Antihyperlipidemic and hepatoprotective effect of *Cucurbita maxima (*Duch) seed in paracetamol induced hepatotoxicity and high fat diet-induced hypercholesterolemic rats.

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ABSTRACT:

Background- Putative conventional medicine has long documented the biological roles of *Cucurbita maxima (D.)* (Family: Cucurbitaceae). This research comprehensively explored the all-inclusive and rigorous antihyperlipidemic and hepatoprotective activities of the seed of *Cucurbita maxima (D.)* employing in-vivo rats models as well as in-vitro tests.

Aim- The current study aims to assess the hepatoprotective and antihyperlipidemic activity of an alcoholic extract of Cucurbita maxima (D.) seed (ACMS).

Method- Seed extracts was administered in the dose of 150mg/kg and 300mg/kg b.w. and toxicity was caused by paracetamol (2 g/kg) on day 5. Silymarin (100 mg/kg b.w) and Atorvastatin10mg/kg (p.o.) was utilised as reference standard. On the 7th day animals were slaughtered and liver function indicators in blood serums and lipid profile were assessed

Result- ACMS may dramatically improve the activities of antioxidative enzymes and successfully eliminate the oxidants in a liver-injury animal study. Furthermore, in a HFD mouse model, ACMS not only notably lowered the levels of total cholesterol, total triglycerides, and LDL cholesterol but also boosted HDL cholesterol. Histopathologic results suggest that ACMS may efficiently prevent excess lipid accumulation in hepatic tissue.

Conclusion- Our observations imply that ACMS may be employed as a helpful, practicable food ingredient for hypocholesterolemic and antihepatotoxicity therapy.

KEYWORDS: *Cucurbita maxima (D.)* seed, Hypercholesterolemic rats, Hepatoprotective, Antioxidant activities, Kaempferol.

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1. INTRODUCTION:

There is a significant chance that hyperlipidemia, which includes high cholesterol and high triglycerides, will lead to the development of cardiovascular disease. The hunt for novel medications capable of decreasing and managing blood cholesterol levels has increased in significance across the decades, in masny papers on substantial natural agent activity. [1]]Plant extracts are interesting choices, yet they might include a complicated blend of numerous different chemicals having different polarities, antioxidants, and pro-oxidant activity.[2]polyunsaturated fatty acids and monounsaturated fatty acids are required for healthy growth and physical development. They have been connected to the regulation of coronary inflammation illnesses and cancer.[3]

Also, a variety of epidemiological research conducted in recent decades has proven a relationship between stress and the occurrence of different disorders,

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emphasizing the requirement of antioxidants in sustaining health. [1, 4]

Depending on their regulation of the body's antioxidative process, providing antioxidant chemicals decreases the atherogenic process in laboratory animals, owing to their ability to scavenge free radical damage. Natural herbs provide a variety of therapeutic properties and are the primary origin of innovative healthcare and medicine items with antioxidant potential.[3] *Cucurbita maxima (D.)*: historically, seeds have been exploited as a traditional meal in the Orient for numerous uses. They may have health benefits and may assist in preventing chronic diseases. However, there is no data on the impact of *Cucurbita maxima (D.)* seed use as nutritional supplements in rats given a high-fat cholesterol diet. Therefore, the objective of this work was to examine in-vivo certain dietary supplement features arising from frequent intake of *Cucurbita maxima (D.)* seed, specifically its possible impact on hyperlipidemia and antioxidant levels in animal studies.[5]

As a consequence, crude alcoholic extracts of *Cucurbita maxima (D.)* Seed revealed hepatoprotective effects against acute liver damage caused by paracetamol in rats. The preventive actions of silymarin and atorvastatin, a popular hepatoprotectant and antihyperlipidemic medication, were studied against paracetamol-induced hepatotoxicity and HFD.[6]

2. MATERIALAND METHODS:

Drugs and Chemicals:

Paracetamol and a high-fat diet were utilized to induce hepatotoxicity and hyperlipidemia in the animals, respectively, while silymarin and atorvastatin were served as reference medicines to compare to the test substance.

Plant material:

Botanical Survey of India Regional Centre, 10 Chatham Lines, Prayagraj, 211002 Dr. Arti Garg recognized the specimen of *Cucurbita maxima (D.)*(D.)Seed collected in April and May.

