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Hepato-restorative Activity of Methanolic Extract of *Argemone mexicana* Linn. (Stem and Stem Callus) in CCl_4-induced Toxicity in Albino Wistar Rats

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Abstract

Background: *Argemone mexicana,* commonly known as prickly poppy, belongs to the *Papaveraceae* family and has several medicinal properties. Liver plays a significant role in the secretion of vitamins, cholesterol and glycogen and also metabolises carbohydrates, lipids, protein and detoxifies the xenobiotics. **Aim:** This study revealed the antioxidant and hepatoprotective efficacy of the motherplant (stem) and stem callus of *A. mexicana*. **Methods:** The callus initiation of the stem explant was obtained at the concentration of 1.0mg/L N NAA +0.5mg/L BAP and 0.5mg/L IAA +1.0mg/L Kn with MS medium. Methanolic extracts of the stem and callus of *A. mexicana* were evaluated against CCl_4-induced toxicity in albino Wistar rats. **Results:** The present study deals with the hepato-restorative activity exhibited by methanolic extracts of *A. mexicana* stem and their corresponding callus against CCl_4-intoxicated albino Wistar rats (1.5 ml/kg bw) and also identified various phytocompounds through GC-MS profiling, which may be responsible for the restorative activity from hepatic-toxicity. The study demonstrated that *A. mexicana* stem and callus methanolic extracts have hepatoprotective potential at 180mg/kg bw treatment with reference to standard (silymarin 100mg/kg bw), showing a marked decline in CCl_4-induced increase in SGPT (Serum Glutamic Pyruvic Transaminase), SGOT (Serum Glutamic Oxaloacetic Transaminase), ALP (Alkaline Phosphatase), Total bilirubin and rise in CCl_4-induced decrease in Total protein compared to normal control and standard (Silymarin). Histopathology also confirmed the recovery from hepatotoxicity by treatment with standard or with stem and callus extracts of *A. mexicana*. **Conclusion:** The present study revealed the antioxidant and hepato-restorative potential of methanolic extracts of the stem and stem callus against CCl_4-induced toxicity in albino Wistar rats. The current study confirms its antioxidant and hepato-restorative potential against liver damage. These findings indicate a possible alternative cost-effective drug formulation to be developed after further experiments to identify specific compounds responsible for the restoration of the liver from potential damage.

Major Finding: Methanolic extracts of the stem and callus of *A. mexicana* demonstrated hepatoprotective activity against CCl_4-induced hepatotoxicity by reducing SGPT, SGOT, ALP, and TB, increasing TP, and gradually restoring normal liver tissue architecture.

Keywords: *A. mexicana* Stem Callus, Gas Chromatography-Mass Spectroscopy Profile, Serum Glutamic Oxaloacetic Transaminase, Serum Glutamic Pyruvic Transaminase

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1. Introduction

Liver is a vital organ of the body that regulates metabolic activity, secretion, detoxification and storage in the body, and hepatic toxicity is often associated with distortion of theses function[1]. Liver cells have many compensatory mechanisms to protect the tissue from reactive oxygen species, among these are the induction

