In vivo and in silico Evaluation of chloroform extract of *Argemone Mexicana* for Antihyperlipidemic Potential: A Comprehensive Approach.

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ABSTRACT: -

Background: In this study we investigate the effect of *Argemone Mexicana* seeds extract on lipidemic mice by using different screening model

Argemone Mexicana, a prickly plant called a prickly poppy is found in sub-tropical regions and it has Several medicinal properties. These plants used in Ayurveda, Unani, Siddha, and Homeopathic practices several times. These plants contain advanced attention linolic acid, oleic acid, and steric acid, which are responsible for Antihyperlipidemic activity and antianginal activity, due to a decrease in the level of cholesterol, it may also be helpful in CAD.

Objectives: - The aim of this study is to investigate the antihyperlipidemic effect of *Argemone Mexicana* seeds extract in High Fat diet and Chlorpromazine induced hyperlipemia in Swiss albino mice

Material and method: - *Argemone Mexicana* seeds were subjected to Soxhlet extraction using chloroform as the solvent to obtain the bioactive extract. The antilipidemic activity of the chloroform extract was evaluated using a High-Fat Diet (HFD) and Chlorpromazine (CPZ)–induced dyslipidaemia model. The HFD consisted of 2% cholesterol, 16% fat, and 0.2% deoxycholic acid supplemented with a regular diet. The extract's effect on lipid profiles was assessed through biochemical and Histopathological parameters.

Result: In hyperlipidaemic mice of both models, oral administration of 400 mg/kg, 200 mg/kg, and 100 mg/kg body weight of the chloroform extract of argemone Mexicana seeds showed a statistically significant decrease ($P<0.01$) in blood lipid parameters such as total cholesterol and triglycerides when compared to hyperlipidaemic control. When compared to negative control groups, these extracts were revealed to have superior antihyperlipidemic potential.

Conclusion: Based on in vivo and Insilco evaluation chloroform extracts of *Argemone Mexicana* seeds containing PUfA shows antihyperlipidemic activity.

Key Words: *Argemone Mexicana*, Hyperlipidaemia, Molecular docking, CAD, Histopathological Study

INTRODUCTION

Hyperlipidemia is a family of disorders that are characterized by abnormally high levels of lipid (fats) in the blood. While fats play a vital role in the body's metabolic processes, high blood levels of fats increase the risk of coronary heart disease (CHD).

It is defined as an increase in one or more triglycerides, phospholipids, cholesterol, or cholesterol esters. The Coronary, cerebrovascular, and even peripheral vascular artery disorders can result from abnormal plasma lipid levels.

Recent studies indicate that high cholesterol levels are present in approximately 25–30% of urban residents in India and in rural populations, the prevalence is slightly lower, ranging from 15–20%.

Argemone Mexicana L., known as Ghamoya (class Magnoliopsida Dicotyledons; class Magnoliidae; order Papaverales; family Papaveraceae;) is an exotic weed indigenous in South America but has wide distribution in numerous tropical and sub-tropical countries including West African [(1)].

Plant Profile

- Domain: Eukaryota
- Kingdom: Plantae
- Phylum: Spermatophyta
- Subphylum: Angiospermae
- Class: Dicotyledonae
- Order: Papaverales
- Family: Papaveraceae
- Genus: Argemone
- Species: *Argemone Mexicana* [(2)]

Chemical constituents: The biological constituents, that are present in argemone

FIG: 1.0

Mexicana linn are responsible for the antihyperlipidemic and antianginal activity that follows.

1) linoleic acid (54% to 61%) – Antihyperlipidemic activity
2) Oleic acid (21% to 33%) -- Antihyperlipidemic activity
3) Steric acid (20.2%to 22%) --- Antihyperlipidemic activity [(3)]

Material and Methods:

Drug and Chemicals: The Fenofibrate and Chlorpromazine was obtained from Matoshri medical Bhor, Pune. The High Fat Diet containing cholesterol, Fat was obtained from Pathozyme Diagnostics Kagal, Dist-Kolhapur, and deoxycholic acid was obtained from matoshree medical bhor, pune, the chloroform used as solvent for extraction.

Molecular Docking:

Using the Argus lab software the Linolic acid and oleic acid a chemical constituent of *Argemone Mexicana* docked with LDL Receptor and HMG CO A, and the docking score of LDLS and linolic acid was -9.53, Linolic acid AND HMG COA WAS -9.67445 kcal/mol, oleic acid and LDL was

-9.75037 kcal/mol, oleic acid with HMG COA was -8.15148 kcal/mol.

Collection of plant: *Argemone Mexicana* Seeds was obtained from Shyam Sundar, Hyderabad, Telangana, 500012(Begum Bazzar, Afzal Gunj, Hyderabad, Telangana, 500012)

Authentication of plant: AMS authenticated at waghire college of arts, commerce and science

Extract preparation: Extraction of AMO

AMSE was extracted from *Argemone mexicana* seeds (AMS) using chloroform in a Soxhlet apparatus at 65 °C. The system included a solvent reservoir, extraction chamber, condenser, and oil collection setup. Oil yield was maximized near the solvent's boiling point, and the extracted oil was weighed and compared to the dry seed weight. Triplicate experiments were conducted, and mean values were used for analysis. The oil was stored at −4 °C without further purification until characterization.

Fig:1.1: Soxhlet extraction of AMS

Oil yield (%) = weight of extracted oil / weight of seed sample × 100

Phytochemical Test:

1)PUFA Test : 1ml Alc solution + 1 gm of Fat/oil+ potasium permagnate solution = decolurisation of permagnate

2)Alkaloids : Extrat + Dil.HCl + Mayur reagent = formation of White precipitate

3)Phenol test : 2ml test solution +feric chlroide solution = Green or red colour indicate phenol is present

FIG : 1.2 Phytochemical Test .

Table No 1.0:

Phytochemical	Test Performed	Result
Alkaloids	Mayer's test	+
Polyunsaturated Fatty acid	Unsaturation Test	+
Proteins	Biuret Test	+
Flavonoids	Ferric Chloride Test	+
Fixed Oil	Spot	+
Amino Acid	Ninhydrin Test	-
	Salkowski's Test	+

Preparation of Emulsion From AMSE

The o/w emulsion was prepared using a selected test dose, with Span 80 as the oil-phase surfactant and Tween 80 with phenoxyethanol caprylyl glycol as the water-phase components. The water phase was gradually added to the oil phase while stirring at 1000 rpm for 30 minutes at room temperature to ensure proper mixing and stable emulsion formation.