The Botanical Survey of India has received a specimen with the voucher number SIP/2022-23/129.

Animals housing and feeding conditions:

Albino Wister rats weighing 160–180 g were obtained from Saha Enterprises in Kolkatta, India. The animals were housed on rice husk bedding in propylene cages that were maintained at 24°C and 30–70% relative humidity. The regular commercial pellet (Hindustan Unilever Ltd., Mumbai Maharashtra, India) and unlimited amounts of purified water were used to maintain a 12:12 light-dark cycle. All experimental techniques and protocols were certified by the Animal Ethical Committee (1632/PO/Re/S/12/CPCSEA) following CPCSEA norms.

Preparation of Plant Extracts:

The seeds of *Cucurbita maxima (D.)* were dried in the shade and pulverized with a machine. Cold maceration was used to extract the methanol from the coarse powder after it had been defatted with petroleum ether. With low pressure and a rotating evaporator, the extract was concentrated.Phytochemical screening was performed on them. They had been dissolved in 0.5% carboxymethyl cellulose and were administered to the individual groups in the appropriate quantities based on their body weights.

Acute oral toxicity studies:

The Swiss albino rats (22–30 g) were in good physical condition and supplied only with water for 3–4 hours before being randomly assigned to one of five (n = 3) groups. They were given extracts orally at a dosage of 5 mg, 50 mg, 300 mg, and 2 gm/kg b.w. (p.o.) with a 0.5 percent CMC control. The research was

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conducted in compliance with OECD norms. (423: acute toxic method). During the first 24 hours, the rats were supervised for indicators of toxicity, morbidity, and death, with particular care paid to the first 4 hours, and their behavioral and neurological profiles were examined. They were also monitored for 72 hours and until the 14-day period ended. The test dose was computed.[7]

Total superoxide dismutase activity(SOD):

Beauchamp and Fridovich(1971) measured the activity of superoxide dismutase (SOD). The reaction mixture hold 50 mM of tissue homogenates using potassium phosphate buffer, EDTA (0.1mM), L-methionine (13mM), 2 lM riboflavin, and 75 lM of Nitro Blue Tetrazolium (NBT). The blue color produced by the reaction was detected at 560 nm. superoxide dismutase activity was measured in units per mg of protein and estimated as the quantity of enzyme required to avoid a 50% decline in nitroblue tetrazolium (NBT).[8]

Catalase enzyme activity (CAT):

The Aebi(1984) approach was used to test the activity of the catalase (CAT). In a medium with phosphate buffer (pH 7.4), with homogenised tissue and the substrate hydrogen peroxide (concentration of 0.5 M) were added to begin an enzymatic reaction. A change in absorbance was measured at 240 nm. Catalase enzyme activity (CAT) activity was determined by counting the milligrams of protein that were exposed to H2O2 each minute.[9]

Glutathione peroxidase activity (GPx):

When GSH reductase and NADPH are present, oxidised GSH is rapidly reduced, and NADPH-NADP+ is also oxidised at the same time. At 340 nm, the reduce in absorbance was detected.[9]

Glutathione (GSH) levels:

The Ellman (1959) method was used to identify GSH in tissues, which was improved by "Jollow et al. (1974)" as indicated by the formation of a yellow colour once "5, 5-dithiobis-2 nitro benzoic acid" (DTNB) was introduced to substances that contained sulfhydryl group.Tissue homogenate in phosphate buffer was mixed with 4 mL of 4% sulfosalicylic acid. After that, the mixture underwent a 15-minute centrifugation at 1600 g. A 500 mL supernatant was collected and mixed with "Ellman's reagent". After 10 minutes, the absorbance at 413 nm was measured. The total amount of Glutathione in the liver was measured in milligrammes per millilitre of plasma and milligrammes per milligramme of protein.[10]

HEPATOPROTECTIVE ACTIVITY:

Paracetamol induced hepatotoxicity inrats:

The rats utilized in the study weighed 150-180 g. The rats were distributed into five groups (n = 3):

Gp.I: Control group rats were given days of distilled water at (4 mL/kg.) (p.o.) for 7days.

Gp.II: Rats were given distilled water with paracetamol at (2g/kg) (p.o.) for 7 days (except for the fifth day)

Gp.III: Rats were given the standard drug silymarin at (100 mg/kg)(p.o.) for 7 days.

