8[a] | 240.4±10.8[a] |
| HFD + 200 mg extract | 162.2±12.2 | 176.8±14.7 | 192.8±16 | 208.0±16.4 | 222.4±16.6 | 236.0±17.0 | 248.4±17.4 | 258.2±18.7 | 263.6±18.76 |
| HFD + 300 mg extract | 162.4±11.6 | 177.6±12.0 | 190.2±12.4 | 205.0±12.5 | 219.4±12.9 | 232.2±12.3 | 244.2±16.0 | 250.1±16.26 | 257.6±17.7[a] |
| HFD + 400 mg extract | 162.0±10.7 | 174.2±11.34 | 189.6±10.9 | 202.8±11.0 | 217.4±12.2 | 231.8±11.7 | 248±11.3 | 248.4±11.8[a] | 246.7±9.3b[a] |

Values are mean±SD ($n$=6). Values are statistically significant at *$P<0.05$ using one-way ANOVA followed by *post hoc* -Tukey test. [a]$P<0.05$ compared with hyperlpidemc control group. HFD: High-fat diet; SD: Standard deviation

**Table 5:** Body mass index of rats on the 8th week during treatment with hydromethanolic extract of *Calpurnia aurea*

| | Normal control | HFD | Atorvastatin (3.5 mg/kg) | HFD + 200 mg extract | HFD + 300 mg extract | HFD + 400 mg extract |
|---|---|---|---|---|---|---|
| BMI (g/cm) on 8th week | 0.65 | 0.77 | 0.67 | 0.73 | 0.70 | 0.68 |