Acute Oral Toxicity Study (AOT):

The OECD 423 standards were followed for performing oral toxicity. To test for

acute oral toxicity, four dose levels were chosen. The dose that was employed was 2000 mg/kg. The OECD 423 guidelines' (Annexure 2d) methodology was used.

Principle: The acute oral toxicity test (OECD 423) determines the LD_{50} of a substance using minimal animals. The test involves dosing three same-sex animals at a time with predefined levels (5, 50, 300, or 2000 mg/kg b.wt.). Based on the presence or absence of mortality, the next step is either to stop testing, repeat the dose with three more animals, or move to a higher or lower dose.

Blood Collection and analysis: For biochemical analysis of cholesterol and triglycerides, blood was collected from the tail vein of mice after 12–16 hours of fasting. The tail was warmed (37–40°C) for 1–2 minutes to enhance blood flow, cleaned with 70% alcohol, and punctured on the ventral side. Blood was collected using a microcapillary tube and sent to Omega Laboratories, Lonand, Satara for analysis.

FIG: 1.3 Blood Collection and analysis

MECHANISM OF LINOLEIC ACID: Linoleic acid is an essential polyunsaturated fatty acid (PUFA) that is present in the Argemone Mexicana plant. In humans, linoleic acid cannot be synthesized and must be acquired through diet. Linoleic acid is used to synthesize a variety of other unsaturated fatty acids, including eicosapentaenoic acid and docosahexaenoic acid. preliminarily, these unsaturated fatty acids have been shown to ameliorate blood pressure, platelet reactivity, thrombosis, triglyceride (TG) situations, vascular reactivity, heart-rate variability, and inflammation.

In another study, linoleic acid was reported to reduce blood cholesterol and play a significant part in precluding cardiovascular conditions. This unsaturated fatty acid plays an important role in LDL-lowering capacity by increasing membrane fluidity, which increases LDL receptor activity and accordingly, decreases LDL apoB and increases LDL catabolism. In addition, it increases CYP7 action, thereby converting cholesterol to bile acids in the

In other studies, linoleic acid consumption has been reported to increase HDL situations. The increase in HDL situations is due to an increase in apolipoprotein A1(ApoA1) expression ApoA1 triggers a response called cholesterol esterification that converts cholesterol to a form that can be completely integrated into HDL and later transported through the bloodstream from the body's towel to the liver.[4],[12]

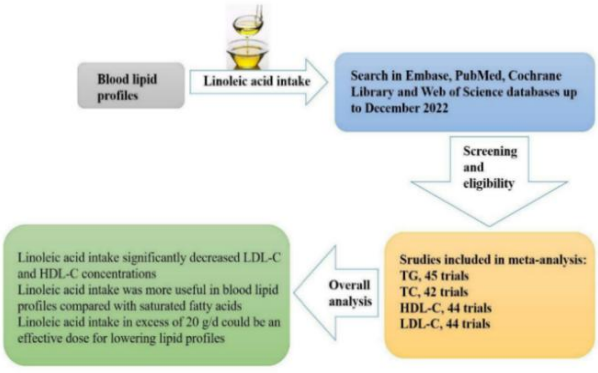


FIG: 1.4 MOA of linoleic acid

Linoleic acid is also used in CVD, Cardiovascular complaint (CVD) is the leading cause of death worldwide. threat factors for developing this complaint include high serum attention of total cholesterol\very low-density lipoproteins

and ldl. decreasing these risk factors involves the replacement of saturated fatty acid with mono or polyunsaturated fatty acid. The essential omega 6 PUFA, linoleic acid (LA), is suggested to drop the threat for CVD by affecting these lipid risk labels [5].

MECHANISM-BASED ON DOCKING BINDING SCORE

The 3D structures of active chemical constituents linolic acid and oleic acid, were downloaded from PubChem which are present in *argemone-Mexicana*. The RCSB PDB protein data repository provided the 3D structures of the Ldl receptor and HMG COA. Subsequently, co-crystallized ligand and crystallographic water were eliminated to produce the results. Argus Lab 4.0.1 was used to simulate molecular docking with the default settings and Discovery Studio was used for visualization

Using the Argus lab software the Linolic acid and oleic acid a chemical constituent of *Argemone Mexicana* docked with LDL Receptor and HMG CO A, and the docking score of LDLS and linolic acid was -9.53, Linolic acid AND HMG COA WAS -9.67445 kcal/mol, oleic acid and LDL was -9.75037 kcal/mol, oleic acid with HMG COA was -8.15148 kcal/mol)
From the docking, it was predicted that the linolic acid receptor might bind to the LDL receptor, which was thought to be responsible for the antihyperlipidemic activity of *Argemone Mexicana* in the following way.

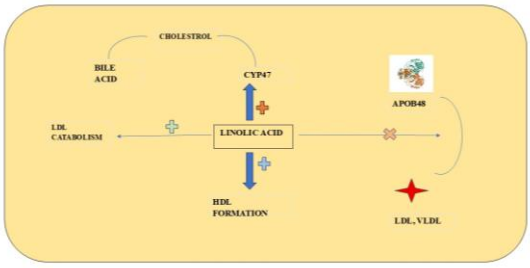


Fig: 1.5 MOA Based on Docking

EFFECT OF OLEIC ACID: Oleate and Other Long Chain Adipose Acids Stimulate Low-density Lipoprotein Receptor activity by Enhancing Acyl Coenzyme A Cholesterol Acyltransferase Activity and Altering Intracellular Regulatory Cholesterol leval[8]. The Oleic acid was the most effective at depressing lipogenesis and cholesterol genesis. A decrease in the label incorporation into cellular palmitic, stearic, and oleic acids was detected, suggesting that an enzymatic step of de novo adipose acid biosynthesis was affected. Oleic acid reduced the of 3- hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) and ACC. The inhibition of ACC and HMGCR conditioning is corroborated by the diminishments in ACC and HMGCR mRNA abundance and protein situations. The down-regulation of ACC and HMGCR conditioning and expression by oleic acid could contribute to reduced lipogenesis and cholesterol genesis [7].

