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# COMPARATIVE ANALYSIS OF ANTIOXIDANT AND LIPID-LOWERING ACTIVITY OF VARIOUS MILLETS

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**Abstract**

Millets, a diverse group of small-seeded grasses, have been cultivated for millennia, serving as essential cereal crops and fodder worldwide. This study assesses the antioxidant properties of extracts from *Pennisetum glaucum*, *Paspalum scrobiculatum*, and *Eleusine coracana*, which contain phenolic compounds such as flavonoids, phenolic acids, and phenolic diterpenes. *In-vitro* antioxidant potential was determined using DPPH and FRAP assays, targeting phytochemicals, including coumarin, steroids, flavonoids, tannins, carbohydrates, and saponins. Liver specimens were examined for pathological changes and the efficacy of extracts in ameliorating these features. Ethanol extracts of *Pennisetum glaucum*, *Paspalum scrobiculatum*, and *Eleusine coracana* demonstrated significant antihyperlipidemic activity, supported by histopathological findings. The present study underscores the potential antihyperlipidemic and hepatoprotective effects of investigated millet extracts.

**Keywords:** Pearl Millets, Finger Millets, Kodo Millets, Lipid Lowering Activity, Antioxidant Study.

## 1. INTRODUCTION

Millets represent a diverse group of small-seeded grasses, extensively cultivated worldwide for their significance as cereal crops, fodder, and human food (Gupta et al., 2023). With a cultivation history spanning over 10,000 years in East Asia, millets have remained pivotal staples in the semi-arid tropics of Asia and Africa, serving as primary sources of energy, protein, vitamins, and minerals for millions of people, particularly in impoverished regions.

Their resilience to dry, high-temperature conditions and short growing seasons make them favored crops in such environments (Saini et al., 2021). Among the various millet species, pearl millet stands out as the most widely grown, holding critical agricultural importance in India and parts of Africa. Additionally, finger millet, Proso millet, and Foxtail millet contribute significantly to global millet cultivation (Ademosun and Oboh, 2015).

Finger millet (Eleusine coracana (L.) Gaertn) is primarily cultivated for its grains, characterized by robust tufted growth reaching up to 170 cm in height (Hegde et al., 2005; Chandra et al., 2016). Its distinctive inflorescence forms a panicle with finger-like spikes, reminiscent of a fist upon maturity, hence earning its name.

While finger millet grains are predominantly utilized for human consumption, they are less favored for livestock than maize, sorghum, and pearl millet. In India, finger millet is occasionally employed to feed young calves, grow animals, and convalesce livestock.

Pearl millet (Pennisetum glaucum (L.)) originates from Central tropical Africa and is widely distributed in drier tropical regions, including India (Singhal et al., 2022; Alagusundaram et al., 2023). Its introduction to Western states dates back to the 1850s when it established itself as a minor forage crop in the Southeast and Gulf Coast states of the United States. Pearl millet thrives in regions characterized by drought, low soil fertility, and high temperatures.

Its adaptability to adverse growing conditions, including high salinity and low pH soils, renders it suitable for cultivation in areas where other cereal crops like maize or wheat struggle to survive. Kodo millet (Panicum miliaceum (L.)) is indigenous to India and is grown predominantly in Uttar Pradesh in the North and Kerala and Tamil Nadu in the South (Dey et al., 2023; Nelson et al., 2023; Dhanoriya et al., 2024).

Notably gluten-free, Kodo millet offers a beneficial dietary option for individuals with gluten intolerance. Regular consumption of Kodo millet has shown promise in benefiting postmenopausal women, particularly in mitigating cardiovascular risk factors such as high blood pressure and elevated cholesterol levels.

Millets serve as excellent sources of micronutrients, including vitamins and minerals, offering a superior amino acid profile to sorghum. They boast significant levels of proteins, carbohydrates, dietary fiber, and minerals, with finger millet containing approximately 5-8% proteins, 65-75% carbohydrates, 15-20% dietary fiber, and 2.5-3.5% minerals (Dubey et al., 2014; Baghel et al., 2016; Jain et al., 2024c).

Additionally, millets are rich in phytochemicals such as tannins, phenolic acids, anthocyanins, phytosterols, and pinacosanols, contributing to their nutritional value and potential health benefits. The ethnobotanical uses of millets have spurred research into their pharmacological activities, with studies investigating various potential therapeutic properties, including cardiovascular disease prevention, anticancer effects, anti-obesity properties, anti-malarial activity, anti-aging effects, antidiabetic properties, and antimicrobial activity.