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of antioxidant enzymes such as catalase, glutathione peroxidase, superoxide dismutase, *etc*. Oxidative stress generated due to the imbalance between the free radicals causes oxidative injury, such as liver damage, cancer, diabetes and inflammation[2]. Reactive oxygen species are generated under a physiological set of conditions and overcome by extracellular and intracellular antioxidant systems[3]. The excess production of reactive oxygen species leads to the degradation of cellular macromolecules (lipid, DNA and protein) and other small antioxidant molecules. Superoxide anion radical O_2^-, hydroxyl radical (OH), hydrogen peroxide (H_2O_2), alkoxyl (RO), hypochloric acid (HOCl) and peroxy (ROO) are important reactive oxygen species. Lipid epoxidation and other oxidative damages are produced by the activity of hepatotoxic chemicals[4]. Liver plays a pivotal role in the removal of toxic substances such as oxidative stress, drugs and xenobiotics from the circulation[5]. Cytochrome P_{450} and GSH-peroxidase are major pathways in liver metabolism. Recently, much attention has been focused on the investigation of naturally occurring compounds and their mechanism of action for hepatoprotective potential. Reliable hepatoprotective drugs are scarcely available at present; some traditional cost-effective medicines are recommended for the treatment of hepatic toxicity. Medicinal plants contain various types of phyto-molecules, including phenolics, flavonoids, terpenoids and steroids. These phytocompounds show a wide array of pharmacological activities such as anticarcinogenic, anti-inflammatory, antiviral and anti-proliferative[6]. Currently, all over the world, medicinal features of the plants are being identified due to their low toxicity, highly potent pharmacological uses and economic reliability compared to synthetic drugs[7].The pharmaceutical, chemical, and clinical studies, too, focus on orally transmitted drugs, chiefly derived from medicinal plants. Many early drugs, such as Quinines (from *Cinchona* bark), Digoxin (from Foxglove), Aspirin (from Willow Bark), Morphine (from Opium poppy), and Pilocarpine (from *Maranham jaborandi*) are used in curing different diseases/disorders in different countries. According to some reports, it is calculated that above 50% of available drugs are derived from medicinal plants[8]. *A. mexicana* is also known as Mexican weed. It is an annual herb and extensively used in the treatment of various disorders, such as leprosy, skin infection, piles, liver infection, malaria fever and inflammation. *A. mexicana* has good antioxidant compounds. Plant-derived antioxidants, such as flavonoids and phenolic compounds, are considered to contribute to the prevention of oxidative stress-induced diseases. In this study, we evaluate the hepatoprotective efficacy of methanolic extracts of the stem and stem callus of *A. mexicana* Linn. in CCl_4-induced toxicity in albino Wistar rats.

2. Materials and Methods

The calli were produced from the stem explant of the mother plant, *A. mexicana*, using MS media supplemented with 1.0mg/L NAA +0.5mg/L BAP and 0.5mg/L IAA +1.0mg/L Knby Singh and Yerramilli[9], of *A. mexicana* was from the Department of Botany, Chaudhary Charan Singh University, Meerut, Uttar Pradesh (Figure 1(a), (b)). The study material was



Figure 1. **(a).** Mother plant of *A. mexicana*; **(b).** Stem callus of *A. mexicana.*

identified by the Botanical Survey of India (BSI), Central National Herbarium, Howrah (BSI, Howrah, Calcutta, India), and deposited as brochure no. CNH/Tech. II/2022/63(Bot/Mahendra/3), BSI India.

2.1 Preparation of Methanolic Extracts from Stem (ARGS) and Callus (ARGSC)

To prepare the extracts, the stem and callus of *A. mexicana* were dried at 50°C in an oven and ground into a fine powder using an electric grinder. The thimble was filled with 1.0g of finely ground stem and callus powder, and it was run in a 95% methanolic solution at 60°C for 48 hours using a Soxhlet. The collected supernatant was filtered using Whatman no. 1.0 filter paper and lyophilised. For additional examination, the lyophilised fine powder was diluted in sterile distilled water to a concentration of 1.0 mg/ml[10].

2.2 Drugs and Chemicals Used

Standard kits for the assay of Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Alkaline Phosphatase (ALP), Total Bilirubin (TB), and Total Protein (TP) were acquired from Span Diagnostic Ltd. in Bhopal, Madhya Pradesh, while silymarin (Sigma-Aldrich, USA) and carbon tetrachloride (CCl_4, Sigma-Aldrich, USA) were donated by PBRI Bhopal.

2.3 Experimental Design

Thirty Albino Wistar rats were split up into seven groups, each with six rats, to test the hepatoprotective efficacy of methanolic extracts of stem and callus. To induce liver toxicity[11] for fourteen days, 1.5 ml/kg bw of CCl_4 diluted in olive oil (CCl_4:Olive oil:1:1) was administered orally. Methanolic extracts of the parent plant parts, callus, and silymarin medication were administered intraperitoneally. Silymarin is a naturally occurring polyflavonolignan molecule that is frequently used as a reference in both scientific and conventional medicine[12].