EFFECT OF STERIC ACID –

The Dietary Stearic Acid Reduces Cholesterol Absorption and Increases Endogenous Cholesterol Excretion[9]. Stearic acid, unlike other saturated fats, does not seem to raise LDL cholesterol levels, the "bad" cholesterol linked to an increased risk of heart disease. Stearic acid, on the other hand, has been shown to have a neutral effect on LDL cholesterol and may even enhance HDL cholesterol, or "good" cholesterol. Because of this distinguishing feature, stearic acid may not have the same harmful influence on cardiovascular health as other saturated fats[10]. the lower concentration of both plasma total cholesterol and hepatic total land esterified cholesterol in hamsters consuming the stearic acid–enriched diet compared with the myristic acid–and palmitic acid-enriched diet groups. Thisoccurredeventhoughthe18:0-enriched

diet contained a substantial amount of 16:0 (20.6g/100g fatty acids) and suggests that under the conditions of this experiment, stearic acid is hypocholesterolaemia relative to myristic and palmitic acids. An increase in bile acid excretion could potentially mediate the relative hypocholesterolaemia effect of stearic acid. they alter the hepatic LDL receptor activity and thereby influence LDL cholesterol concentration. they also decrease cholesterol synthesis [7].

Docking: A molecular docking investigation was conducted under the assumption of a model in which the docking process treated the protein and ligand as flexible entities. estimated docking score presented in Tables

Table NO 1.2

Interaction between chemical constituents of *Argemone Mexicana* and LDL RECEPTOR (PDB-1YA9 https://doi.org/10.2210/pdb1YA9/pdb)

DRUG	Docking score / binding energy (Kcal/mol)	Van Der waals	H bond	Pi Alkyl	Alkyl
LINOLIC ACID	-9.53	GLU A:19, GLNA 20, ASN A23 LEUA:29 LEU A:96 MET A: 100, ARG A:137 ARG A:142 GLU A:145	TRP A 26	ALA A:144	ALA A:144
OLEIC ACID	-9.75037 kcal/mol	GLU A:19 ASN A:23 PHE A:25 LEU A:96 MET A:100 GLU A:145 LEU A:147	TRP A:26	ALA A:144 LEU A:22 MET A:141	ALA A:144 LEU A:22 MET A:141

Table 1.3:

Interaction between chemical constituents of *Argemone Mexicana* and

HMG CO A (PDB A8DJK, HMGCR-UBIAD1 Complex State 2 Chen, H., QI, X., LI, X)

DRUG	Docking score / binding energy (Kcal/mol)	Van Der waals	H bond	Pi Alkyl	Alkyl
LINOLIC ACID	- 9.67445 kcal/mol	TRP B:56 LEU B:229 THR B:319 ASP B:322 ASP B:394 LYS B:388 ASN B:433	ARG B:54 SER B:318	TYR B:321 ILE B:327 PHE B:397 VAL B:390 ALA B:391 ULEB:445	TYR B:321 ILE B:327 PHE B:397 VAL B:390 ALA B:391 ULEB:445

		ASP B:440			
OLEIC ACID	-8.15148 kcal/mol	LEU B:229 GLY B:314 TYR B;321 ASP B:322 SER B:324 LYS B:324	ASP B 394 SER B 318 ASN B 315	ARG B :54 TRP B: 56 ILE B:327 VAL B:390 ALA B :391	ARG B :54 TRP B: 56 ILE B:327 VAL B:390 ALA B :391 ILE B;445

DOCKING IMAGE:

1.1 LINOLIC ACID AND LDL RECEPTOR

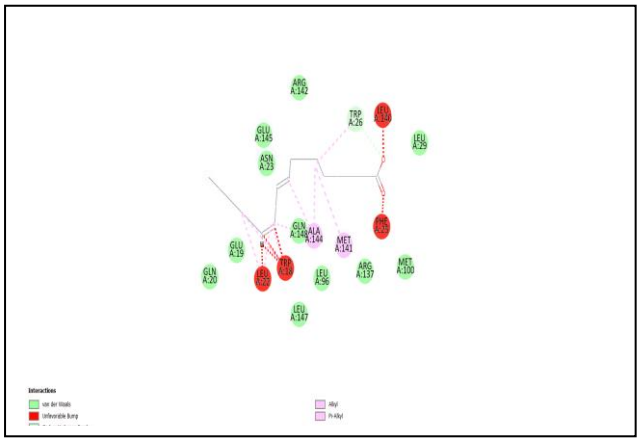


FIG:1.6

1.2 LINOLIC ACID AND HMG COA

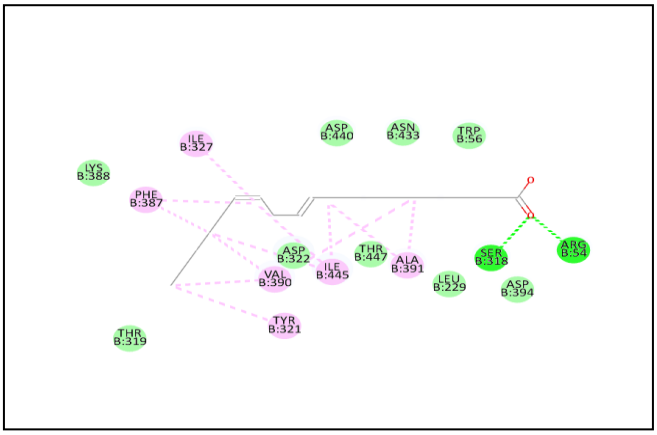


FIG:1.7

1.3 OLEIC ACID WITH LDL RECEPTORS

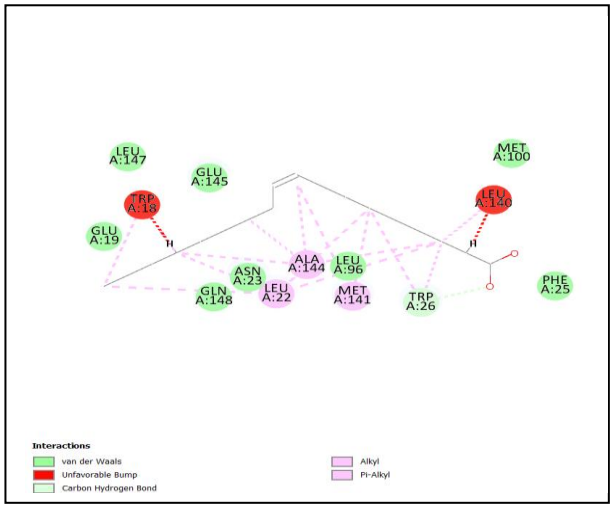


FIG: 1.8

1.4 OLEIC ACID AND HMG COA

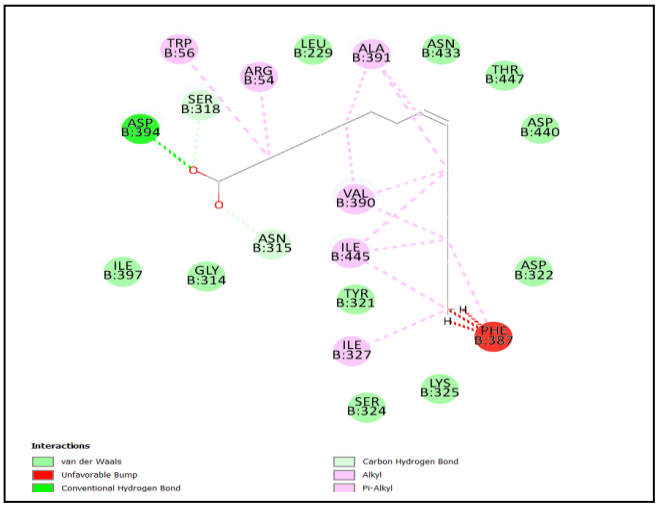


FIG: 1.9

DETERMINATION OF ANTIHYPERLIPDMIC ACTIVITY

High Fat Diet (HFD) induced hyperlipemia:

1) Preparation of HFD: The high-fat diet was prepared according to the protocol described by Harnafi et al. (2009) [11]; this diet consisted of the regular chow diet, cholesterol 2%, fat 16%, and deoxycholic acid 0.2%[11].

Experimental Protocol Design

After two weeks of acclimatization, the mice were divided into six equal groups (n = 6) and treated as follows:

• A standard control group (NCG) received only distilled water at 10 mL/kg body weight.

• The Hyperlipidaemic Control Group (HCG) freely received the high-fat diet and received distilled water daily (10 mL/kg).

• The Standard Group Received Fenofibrate (200mg/kg) and then High Fat Diet

• Three treated groups received 100, 200 and 400 mg/kg of *Argemone Mexican* seeds extract and then high-fat diet for 30 days of the treatment.

At the end of the treatment, Blood samples were collected from the animals under mild ether anaesthesia from tail vein 1 h after drug administration and then will be given for biochemical estimation of cholesterol and Triglycerides

CHLORPROMAZINE (CPZ) INDUCED HYPERLIPDMIA:

Male Swiss albino mice were used at 8 weeks of age.

The positive control group received vehicle (Distilled water, 10ml/kg, p.o.) for 7 days

The negative control group received 10mg/kg CPZ and vehicle (Distilled water) for 7 days

In Test Group Mice were administered chlorpromazine (10mg/kg, p.o.), concomitantly z

The Standard Groups received CPZ and fenofibrate (100mg/kg p.o.) for 7 days.

At the end of treatment, the Blood samples collect in the fed state from the animals under mild ether anaesthesia from tail vein 1 h after drug administration and given for biochemical estimation of Cholesterol and Triglyceride.

Effect of AMSE on body weight in HFD

The effect of AMSE on body weight was evaluated over 31 days in an HFD-induced hyperlipidaemic mice model. The negative control group showed the highest weight gain (12.50 g), while the normal group gained 11.30 g. The standard group (fenofibrate) showed reduced gain (7.30 g), indicating strong antihyperlipidemic activity. Test Group 1 gained 10.07 g, and Test Group 2 gained 10.00 g, both showing moderate efficacy. Test Group 3 had a gain of 8.70 g, indicating better control than other test groups and closer effect to the standard treatment.

Table No 1.4: Body Weight check

Groups	Day 1	Day 31	Weight gain
NCG	20.00 g	31.30 g	11.30 g
HCG	23.00 g	35.50 g	12.50 g
Standard	19.70 g	27.00 g	7.30 g
AMSE 1	24.00 g	34.07 g	10.07 g
AMSE 2	26.00 g	36.00 g	10.00 g
AMSE 3	20.30 g	29.00 g	8.70 g

(Table no. Effect of *Argemone Mexicana Seeds* on Body weights, AMSE: *Argemone Mexicana Seeds* Extract, NCG: Normal Control Group, HCG: Hyperlipidemic control Group)

Effect of AMSE on body weight in CPZ

The study evaluated the effect of treatments on body weight in CPZ-induced hyperlipidaemic mice over 31 days. The Normal group showed a 12.30 g weight gain, while the Negative control group had the highest gain of 14.50 g, confirming the impact of CPZ. The Standard group (fenofibrate) showed a lower gain of 8.30 g, indicating strong efficacy. Test Group 1 gained 11.07 g, showing partial effect; Test Group 2 gained 9.60 g, showing moderate efficacy; and Test Group 3 gained 9.00 g, indicating better weight control than the other test groups and closer to the standard group.