Gp.IV: Rats were given the test drug (*Cucurbita maxima*) at low dose (150 mg/kg.) (p.o.) for 7 days.

Gp.V: Rats were given the test drug (*Cucurbita maxima*) at high dose (300 mg/kg.) (p.o.) for 7 days.

All rats in groups Gp.III-Gp.V received their drugs for 5 straight days. All rats except those in the Gp. I was given paracetamol (2g/kg) body weight on the fifth day rats were anesthetized with a diethyl ether inhalation jar after 2 hours of respective treatments. The

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serum was separated after blood was taken via heart puncture.

Analysis of key biomarkers of liver function:

Biomarkers of liver function such as Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, and total protein were determined in the blood of treated rats using standard kits according to the supplier's requirements.

ANTIHYPERLIPEDEMIC ACTIVITY:

High – Cholesterol diet model:

Rats weighing 160–200 gm used in the experiment. The rats were distributed into five groups (n = 3), as shown below.

Gp.I: Control group rats were given distilled water at 4 mL/kg p.o. for period of 5 weeks.

Gp.II: High-fat diet was given as hyperlipidemic control for period of 5 weeks.

Gp.III: Rats were given the atorvastatin as a standard drug at (10 mg/kg) p.o. **(**After 3 weeks of feeding HFD, continued until remaining 2 weeks).

Gp.IV: Rats were given the test drug (*Cucurbita maxima*) at low dose (150 mg/kg.) p.o. **(**After 3 weeks of feeding HFD, continued until remaining 2 weeks).

Gp.V: Rats were given the test drug (*Cucurbita maxima*) at high dose (300 mg/kg.) p.o. **(**After 3 weeks of feeding HFD, continued until remaining 2 weeks).

HISTOPATHOLOGICAL STUDIES:

After sacrificing the animals, the livers were removed and instantly washed with buffer and stored in a 10% formalin solution.After that they were dehydrated in a alcohol series, cleaned up in xylene, and ingrained in paraffin wax.Microtome sections with a thickness of 4–5 mm were created and stained with hematoxylin and eosin.Microscope was used to examine the histopathological changes, with images captured at total magnification of 10 and 40 X.

3. STATISTICAL ANALYSIS:

The results were analysed using Graphpad instat (3.0 software) and one-way ANOVA, the data was expressed as SEM after Dunnett's multiple comparison test and P values lower than 0.01 were considered significant.

4. RESULTS:

Extraction of *cucurbita maxima* seed:

The percentage yield of the *Cucurbita maxima* (D.) seed was determined using several solvents, as follows:

Table 1: The extraction value of *Cucurbita maxima* (D.) seed powder by hot extraction method.

S.N.	Nature of extract	Values (% w/w) by hot extraction
01	Petroleum ether	3.80
02	Chloroform	2.39
03	Ethanol	2.10
04	Methanol	13.25
05	Aqueous	2.87

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Hepatoprotective activity of alcoholic extract of cucurbita maxima seed (ACMS) in paracetamol induces rats:

Table 2 shows the results of *Cucurbita maxima (D.)* hepatoprotective efficacy. This demonstrates that when a plant extract is administered, the levels of ALP, AST, total bilirubin, total protein, and AST are significantly lower than when paracetamol is administered.

Table-2 Effect of ACMS on Hepatoprotective in paracetamol induced hepatotoxicity

S.NO.	Treatment	SGPT (U/L)	SGOT (U/L).	ALP (U/L)	Total Bilirubin (mg/dl)	Total Protein (gm/dl).
1.	Normal Control	40.6±0.347	41.7±0.265	99.9±0.543	0.82±0.042	6.3±0.234
2.	Paracetamol 2g/kg (p.o.)	125±0.229	150.7±0.388	310.5±0.347	3.7±0.248	11.82±0.411
3.	Silymarin100mg/kg+paracetamol (p.o.)	38.7±0.183	50.0±0.250	110.0±0.451	0.87±0.013	7.537±0.197
4.	ACMS 150mg/kg+ Paracetamol (p.o.)	52±0.290	55.5±0.471	115.5±0.271	0.84±0.020	9.355±0.224
5.	ACMS 300mg/kg+ Paracetamol (p.o.)	49.9±0.350	53.8±0.157	111.5±0.281	0.55±0.023	8.067±0.171