These findings underscore the multifaceted significance of millets beyond their traditional roles as food and fodder crops, highlighting their potential to promote human health and well-being.

## 2. MATERIAL AND METHODS

### 2.1 Plant collection and authentication:

Eleusine coracana, Pennisetum glaucum, and Paspalum scrobiculatum seeds were collected from the local markets at Sagar, Madhya Pradesh. The sample was authenticated at the Department of Botany, Dr. H.S. Gour Vishwavidyalaya, Sagar, Madhya Pradesh. Herbarium files were submitted for record at the department (Bot./H/03/47/13, Bot./H/02/27/10, and Bot./H/06/57/42, respectively).

### 2.2 Preparation of extracts and phytochemical screening

Eleusine coracana, Pennisetum glaucum, and Paspalum scrobiculatum seeds were dried, crushed into powder, and defatted with petroleum ether, followed by diethyl ether and ethanol extraction. The resulting ethanolic extracts were concentrated and dried to a constant weight.

Further, qualitative phytochemical screening was performed on the ethanolic extract of various millets using standard tests for analyzing alkaloids, tannins, glycosides, flavonoids, amino acids, proteins, and carbohydrates (Khan et al., 2022; Wal et al., 2023b, 2023a, 2024).

### 2.3 In-vitro Antioxidant activity:

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was assessed by measuring the ability to decolorize a purple-colored ethanol solution of DPPH. Briefly, varying concentrations (200–1000 µg/mL) of the samples dissolved in ethanol (2 mL) were combined with 2 mL of a 0.2 mM DPPH solution in ethanol (Prevc et al., 2013; Dey et al., 2023).

Following a 30-minute incubation period at room temperature, the absorbance was measured against a blank at 517 nm. The inhibition rate (% I) of the DPPH radical was calculated using the formula:

$$\textbf{Percentage DPPH inhibition (\%I)} = \frac{(Ao-As)}{Ao} X\ 100$$

### 2.4 Animals:

Albino rats weighing 150-180g of either sex were used for the study. They were housed in a hygienic facility with controlled temperature, humidity, and light-dark cycles. The rats were provided with a standard pelleted diet and water ad libitum.

Ethical clearance was obtained from the Institutional Animal Ethics Committee of the Department of Pharmaceutical Sciences, Dr. Harisingh Gour Central University, Sagar, Madhya Pradesh, under reference No.379/CPCSEA/IAEC-2019/32, dated January 25, 2019, and the studies were conducted following CPCSEA guidelines.

### 2.5 In-vivo Anti-hyperlipidemic study:

In this study, a Triton-induced hyperlipidemic model was used (Parwin et al., 2019; Wal et al., 2022). The animals were fasted for 24 hours and then injected with a saline solution of Triton at a dose of 10 mg/kg intraperitoneally.

Following this, the rats were given various millet extracts suspended in 0.2% tween 80 at a dose of 100mg/kg body weight once daily in the morning through gastric intubation for 7 consecutive days. Atorvastatin 10 mg/kg was used as the reference standard drug.

**2.5.1: Study design and Dose regimen**: Albino rats (Wistar strain) of either sex weighing 90- 120 gm were divided into 6 groups (**Table 1**) and administered with test and standard drug as per the dose regimen demonstrated in **Fig. 1**.

**Table 1: Group representation for animals**

| | |
|---|---|
| Group I | Normal healthy rats |
| Group II | Hyperlipidemic control |
| Group III | Standard (atorvastatin) |
| Group IV | Kodo millets |
| Group V | Pearl millets |
| Group VI | Finger millets |