2.4 Dose Design

Group I (Controlas saline water): No treatment

Group II (Carbon-tetrachloride+Olive oil): 1.5ml/kg bw/day

Group III (Carbon-tetrachloride+Silymarin): 100mg/kg bw/day

Group IV (Carbon-tetrachloride+ARGS): 180mg/kg bw/day

Group V (Carbon-tetrachloride+ARGSC): 180mg/kg bw/day

Upon completing the dosage, the models (albino Wistar rats) were dissected, and the animals were euthanised by decapitation 24 hours after CCl_4 administration. Following anaesthesia, the liver was immediately removed and rinsed in cold saline at 4°C, then blotted dry. The cleaned and isolated liver portion was utilised for histopathological studies, while blood samples were collected via retro-orbital puncture and centrifuged at 3000 rpm for 10 minutes at 4°C to isolate the serum. The serum obtained was stored at -20°C for future biochemical analysis.

2.5 Experimental Animal (Models)

The experimental model (Male albino Wistar rats, 200±20g-bw) was acquired from PBRI Bhopal, India. All the rats were protected in pure polypropylene cages and maintained at 25±2°C temperature, with 12-hours light/dark cycle in pathogen free environment. Food was provided ad libitum as pellets and water. The experimental work on Wistar albino rats was started after seeking permission from the Institutional Animal Ethical Committee (Department of Zoology, C.C.S University, Meerut, U.P). The experimental study was initiated after 12 days of acclimatisation of six rats in each group. The dosages of rats (each group) were determined according to OECD guidelines, 2000[13].

3. Biochemical Assays

3.1 Estimation of Serum Glutamic Pyruvic Transaminases (SGPT) Assay

SGPT is located in the cytosol of liver cells and is also known as alanine transaminase. With an increased permeability of the cell membrane due to inflammation and liver cell stress, SGPT were liberated in the blood circulation. Therefore, an increased level of SGPT is a sign of liver damage. Pyruvate and L-glutamate were produced from the catalysis of SGPT and L-lactate by the reaction of pyruvate and NADH. Pyruvate and NADH produced L-lactate. The decrease in the value of NADH at 340nm indicates that oxidation of NADH is equivalent to the potency of SGPT action[14].

3.2 Estimation of Serum Glutamic Oxaloacetic Transaminase (SGOT)

Serum glutamic acid transaminase enzyme is found mostly in the heart and liver cells. The low value of SGOT in blood was an indication of a healthy liver, but injured liver cells or muscles showed a high level of SGOT in blood. SGOT is the most potent test for detecting liver damage due to toxic drugs, cirrhosis, alcoholism and hepatitis. SGOT level increased in injured liver cells. The detection of SGOT in a sample involves the conversion of L-aspartate into oxaloacetate, which is then reduced by malate dehydrogenase to produce malate, accompanied by the oxidation of NADH to NAD. The resulting decrease in value is measured spectrophotometrically at 340 nm[15].

3.3 Estimation of Alkaline Phosphatase Activity (ALP)

The colourless p-nitrophenyle phosphate hydrolyses into yellow colored p-nitrophenol and phosphate by alkaline phosphatase due to the oxidising agent Mg^{++} at pH-10.3. The reaction is measured as absorbance at 405nm and is proportional to ALP activity in sample[16].

3.4 Estimation of Total Bilirubin

According to the Jendrassik and Grof method, serum was determined by coupling with diazotised sulphanilic acid after addition of sodium benzoate, sodium acetate, caffeine and blue Azobilirubin is formed in alkaline Fehling solution II. The absorbance was measured against a blank using a spectrophotometer at 546nm wave length[17].

3.5 Estimation of Total Protein

The peptide bonds in proteins interact with copper ions, resulting in a blue-violet complex in an alkaline solution (Biuret reaction). Each copper ion forms complexes with either 5 or 6 peptide bonds. The addition of tartrate serves as a stabiliser until iodide is introduced to inhibit the auto-reduction of the alkaline copper complex, and the resulting colour intensity is proportional to the concentration of protein. The absorbance was recorded at a wavelength of 546 nm, referenced against a blank[18].