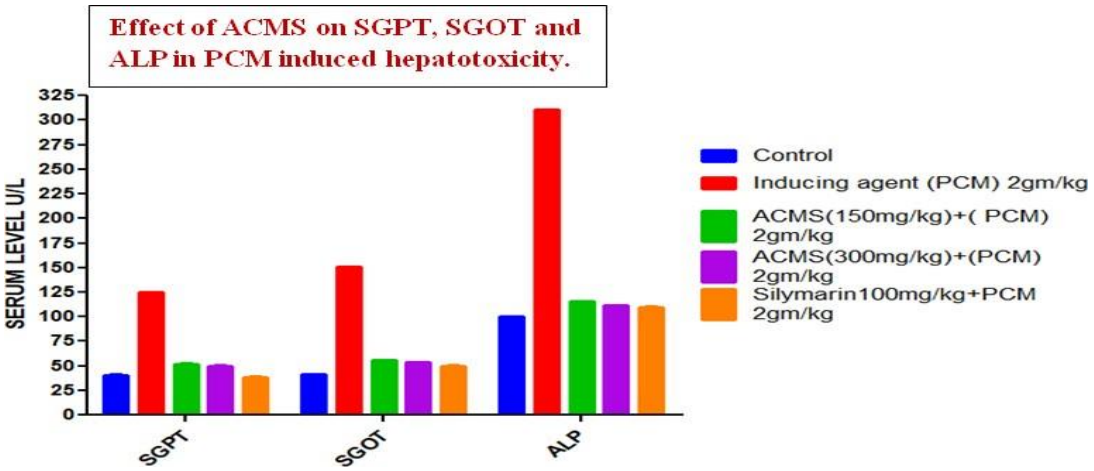

Figure 1. Effect of ACMS on SGOT, SGPT and ALP in paracetamol induced hepatotoxicity.

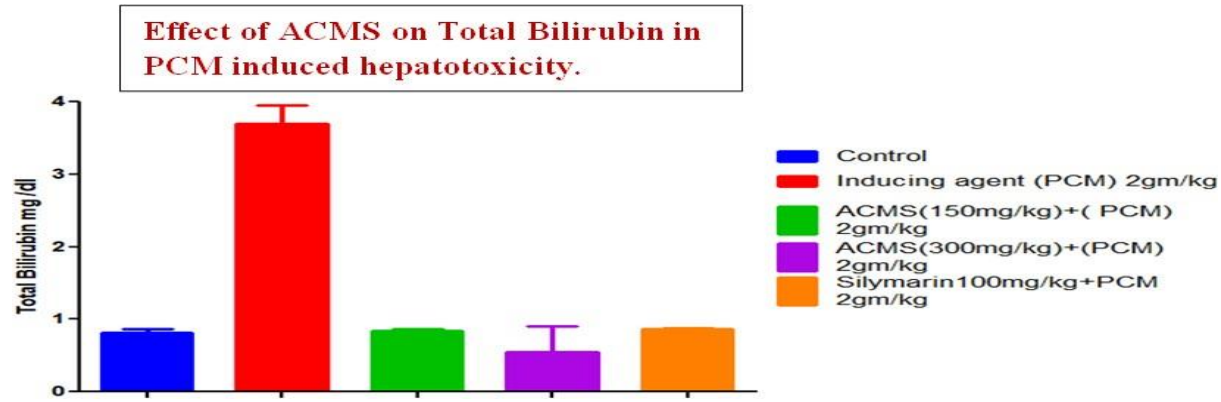

Figure 2. Effect of ACMS on total bilirubin in paracetamol induced hepatotoxicity.