Table No 1.5: Effect of AMSE on body weight in CPZ induced Model

Group	Day 1	Day 31	Weight Gain (g)
+NC	20.00 g	32.30 g	12.30 g
- NC	22.00 g	36.50 g	14.50 g
SD	19.70 g	28.00 g	8.30 g
T1 group	24.00 g	35.07 g	11.07 g
T2 group	26.40 g	36.00 g	9.60 g
T3 group	20.g	29.00 g	9.00 g

Effect of AMSE on cholesterol and triglycerides in HFD model:

Administration of a high-fat diet (HFD) led to a significant rise in serum lipid levels in the negative control group (TC: 193.33 ± 1.12 mg/dL, TG: 191.44 ± 0.58 mg/dL). The standard group treated with fenofibrate showed a marked reduction (TC: 132.25 ± 0.48, TG: 143.23 ± 0.92). AMSE Test 1 (100 mg/kg) and Test 2 (200 mg/kg) groups showed moderate lipid-lowering effects, while Test 3 (400 mg/kg) significantly reduced TC (134.65 ± 1.14) and TG (144.42 ± 0.72), comparable to the standard. Thus, fenofibrate and AMSE Test 3 showed strong anti-hyperlipidaemic activity, with Test 1 and 2 showing moderate effects.

Table No 1.6 : Effect of AMSE on total cholesterol in HFD-induced hyperlipidaemia model.

Sr. no	Group	Treatments	Dose	Means Cholesterols
1	+NC	Distilled water	10Ml/kg	190.97±1.33
2	- NC	Water +HFD	HFD	193.33±1.12
3	SD	Fenofibrate	200	132.25±0.48 ***
4	Low	AMSE (T1)	100	188.73±0.99 *
5	MD	AMSE (T2)	200	187.00±0.78 **
6	High	AMSE (T3)	400	134.65±1.14 ***

The data was presented as mean ± SEM (n=6). One-way analysis of variance (ANOVA) following multiple comparison Dunnett test. *p<0.05, **p<0.01, **p<0.001 as compared with negative control group.

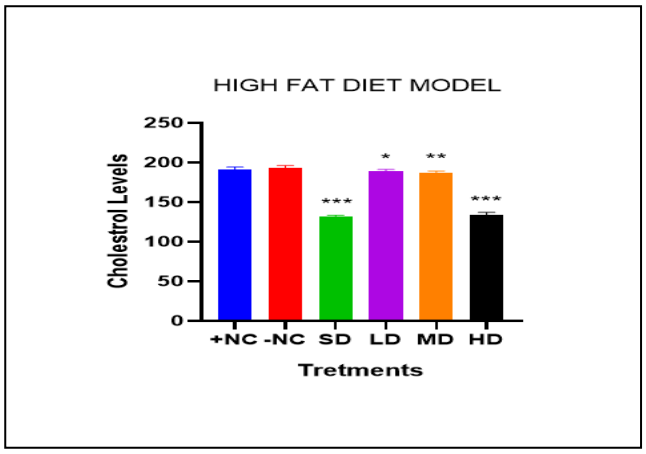


FIG 1.10: Effect of AMSE on Cholesterol in HFD

Table No1.7 : Effect of AMSE on triglycerides in HFD-induced hyperlipidaemia model.

Sr.	Group	Treatments	Dose	Triglycerides
1	+NC	Distilled water	10Ml/kg	188.48±0.57*
2	-NC	Water +HFD	HFD	191.44±0.58
3	SD	Fenofibrate	200mg/kg	143.23±0.92***
4	Low	AMSE (T1)	100mg/kg	187.63±0.83**
5	MD	AMSE (T2)	200mg/kg	187.41±0.87**
6	High	AMSE (T3)	400mg/kg	144.42±0.72***

The data was presented as mean ± SEM (n=6). One-way analysis of variance (ANOVA) following multiple comparison Dunnett test. *p<0.05, **p<0.01, **p<0.001 as compared with negative control group.

FIG: Effect of AMSE on triglycerides in HFD model

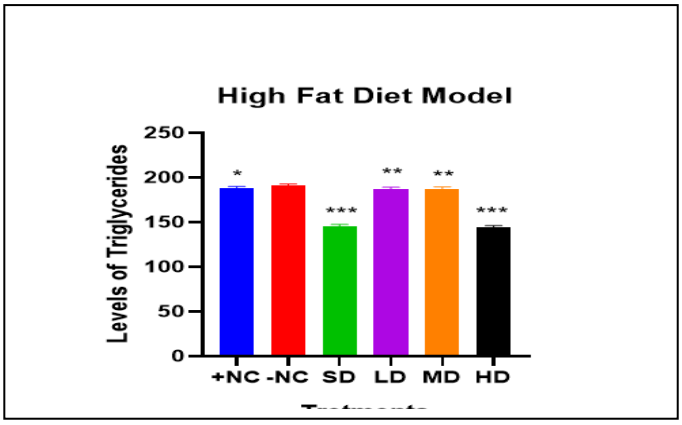


FIG 1.11: Effect of AMSE on Tg in HFD

Effect of AMSE on cholesterol and triglycerides in CPZ induced Model:

Chlorpromazine (CPZ) administration significantly elevated serum lipid levels in the negative control group (TC and TG: 193.33 ± 1.12 mg/dL). The standard group (Fenofibrate 200 mg/kg) showed a marked reduction (TC: 132.20 ± 1.50, TG: 144.96 ± 1.50). AMSE Test 1 (100 mg/kg) and Test 2 (200 mg/kg) groups showed moderate decreases in TC and TG, while Test 3 (400 mg/kg) showed significant lipid-lowering effects (TC: 141.41 ± 1.11, TG: 157.15 ± 1.11), comparable to the standard. Thus, Fenofibrate and AMSE Test 3 demonstrated strong anti-hyperlipidaemic activity.

Table No 1.8: Effect of AMSE on triglycerides in CPZ-induced hyperlipidaemia model

Sr	Group	Treatments	Dose	Means TG
1	+NC	Water	10Ml/kg	189.22±1.06*
2	- NC	CPZ	10mg/kg	193.33±1.12
3	SD	Fenofibrate	200mg/kg	144.96±1.50***
4	Low	AMSE (T1)	100mg/kg	188.73±0.99*
5	MD	AMSE (T2)	200mg/kg	187.31±0.78**
6	High	AMSE (T3)	400mg/kg	157.15±1.11***

The data was presented as mean ± SEM (n=6). One-way analysis of variance (ANOVA) following multiple comparison Dunnett test. *p<0.05, **p<0.01, **p<0.001 as compared with negative control group.