3.6 Histopathological Analysis

After the experimental model was anaesthetised, blood samples were obtained via retro-orbital puncture, and

Table 1. Selected phytocompounds identified by GC-MS from methanolic extracts of ARGS and ARGSC

Sr. No.	Name of the Compounds	Chemical Structure	Retention Time (RT)	Molecular Mass (mg/mol)	Area (% Content)	
					Stem	Stem Callus
ARGS						
1	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy	$C_{18}H_{28}O_3$	18.053	292	2.83	2.83
2	Stigmast-5en-3ol, (3-Beta)	$C_{29}H_{50}O$	32.185	414	4.08	5.70
3	10-Nonadecanol	$C_{19}H_{40}O$	32.493	284	71.08	
4	Clionasterol	$C_{29}H_{50}O$	36.902	414	9.11	
5	Benzenepropanoic acid, 3,5-bis (1,1-dimethylethyl)-4-hydrox	$C_{18}H_{28}O_3$	18.053	292	2.83	
6	Dibutyl phthalate	$C_{16}H_{22}O_4$	18.343	278	2.55	2.55
ARSC						
7	1,3-propanediol,2 (hydroxymethyl)-2-nitro-	$C_4H_9NO_5$	12.862	151		25.88
8	Oxalic acid, monoamide, N-allyl-dodecyl ester	$C_{17}H_{31}NO_3$	14.531	297		5.40
9	Squalene	$C_{30}H_{50}$	28.298	410.7		1.29
10	Vitamin E	$C_{29}H_{50}O_2$	32.867	430		0.66
11	3A,4,5,5B,7A,12A,12B,13,14,14A,14B-Dodecahydro	$C_{20}H_{17}NO_4$	33.786	335		38.20
12	D:A-friedoolean-6-ene	$C_{30}H_{50}$	36.541	410		11.5

the liver was immediately excised and immersed in a 10% formalin solution for histopathological analysis. The tissues underwent dehydration through a series of alcohols, followed by clearing with toluene and infiltration with molten paraffin wax for a specified duration. Once processed, the tissues were embedded in fresh paraffin wax and allowed to set. The embedded tissues were sliced and stained with eosin for structural identification purposes. The stained tissues were dehydrated using a series of alcohols, then cleared in xylene, and ultimately mounted in Canada balsam. The prepared slides were examined under a microscope with a 10X objective lens. Finally, the stained slides were scrutinised microscopically for histopathological alterations[19].

3.7 Statistical Examination

All the research results were compared as Mean ± Standard deviation and analysed through the statistical package program version (SPSS-25.0). Bonferroni test significant level has been expressed as **P<0.001.

4. Results and Discussion

Phenolics are a major class of secondary metabolites that are found in free or bound form in plants[20,21]. Phenolic and flavonoid compounds are known as antioxidants that can reduce the capacity of free radicals[22]. Flavonoids, being polyphenolic[23] have several Hydroxyl groups, with free radical scavenging properties (antioxidant properties). The previous screening of phenolics and flavonoids of *A. mexicana* (stem and callus) revealed the antioxidant potential[9]. GC-MS profiling showed the presence of a variety of compounds such as aromatic hydrocarbons, phenolics, flavonoids, terpenoids, alcohol, fatty acid, ester and aldehydes, *etc.* In GC-MS analysis, forty-seven compounds were identified in methanolic extracts of the callus and stem of *A. mexicana*. GC-MS identified compounds exhibited various phytochemical activities and may be predominantly responsible for the antioxidant and hepatoprotective activity; some significant phytocompounds are listed in Table 1[9].