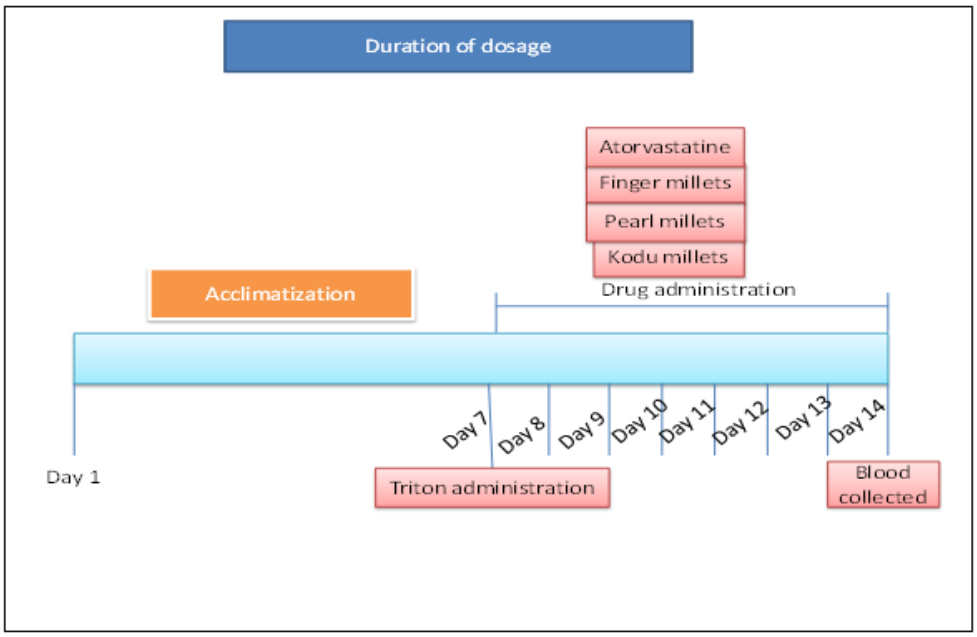


**Fig 1: Dose regimen**

### 2.5.2: Blood collection and Biochemical analysis

Blood was collected from the rat's orbital plexus under ether anesthesia and then centrifuged to obtain serum for the study of various biochemical parameters such as total cholesterol, triglycerides, phospholipids, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) using standard protocol methods (Sciot et al., 1990; Jain et al., 2024a).

### 2.5.3 Histopathological examination

The liver samples were taken at the end of 14 days and examined to demonstrate any pathological changes and the extract's effectiveness in alleviating these changes (Kazi, 1991). The specimens were fixed in formalin, dehydrated, cleared, and processed to prepare sections, which were then stained with hematoxylin and eosin at Kavya Histology Lab in Bhopal, Madhya Pradesh. The stained sections were examined using light microscopy at a magnification of X10 to assess hepatic pathological changes and the extract's efficacy in ameliorating these features.

## 3. RESULTS AND DISCUSSION

Phytochemical screening revealed the presence of amino acids, carbohydrates, flavonoids, glycosides, tannins and proteins in all three millets extracts (Jain et al., 2022b, 2022a, 2024b). A free radicals are implicated in various pathological conditions such as bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging, and neoplastic diseases (Chethan et al., 2008; Jain and Baghel, 2019; Jain et al., 2020, 2023; Jain and Tailang, 2023).

Additionally, they are implicated in autoimmune disorders like rheumatoid arthritis, among others. Our findings revealed that the ethanolic extracts of seeds from Eleusine coracana, Pennisetum glaucum, and Paspalum scrobiculatum exhibit strong antioxidant activity DPPH assay (**Table 2, Fig. 2**).

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**Table 2: Antioxidant activity of ethanolic extracts of various millets**

| S. No | Sample concentration (µg/ml) | Kodo seeds % of inhibition | Ragi seeds % of inhibition | Pearl seeds % of inhibition | Ascorbic acid % of inhibition (Control) |
|---|---|---|---|---|---|
| 1 | 200 | 54.21 ± 0.66 | 23.4 ± 0.33 | 33.5 ± 0.34 | 36.24 ± 0.31 |
| 2 | 400 | 90.86 ± 0.37 | 56.52 ± 0.38 | 60.56 ± 0.37 | 42.21 ± 0.38 |
| 3 | 600 | 93.25 ± 0.62 | 75.49 ±0.45 | 80.65 ± 0.51 | 49.39 ±0.34 |
| 4 | 800 | 96.3 ±0.51 | 95.36 ± 0.47 | 94.54 ± 0.50 | 42.16 ± 0.40 |
| 5 | 1000 | 97.99 ± 0.36 | 96.44 ± 0.33 | 96.47 ± 0.34 | 57.15 ± 0.24 |