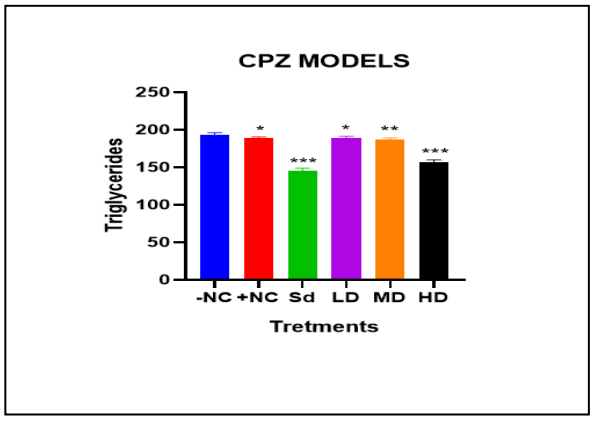


Fig1.12: Effect of AMSE on triglycerides in

Table No1.9: Effect of AMSE on total cholesterol in CPZ-induced hyperlipidaemia model

Sr	Gr.	Treatments	Dose	Means Cholesterols
1	-NC	Water+CPZ	10Ml/kg	193.33±1.12
2	+NC	Distilled water	10Ml/kg	190.35±1.05
3	SD	Fenofibrate	200mg/kg	132.20±1.50***
4	Low	AMSE (T1)	100mg/kg	188.73±0.99*
5	MD	AMSE (T2)	200mg/kg	185.55±1.02**
6	High	AMSE (T3)	400mg/kg	141.41±1.11***

The data was presented as mean ± SEM (n=6). One-way analysis of variance (ANOVA) following multiple comparison Dunnett test. *p<0.05, **p<0.01, **p<0.001 as compared with negative control group

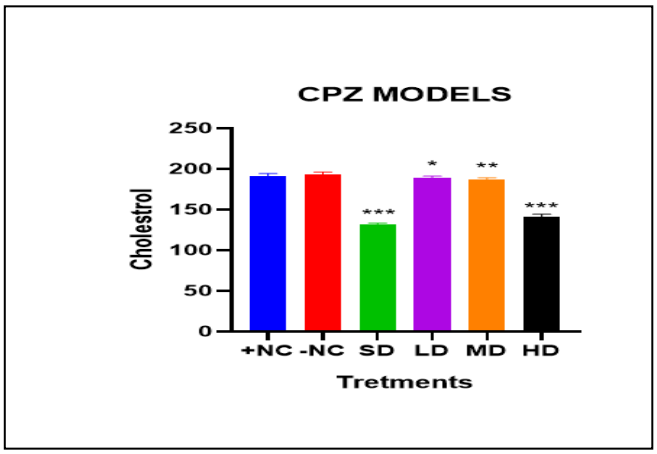


FIG 1.13: Effect of AMSE on cholesterol in CPZ model

Histopathological Evaluation:

Animal were euthanized under ether anaesthesia and liver was dissected out immediately and weighted. For Histopathological analysis, the liver was fixed in 10 %formalin at room temperature. the tissue was embedded in paraffin, sectioned into 3-4 µm thickness and mounted on the glass microscopes slide slides using standard histological techniques. The sections were stained with haematoxylin–eosin and examined using light microscopy at 200× magnitudes. These light microscopic fields were assessed by an image analyser on each section.

FIG:1.14

Table No 8: Histopathological score

FIG: 1.10 Histopathological Evaluation of chloroform extract of Argemone Mexican

Group	Vascular changes	Cellular infiltration	Fatty changes	necrosis
NC	1	1	1	1
-NC	3	3	3	3
SD	2	1	1	1
Low	2	2	2	2
Medium	1	2	2	1
High	1	2	1	1

NC: Normal control, -NC: Negative Control, SD: Standard Deviation

Histological Image of Normal Control:

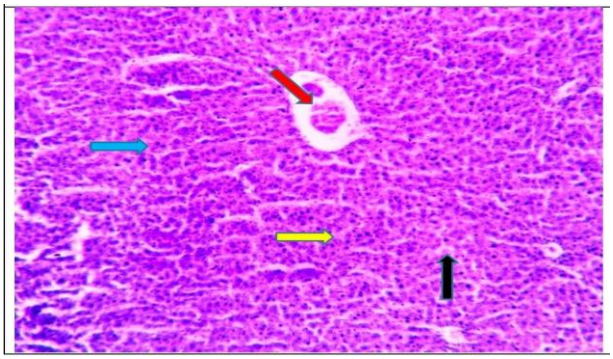
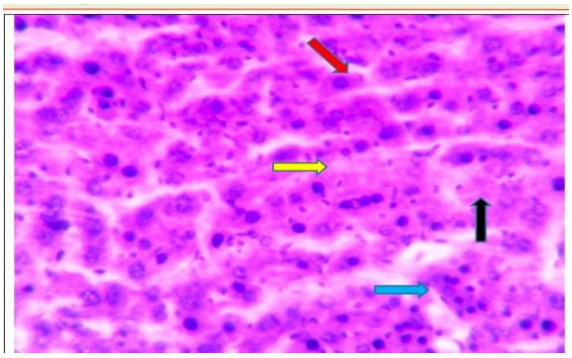


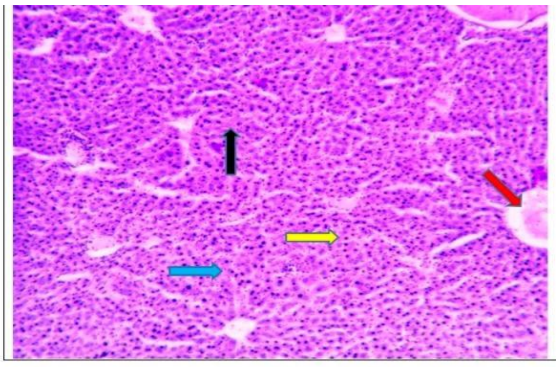
FIG: 1.15 Group Normal Control

Photograph showing fatty changes (Yellow arrow), vascular changes (red arrow), necrosis (Black arrow), cellular infiltration (blue arrow), H& E Stain 100X