In all the test samples (callus and stem), most of the phytochemicals were different, but some phytochemicals were common with low or high amounts. Those compounds which are present in highest quantity in callus extract were 3A, 4, 5, 5B, 7A, 12 A, 12B,13,14,14A,14B-Dodecahydro (38.20%); 1,3-propanediol, 2-(hydroxymethyl)-2-nitro- (25.88%); Oxalic acid, monoamide, N-allyl-dodecyl ester (5.40%); D:A-friedoolean-6-ene (11.35%); Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydrox (2.83%) and Squalene (1.29%), whereas stem of *A. mexicana* showed high area% of 10-Nonadecanol (70.25%), Gamma-sitosterol (9.11%), Oxirane-hexadecyl- (4.01%), Phytyl decanoate (4.01%), 3,4-Dimethoxy-6-methyl5,7,8,15tetrahydrob (1.58%), 26,27 Dinorcholesta-5,22-Dien-3-ol, (3-beta, 22 E (1.21%). Both tissues (stem and callus) showed common compounds in the extracts such as Stigmast-5en-3ol, (3-beta.)-; Dibutyl phthalate; Benzenepropanoic acid, 3,5-bis (1,1-dimethylethyl)-4-hydrox and 1(2H)-nepthhalenone, octahydro-4a, 8a-dimethyle-7(1-methyle) which exhibited high and low area % in callus and stem genotypes of *A. mexicana*[24]. 1,3-propanediol,2-(hydroxymethyl)-2-nitro-; squalene; dibutyl phthalate; 6-alpha-cadina-4,9-diene,9,(-)-; stigmast-5-en-3-ol, (3.beta.)-; vitamin E; Neophytadiene; Dibutyl phthalate; Phytol; dioctyl phthalate; Stigmast-5en-3ol, (3-beta.); gamma-sitosterol etc. (NIST and PUBCHEM),1,3-propanediol,2-(hydroxymethyl)-2-nitro-; Stigmast-5-en-3-ol, (3-beta.)-; vitamin-E (Table1), which are useful in the treatment of diseases as antimicrobial, antioxidant, anti-inflammatory, anti-arthritic, antiasthma, diuretic, analgesic, anti-diabetics, anti-dermatitis, antileukemic, antitumor, anticancer, hepatoprotective, anti-spasmodic agents[24]. Squalene and 1,2-benzendicarboxylic acid, diisooctyls and valeranone are also good antioxidant[21]. All the compounds of callus were pharmacologically very important because earlier research is evident that these compounds are most important for drug development. Callus of *A. mexicana* GC-MS profiling revealed the various novel antioxidant compounds, which are used in curing various acute and chronic diseases (liver injury, cancer, *etc.*). 1,3-propanediol,2-(hydroxymethyl)-2-nitro- or isobutylglycerol, nitro-; squalene; dibutyl phthalate; 6-alpha-cadina-4,9-diene,9,(-); stigmast-5-en-3-ol, (3.beta.)-; vitamin E; 1(2H)-Naphthalenone, octahydro-4a, 8a-dimethyle-7(1-methyle)/valeranone; 1,2-benzendicarboxlic acid and diisooctyles are chief bioactive compounds of antioxidants in methanolic extract of callus (Figure 2).