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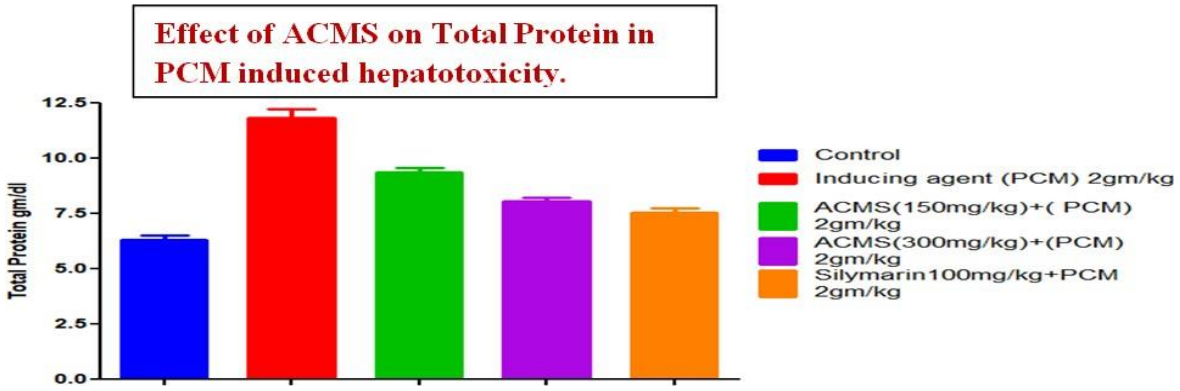

Figure 3. Effect of ACMS on total protein in paracetamol induced hepatotoxicity.

Antihyperlipidemic activity of alcoholic extract of *cucurbita maxima* seed (ACMS) in HFD rats:

Table 3. Effect of ACMS on Lipid Profile in High-fat Diet Induced Hyperlipidemia .

S.N.	Group	Total Cholesterol (Mg/dl).	HDL (mg/dl).	Triglycerides (mg/dl).	VLDL(mg/dl)	LDL (mg/dl).
1.	Control group	81.1±2.78	41.12±1.23	67.89±1.62	13.79±0.13	25.39±3.34
2.	HFD	140.72±2.5	35.19±2.82	139.18±3.80	26.83±0.75	63.35±2.82
3.	Atorvastatin10mg/kg p.o.	86.92±1.76	45.34±2.02	98.1±2.35	21.53±0.64	20.23±2.42
4.	ACMS 150mg/kg p.o.	108.42±2.35	40.81±2.40	116.02±2.10	23.5±0.42	44.73±2.33
5.	ACMS 300mg/kg p.o.	92.35±1.63	42.49±2.18	105.06±2.89	21.6±0.60	28.59±2.02

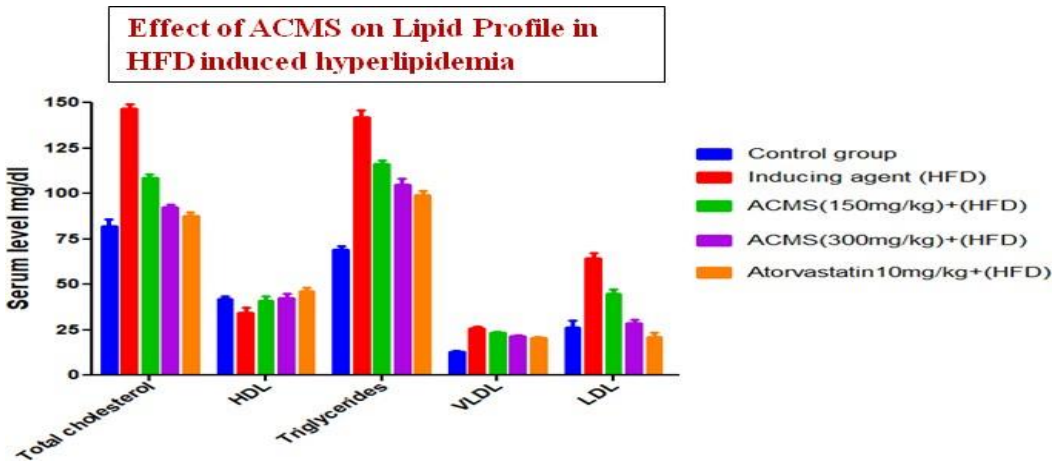

Figure 4.Effect of ACMS on Lipid Profile in High-fat Diet Induced Hyperlipidemia.

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HISTOPATHOLOGICAL OUTCOMES:

Histopathological studies confirmed that *Cucurbita maxima (D.)* has hepatoprotective action.