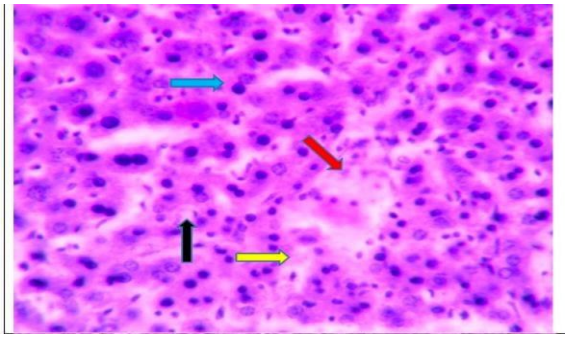


2)FIG1.16: Group Normal Control: Photograph showing fatty changes (Yellow arrow), vascular changes (red arrow), necrosis (Black arrow), cellular infiltration (blue arrow), H& E Stain 400X

Histological Image of -NC

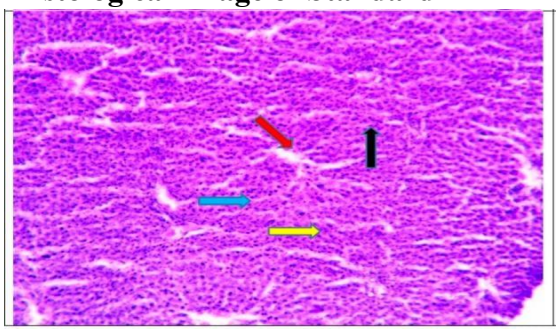


1) FIG1.17: Negative Control: Photograph showing fatty changes (Yellow arrow), vascular changes (red arrow), necrosis (Black arrow), cellular infiltration (blue arrow), H& E Stain 100X

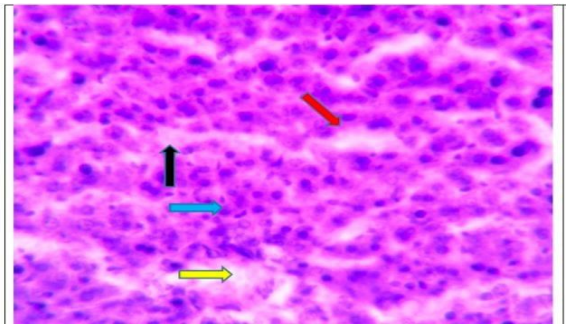


2)FIG:1.18 Negative Control: Photograph showing fatty changes (Yellow arrow), vascular changes (red arrow), necrosis (Black arrow), cellular infiltration (blue arrow),
H& E Stain 400X

Histological Image of Standard

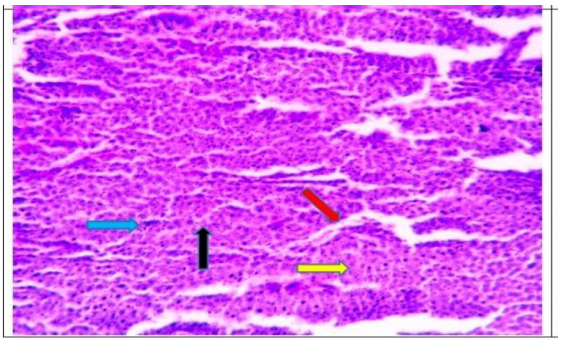


1) FIG:1.19 Standard Group: Photograph showing fatty changes (Yellow arrow), vascular changes (red arrow), necrosis (Black arrow), cellular infiltration (blue arrow), H& E Stain 100X

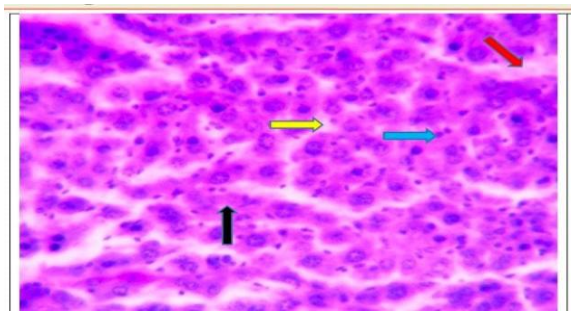


2) FIG:1.20 Standard Group: Photograph showing fatty changes (Yellow arrow), vascular changes (red arrow), necrosis (Black arrow), cellular infiltration (blue arrow), H& E Stain 400X

Histological images Low Group:

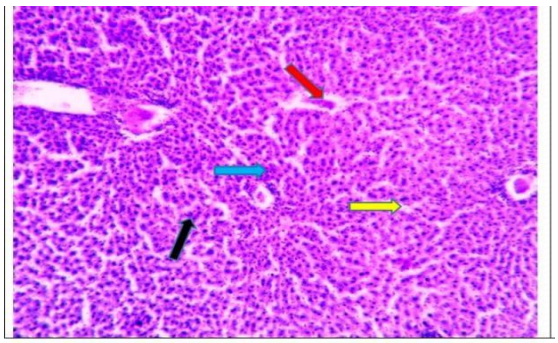


1) FIG: 1.21 Low group: Photograph showing fatty changes (Yellow arrow), vascular changes (red arrow), necrosis (Black arrow), cellular infiltration (blue arrow), H& E Stain 100X

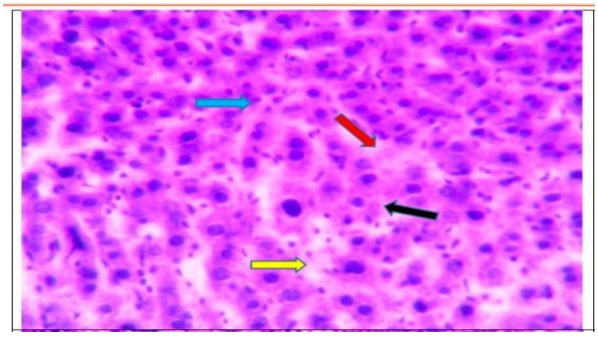


2) FIG:1.22 low group: Photograph showing fatty changes (Yellow arrow), vascular changes (red arrow), necrosis (Black arrow), cellular infiltration (blue arrow), H& E Stain 400X

Histological Image of Medium Test Group:

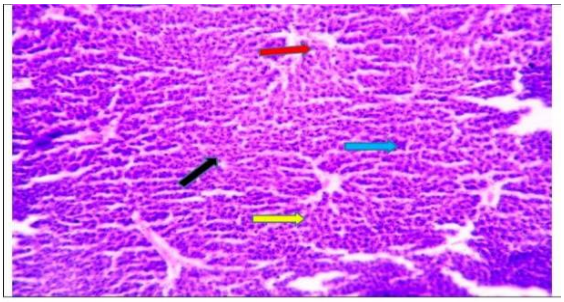


1)FIG:1.23 Medium group: Photograph showing fatty changes (Yellow arrow), vascular changes (red arrow), necrosis (Black arrow), cellular infiltration (blue arrow), H& E Stain 100X



2) FIG:1.24 Medium group: Photograph showing fatty changes (Yellow arrow), vascular changes (red arrow), necrosis (Black arrow), cellular infiltration (blue arrow), H& E Stain 400X

Histological Image of High-Test Group:



1)FIG 1.25 : Medium group Photograph showing fatty changes (Yellow arrow), vascular changes (red arrow), necrosis (Black arrow), cellular infiltration (blue arrow), H& E Stain 100X

Discussion: Hyperlipidemia is a medical condition characterized by an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters, phospholipids and this cholesterol which is present in the the form of lipoprotein for transport. Cholesterol which is present in the form of cholesterol ester in lipoprotein by using cholesterol acetyl transferase.

Argemone Mexicana, commonly known as Poppy Plant, is a medicinal plant traditionally used for its anticancer, antioxidant, hepatoprotective and Antidiabetics. Recent studies have focused on its potential antihyperlipidemic activity, particularly using its Chloroform extract in various animal models.

The Chloroform Extract of *Argemone Mexicana* Shows antihyperlipidemic activity by acting as HMG COA Reductase and Apo B48 inhibitors, and simultaneously increase the HDL or Good Cholesterol

The *Argemone Mexicana* contain polyunsaturated fatty acid like linolic acid, oleic acid, Steric acid which are responsible for Antihyperlipidemic activity. The linolic acid they have multiple mechanism they increase the level HDL cholesterol also they show HMG COA Reductase inhibitory Activity and the linolic acid also inhibit the Apo B 48. The Apo B 48 Which are responsible for receptor Recognition.

The plant selected for present study was Argemone Mexican also called Mexico Plant native from tropical and Subtropical regions worldwide which a traditionally used plant. In present investigation

Antihyperlipidemic activity of a rare species of Argemone belonging to Papaveraceae family named. A phytochemical investigation was performed to screen for the presence or

absence of Polyunsaturated Fatty Acids. Acute oral toxicity was performed according to the procedure given in OECD guidelines 423 of the Argemone Mexican Chloroform Extract was safe up to the dose of 2000 mg/kg Hence, 1/10th of 2000 mg/kg, i.e., 200 mg/kg, is a safe dose.

Thus, this was considered as Not Observed Adverse Effect Limit (NOAEL) in the 2000mg/kg dose. We selected 1/10th i.e. 200mg/kg as the higher dose, 100mg/kg as the medium dose and 50mg/kg as the low dose for further investigations in the current study. Antihyperlipidemic activity was assessed using the standard procedure given High fats Diets (HFD) Induced Hyperlipidaemia and Chlorpromazine induced hyperlipidaemia

The High Fat Diet Model involves regular Diet With 2% cholesterols+16 % Fat+0.2% deoxycholic acid. These are the 30 days model, which increase the level of cholesterol and fats. The deoxycholic acid is a secondary bile acid produced in the intestine by bacterial

metabolism of primary bile acid like cholic acid. the deoxycholic acid is reabsorbed in the intestine and transported back to liver via portal vein. The DCA inhibit the negative feedback mechanism of cholesterol to bile acid synthesis, because the DCA Which act as bile acid when increase the level of bile acid, decrease the negative feedback and increase the level of cholesterol. The DCA which also reduced the excretion of cholesterol, less cholesterol is converted into bile acid and less cholesterol is excreted from the body, this can contribute the elevated level of serum cholesterol level. In the test group We selected 1/10th i.e. 200mg/kg as the medium dose, 400mg/kg as the higher dose and 100mg/kg as the low dose for further investigations in the current study. The standard group given HFD and Fenofibrate for the 31 Days of the treatments. The control group received HFD and distilled water for the 31 days of the treatment

The chloroform extract of argemone Mexican (100mg,200mg,400mg/kg) shows decrease the levels of cholesterol (<0.001) and triglyceride (P<0.001) as compared to the normal control group.

However, the daily intake of chloroform extract of argemone Mexican at 100mg,200mg,400mg/kg during 31 days of the treatment with High fat diet, decrease the the levels of cholesterol and triglyceride as compared to the Negative control group.

In chlorpromazine induced hyperlipidaemia the chlorpromazine used as inducer drug, CPZ increase the levels of cholesterol by inhibition of enzyme involved in the cholesterol metabolism (Acyl cholesterol acetyl transferase). These are 7 days model, CPZ increase the levels of total cholesterol. In the test group We selected 1/10th i.e. 200mg/kg as the Medium dose, 400mg/kg as the higher dose and 100mg/kg as the low dose for further investigations in the current study. The standard group given CPZ and Fenofibrate for the 7days of the treatments. The control group received CPZ and distilled water for the 07 days of the treatment.

At the end of the study, the blood were collected from tail veins for biochemical estimation of cholesterol and triglycerides The chloroform extract of argemone Mexican (100mg,200mg,400mg/kg) shows decrease the levels of cholesterol (<0.001) and triglyceride (P<0.001) significantly as compared to the negative control group in chlorpromazine induced hyperlipidaemia.

Conclusion:

In conclusion, the present results suggest that the Chloroform extract of argemone Mexican seeds exerts considerable antihyperlipidemic activities in the mice model. This effect could be due to their chemical constituent like polyunsaturated fatty acid like linoleic acid, oleic acid, steric acid. These chemical constituent shows antihyperlipidemic effect due to inhibition of apoB48, catabolism of LDL, and these also increase HDL formation Therefore, the Chloroform extract of *Argemone Mexicana* could be exploited as a dietary supplement for subjects with hyperlipidaemia.

Institutional Animal Ethics Committee (IAEC): This study was conducted according to guideline of IAEC

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