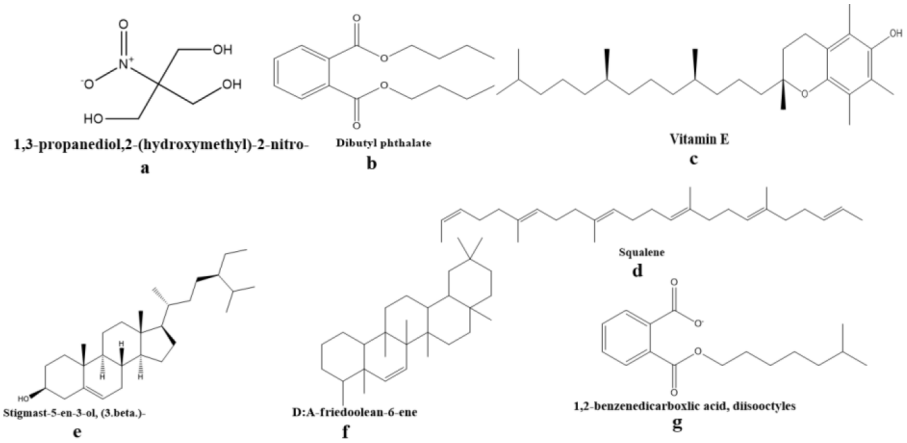


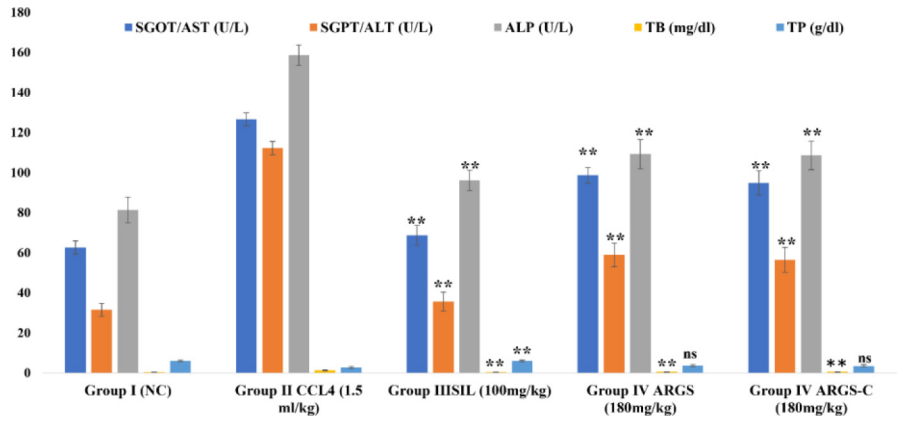
Figure 2. Structure of some valuable novel bioactive compounds from calli of *A. mexicana;* **(a).** 1,3-propanediol,2-(hydroxy)-2-nitro$^-$; **(b).** Dibutyl Phthalate; **(c).** Vitamin E; **(d).** Squalene; **(e).** Stigmast-5-en-3-ol (3-beta); **(f).** D:A-friedoolean-6-ene; **(g).** 1,2-benzenedicarboxylic acid, diisooctyl.

10-Nonadecanol is a major alcoholic bioactive compound, which has a maximum area 71.08%, but its biological activity is not found in literature (Table 1). It may be useful in treating certain disorders. Nonane,3,7-dimethyl- is an ester bioactive compound which has the potential to protect from various disorders like antitumor and antimicrobial activity because it is a good antioxidant[25]. Bicyclo (7.2.0) undec-4-ene,4,11,11-trimethyl-8-/Beta-caryophyllene has the lowest area 0.43%, but interestingly most important sesquiterpene phytochemical; however, earlier research revealed that it is important for various pharmacological activities like neuroprotective effect, nephroprotective, anti-microbial, immune modulatory activity, hepatoprotective, gastro protective, anticancer, cardio protective, anti-inflammatory and antioxidant[26]. 1(2H)-Naphthalenone, octahydro-4a,8a-dimethyl-7(1-methyl)/valeranone was also known as "valerian" which has been used as a remedy for epilepsy, insomnia and tranquilizer[27]. Gamma-sitosterol/ Clionasterol, stigmast-5en-3ol, (3-beta.) exhibited an important role in various disorders like antibacterial, anti-inflammatory, anti-hypercholesterolemic, anti-protozoal (Leishmania, Lipid metabolism regulator, Antiparasitic, Anti-helmintic[28], while stigmast-5en-3ol, (3-beta.) also known as β-Sitosterol, is a phytosterol. Stigmast-5en-3ol, (3-beta)- is mainly used as cholesterol lowering agent, and earlier studies revealed other uses of stigmast-5en-3ol, (3-beta) like antioxidant activity, prostatic enlargement and reducing risk of cancer[29]. Neophytadiene, Benzenepropanoic acid, 3,5-bis (1,1-Dimethylethyl)-4-hydrox and dioctyl phthalate are also very important compounds which have the capacity to defense from following disorders because it is a good antioxidants. Today, we need the discovery of new strong antioxidants with low toxicity and high efficiency in the field of medicinal chemistry. In the GC-MS report, noble phytochemicals were exhibited, which have a significant role in antioxidant activities.