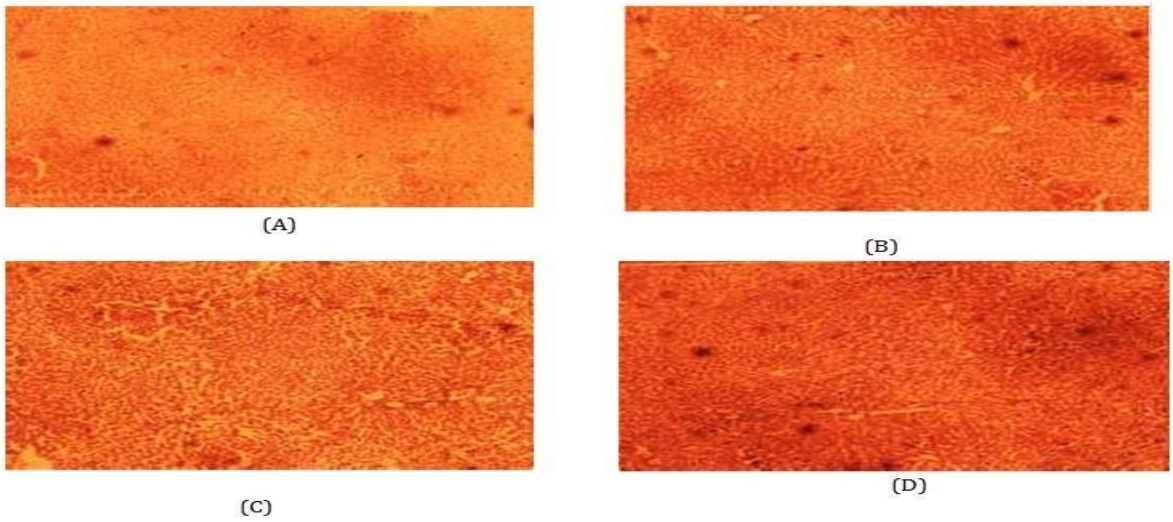

Figure 5. Histopathological outcomes: (A)Normal group with distinct sinusoidal space and central vein architecture.(B) Typical silymarin (100 mg/kg) the treated group had fewer disorganised hepatocytes as well as significant regeneration activity.(C) ACMS low dose (150mg/kg) treated group with less hepatocyte disarrangement.(D) Normal architecture with regeneration activity after a high dose of ACMS (300mg/kg).

5. DISCUSSION:

The current study was designed to assess the effects of cucurbita maxima (D) seed on the lipid profile and antioxidant activities in plasma and liver in paracetamol Induced Hepatotoxicity and HFD induced hypercholesterolemic rats. Rats fed a high-cholesterol diet had higher levels of TG, TC in plasma and liver, and LDL, with lower levels of circulating HDL, providing a model for dietary hyperlipidemia[11]·Paracetamol is a well-known analgesic and antipyretic.When taken as recommended, the drug is harmless; an overdose causes hepatotoxicity because of the development of N-acetyl-p-benzoquinone imine, a toxic metabolite (NAPIQI), which is generated by liver cytochrome P450 enzymes.[6] During the study, a high level of all indicators was seen in the group of rats given paracetamol, indicating severe liver injury. There was a substantial drop in the levels of markers in the rats when they were given silymarin and an alcoholic extract of Cucurbita maxima. Herbal medications with hepatoprotective effects are mostly attributable. Due to the presence of various chemical components like flavonoids, phenols, essential oils, lignans, and so on.[12] There are Kaempferol present, according to a phytochemical investigation into the alcoholic extract of *Cucurbita maxima (D.)*.[4][12] This may be responsible for the significant reduction in ALT, AST, ALP, total protein, and bilirubin levels.[13]In addition, a review of literature found antioxidant efficacy against the free-radical scavenging activity of 1,1-di-phenyl-2-picrylhydrazyl (DPHH).[11] As a result, *Cucurbita maxima (D.)* have hepatoprotective and antihyperlipidemic properties against paracetamol-induced liver injury and HFD induced hyperlipidemia, as demonstrated by a decrease in marker

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enzymes that indicate hepatocyte regeneration activity. These discoveries during the investigation backed up the study's findings.

6. CONCLUSION:

The current investigation found that an alcoholic extract of seed of *Cucurbita maxima (D.)* protects the liver from paracetamol damage. Wistar albino rats were tested for hepatoprotective and antihyperlipidemic activity. The dose levels were given as 150 mg/kg and 300mg/kg body weight, with the latter showing much more hepatoprotective and antihyperlipidemic activity than the former.

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