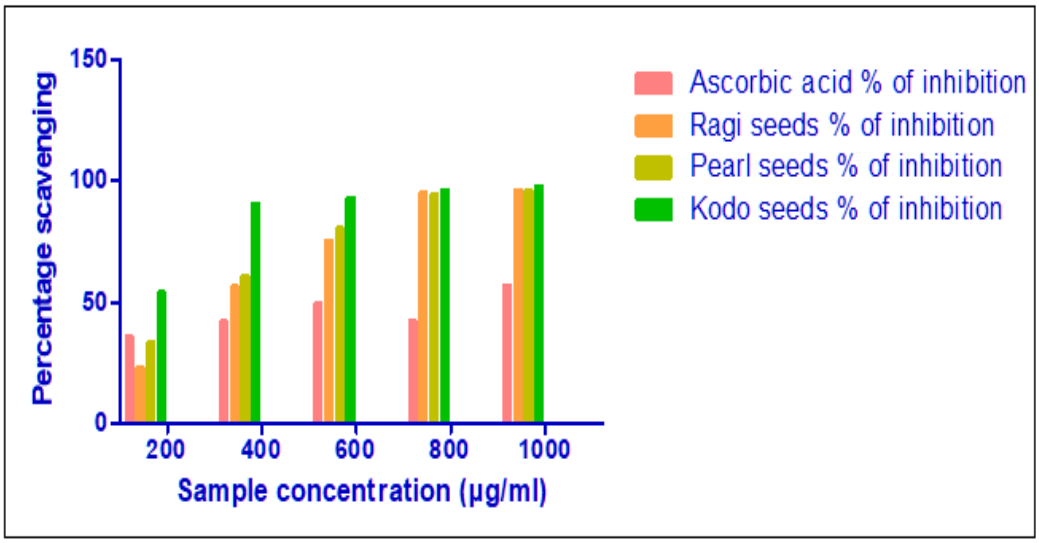


**Fig 2: antioxidant activity of extracts of various millets at different concentration**

There was a notable increase in total serum cholesterol levels, triglycerides, phospholipids, LDL, and VLDL, as well as a decrease in the level of the cardio-protective protein HDL in animals treated with triton. Elevated blood cholesterol levels, particularly LDL, posed a significant risk factor for coronary heart disease, while HDL functioned as a protective factor.

Treatment with various millet extracts (100 mg/kg) markedly decreased cholesterol levels, triglycerides, phospholipids, VLDL, and LDL compared to the hyperlipidemic control.

Additionally, there was a significant increase in HDL compared to the control group. This effect could be attributed to the increased activity of lecithin: cholesterol acetyltransferase, which incorporates free cholesterol and LDL into HDL, transferring them back to VLDL and intermediate-density lipoprotein.

Serum lipid profiles in each group indicated significantly lower triglyceride levels in group V compared to groups I, II, III and IV with total cholesterol levels significantly higher in group I compared to the other groups. Moreover, HDL and LDL-cholesterol levels were significantly higher in group II than in the other groups (**Table 3**, **Fig. 3**).

**Table 3: Effect of millets extracts on serum total cholesterol, triglycerides and phospholipids level in triton-induced hyperlipidemic rats**

| Parameters | Group I | Group II | Group III | Group IV | Group V | Group VI |
|---|---|---|---|---|---|---|
| TG (mg/dL) | 148.76 ± 9.93 | 247.94 ± 11.88 | 164.19 ± 10.95 | 153.96 ± 7.33 | 78.35 ± 4.21 | 142.36 ± 13.25 |
| TC (mg/dL) | 98.64 ± 5.79 | 145.12 ± 10.04 | 76.74 ± 2.90 | 112.94 ± 7.0 | 111.12 ± 4.35 | 115.73 ± 5.69 |
| HDL-C (mg/dL) | 43.25 ± 1.57 | 61.03 ± 5.63 | 37.92 ± 2.73 | 52.56 ± 5.60 | 58.72 ± 3.23 | 42.31 ± 7.61 |
| LDL-C (mg/dL) | 14.18 ± 6.61 | 34.45 ± 5.73 | 14.01 ± 0.49 | 29.58 ± 4.70 | 10.59 ± 1.02 | 12.26 ± 0.01 |
| VLDL-C (mg/dL) | 18.71 ± 2.09 | 18.80 ± 1.14 | 18.70 ± 1.11 | 20.96 ± 2.09 | 16.25 ± 2.35 | 19.36 ± 2.68 |