4.1 Hepatoprotective Potential

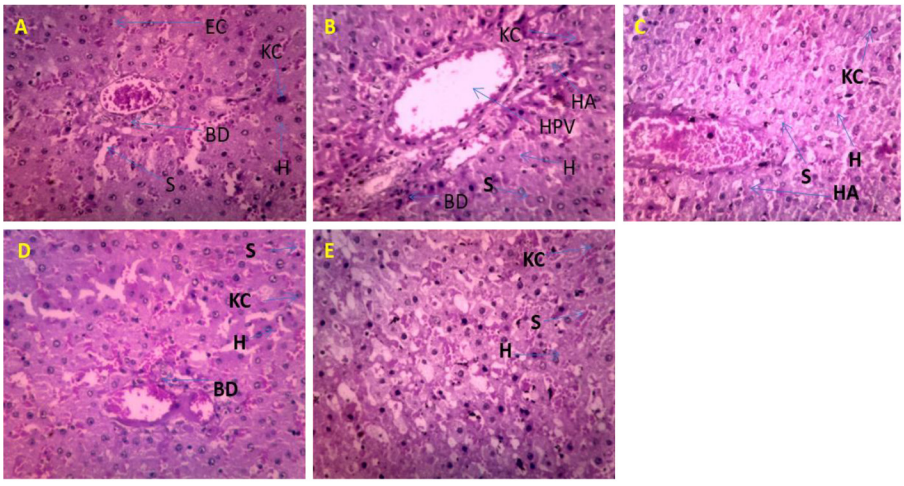
The liver is a vital organ of our body that helps in metabolism and detoxification of xenobiotic agents[30]. Several exogenous and endogenous agents, such as anabolic steroids, alcohol and myriad other causes, can lead to hepatocellular damage, consequently liver disease[31]. CCl_4 is a common hepatic agent that causes liver damage in experimental animals, and the hepatoprotective action of natural components against CCl_4-induced rat[32] liver injury/fibrosis is often caused by viral hepatitis in animals[33]. $CCl_4$is received as a foreign agent and reacts with monooxygenase P_{450}, CCl_4 is metabolised by P_{450} and releases free radicals ($CCl_3^{\bullet}$+$CCl_3OO^{\bullet}$). CCl_4 caused oxidative stress and started oxidation of unsaturated lipids[34]. Oxidised unsaturated fatty acid is a confirmation of hepatic injury. CCl_4 caused injury in hepatocytes of cells and resulted in leakage of hepatic enzymes[35]. CCl_4 generates free radicals and binds with cellular molecules; these free radicals cause lipid peroxidation and affect the fraction of mitochondrial permeability and endoplasmic reticulum, which leads to hepatic