BMI: Body mass index

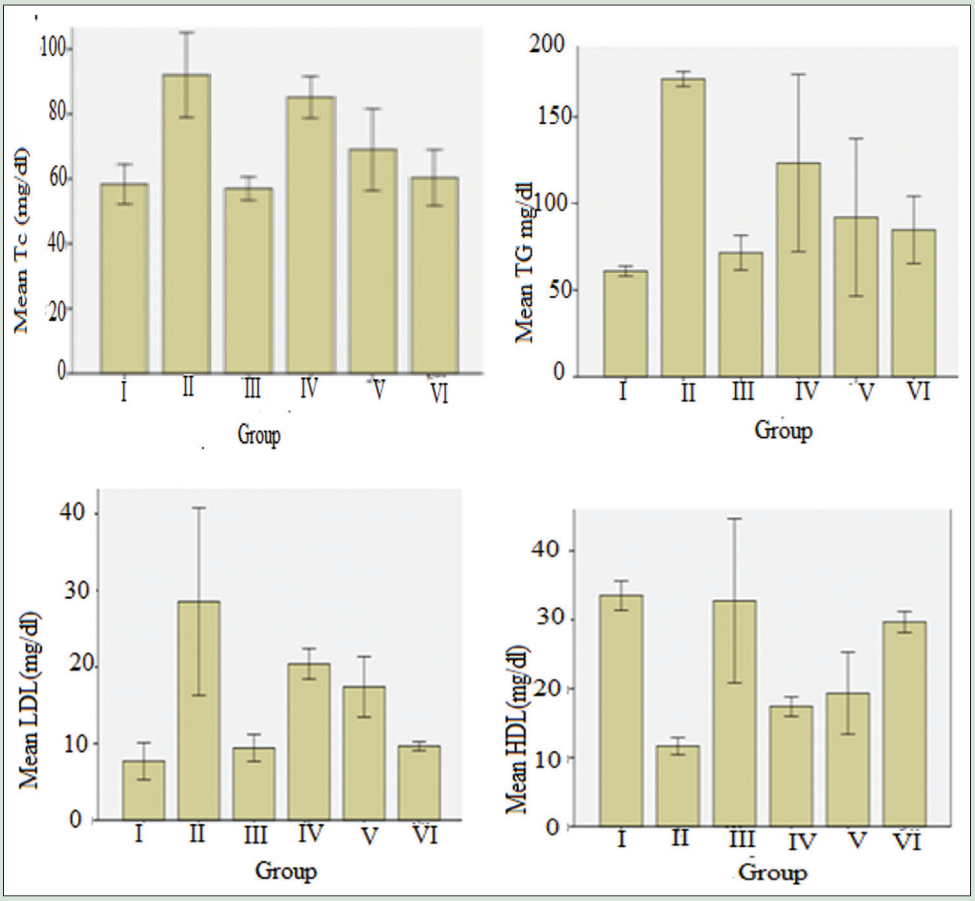


**Figure 4:** Effect of different dose of methanolic extract of *Calpurnia aurea* on serum lipid profiles

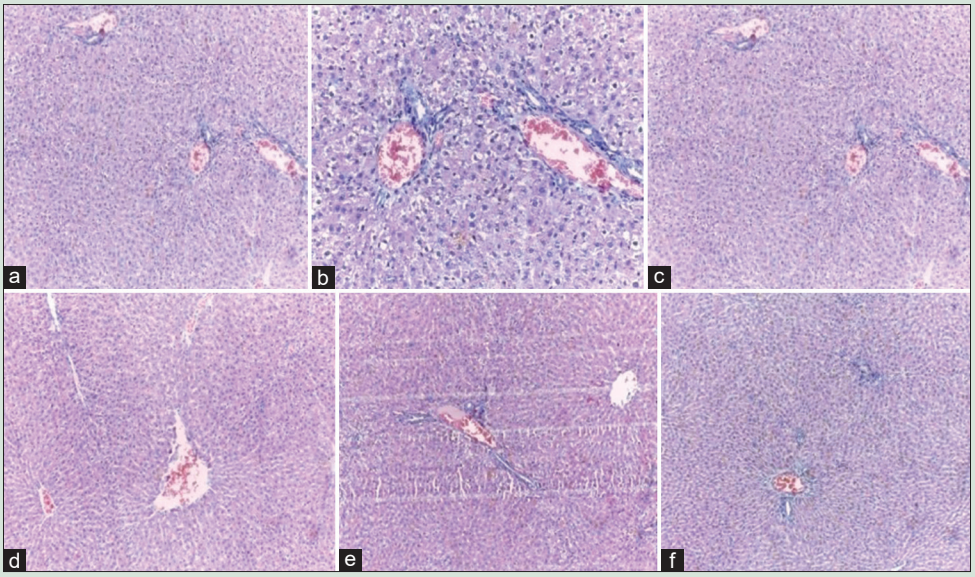


**Figure 5:** Normal liver histology with no lipid deposition in the hepatocytes of the liver tissue. (H and E, ×100). (a) Normal control, (b) high-fat diet control, (c) high-fat diet + atorvastatin (3.5 mg/kg b.w), (d) high-fat diet + hydromethanolic extract of *Calpurnia aurea* (200 mg/kg b.w), (e) high-fat diet + HMECA (300 mg/kg b.w) and (f) high-fat diet + HMECA (400 mg/kg b.w) fed for 8 weeks

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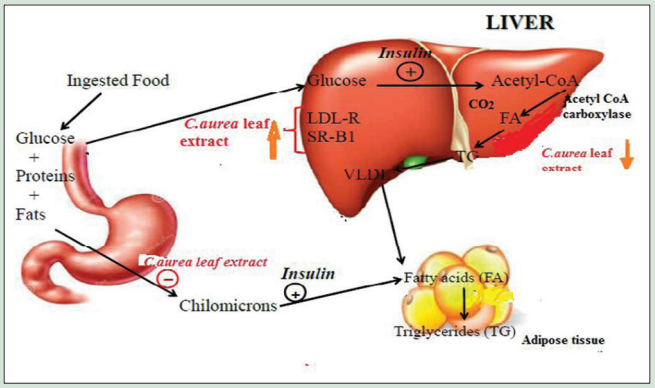


**Figure 6:** Hydromethanolic extract of *Calpurnia aurea* reduces plasma lipid and hepatic triglyceride concentrations and increases cholesterol uptake in the liver via upregulation of low-density lipoprotein receptor and scavenger receptor Class B member 1, downregulate acetyl COA carboxylase which is the regulated step during fatty acid synthesis and influenced cholesterol-regulating enzymes activities, 3-hydroxy-3-methylglutaryl-CoA reductase

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## Conflicts of interest

There are no conflicts of interest.

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