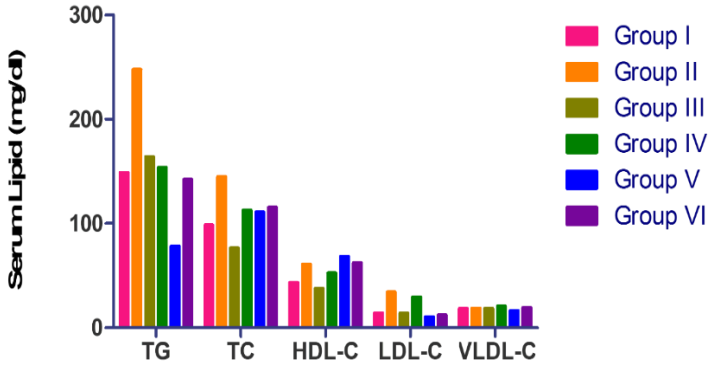


**Fig 3: Effect of extract on serum lipid parameters in hyperlipidemic rats**

Microscopic examination of liver sections from the healthy group revealed a normal arrangement of hepatocytes with a clear central vein at the portal layer. In contrast, liver sections from the triton (10mg/kg) treated group (**Fig. 4b**) exhibited various pathological changes, including centrilobular fatty degeneration, cloudy swelling, and necrosis of hepatic cells. Conversely, liver sections from the groups treated with *Pennisetum glaucum (*Test 1*), Paspalum scrobiculatum (*Test 2*)*, and *Eleusine coracana* extracts (Test 3) (100mg/kg) (**Fig. 4d, e, & f**) displayed normal hepatic cells and central veins, comparable to the atorvastatin-treated group (**Fig. 4c**). The histopathological analysis indicated a restoration of damaged liver cells in the drug-treated groups, with reputed cells of the intoxicated liver being reformed. Furthermore, a reduction in vascularization was observed compared to the hyperlipidemic group.

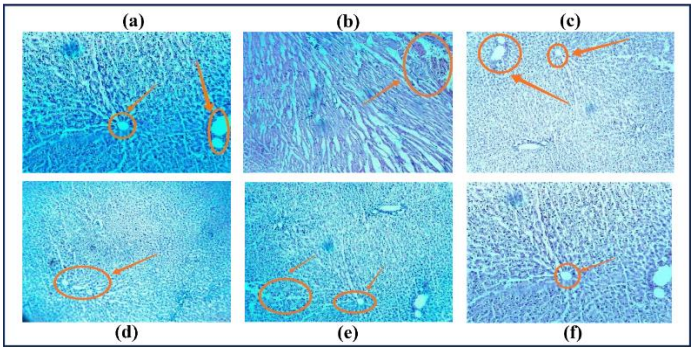


**Figure 4: Microscopical examination of liver section. (a) Normal group, (b) Induction group, (c) Standard group, (d) Test 1 group, (e) Test 2 group, and (f) Test 3 group.**

Increase in serum lipid profile with triton treatment significantly decreased with test drugs and atorvastatin. This activity may be attributed to the presence of saponins, which act as bile acid sequestrants. Administration of *Pennisetum glaucum*, *Paspalum scrobiculatum*, and *Eleusine coracana* extracts significantly reduced fat deposition, serum cholesterol, and increased serum HDL levels in extract-treated groups compared to induction groups. Furthermore, a decrease in body weight was observed in animals treated with ethanolic extracts of *Pennisetum glaucum*, *Paspalum scrobiculatum*, and *Eleusine coracana* compared to the induction group. Histopathological examination of the induction group revealed fat deposition in the liver and hemorrhage, consistent with fat deposition observed in human hyperlipidemia. Administration of extracts significantly decreased fat deposition, indicating the ability of seed extracts of *Pennisetum glaucum*, *Paspalum scrobiculatum*, and *Eleusine coracana* to reduce fat accumulation. These histological findings corroborate the data on fat deposition in the liver, demonstrating the significant antihyperlipidemic activity of the ethanolic seed extracts of *Pennisetum glaucum, Paspalum scrobiculatum,* and *Eleusine coracana*.

## 4. CONCLUSION

The findings from pharmacological screening suggest that ethanolic extracts of various millets possess significant antioxidant and antihyperlipidemic activity. Therefore, they could serve as therapeutic agents or adjuvants in existing therapies for treating hyperlipidemia.

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