injury[36]. In this investigation, we have studied the hepatoprotective effect of *A. mexicana* stem and stem callus in albino Wistar rats against the CCl_4 agent. The albino Wistar rats treated with CCl_4 (1.5ml/kg/bw) once per day for fourteen days, after fourteen days, showed hepatic injury and fibrosis in rats, as confirmed by the hepatic markers. In Wistar rats, hepatotoxicity was confirmed by the increased levels of SGOT, SGPT and ALP activity of hepatic cells. SGOT and SGPT are significant markers to recognise the injury of the liver, as reported by Khadijah *et al.*[37], in rats. The structural pattern of hepatic tissue and cellular damage of cells that have been treated with CCl_4 is seen in Figure 4. Atawia *et al.,*[38] reported that an increased level of SGOT and SGPT was an invariable finding in the CCl_4-induced rats. SGOT and SGPT are cytoplasmic amino transferase enzymes released extracellularly due to damaged hepatocytes[39]. The secretion of SGOT and SGPT is strongly recovered by methanolic extracts of the stem and stem callus in serum (Figure 3). These findings showed the effective results as reported by previous studies that exhibited administration of plant extracts and drugs significantly recovered the SGOT and SGPT levels in serum in hepatotoxic rats[40]. The leakage of lipid peroxidation is a result of free radicals that are responsible for the damage of the cell membrane by marker enzymes, which indicate liver toxicity as reported by Liu *et al*[41]. Hamed *et al.,*[36] reported that an elevated level of TBAR (hepatic thiobarbituric acid reactive substance) in hepatic cells indicates hepatic damage in rats. The products of CCl_4 react with polyunsaturated fatty acids and formed covalently attached with proteins and lipids, causing lipid peroxidation and destruction of hepatocytes[42]. Treatment with stem and stem callus methanolic extracts recovered from the lipid peroxidation, which could be a result of antioxidant compounds that are present in extracts of stem, leaf and stem as reported by Ghalia *et al*[43]. Phenolic and flavonoid are potent antioxidants that are abundantly found in the stem and stem callus extracts, which increase the process of regeneration of the integrity of lipid membrane[36]. The increased level of markers (SGOT, SGPT and AST) in CCl_4-treated rats was inhibited by stem and stem callus of *A. mexicana* as reported by Bouhrim et al.,[10] in *Opuntia dillenii* seed oil. Heinonen *et al.,*[44] reported that phytocompounds of higher and lower plants have potential against chronic and degenerative diseases. ALP and TB levels are indicators of necrosis and biliary pressure of liver dysfunction. The increased level of TB shows hepatobiliary disease and structural changes in liver tissue, while the ALP level increases with an increased level of biliary pressure[45]. In the hepatotoxic rat, increased serum TB concentration may be related to CCl_4- induced liver toxicity that is caused by impaired uptake and secretion of bile[46]. Reduction in protein levels of CCl_4-induced rats indicates a decrease in the number of hepatic cells, which may lead to a diminished hepatic ability to synthesise protein[47]. In this investigation, CCl_4 elevated the level of SGOT, SGPT and ALP in hepatotoxic rats; however, treatment of ARGS and ARGSC extracts, at a 180mg/kg dose,



SGPT: Serum glutamic pyruvic transaminases; SGOT: Serum glutamic oxaloacetic transaminase; ALP: Alkaline phosphatase activity; TB: Total bilirubin; TP: Total protein; NC: Normal control; Corbon tetra chloride; SIL: Silymarin: ARGS; *Argemone mexicana* stem: ARGS-C; *Argemone mexicana* stem callus (180mg/kg); Bonferroni test significant level has been expressed as **P<0.001.

Figure 3. Hepatoprotective potential of ARGS and ARGSC.



(A). Normal architecture incontrol groups. **(B).** The CCl_4-treated group shows abnormalityin hepatocytes and parenchymal cells. **(C).** Silymarin-treatedgroups showed normal architecture as the normal control. **(D).** ARGS; *Argemone mexicana* stem and **(E).** ARGSC; *Argemone mexicana* stem callus; Representative section treated with ARGS and ARGSC extracts that show normal cell architecture. Kupffer cell (KC); Hepatocytes (H); Sinusoids (S); Hepatic artery (HA); Central vein (CV); Epithelial cell (EC); Bile duct (BD); Hepatic portal venule (HPV); Branch of hepatic artery (BHA).

Figure 4. Effects of methanolic extracts of the stem and callus stem of *A. mexicana* on structural variation in liver tissue of control and treated groups of experimental models.

significantly recovered the enzyme activity in CCl_4-induced rats (Figure 3). Silymarin showed significant recovery in hepatotoxicity-induced enzymes (SGOT, SGPT and AST) with respect to the negative control. TP and TB decide the secretion and synthetic function of liver tissue and help to examine liver injury[48]. ARGS and ARGSC (180mg/kg) methanolic extracts significantly recover and reduce the liver injury in rats; therefore, improvement in excretion, secretion, and synthesis of liver cells during the experiments is noted.

The reduced level of total protein showed chronic liver damage, and recovery of the protein synthesis has been regarded as a stimulation of the synthesis of liver cells, which assures the promotion of hepatocytes[49]. The TP significantly increased in the silymarin-treated group but insignificantly increased in the ARGS and ARGSC extract-treated groups within 14 days due to crude extract instead of any purified compound (Figure 3). Stem and callus extracts (methanolic) stimulated the protein synthesis and renewal of hepatic cell production as reported by Khadijah *et al.*,[37] in (-) Epicatechin. The increased activity of enzymes such as SGOT, SGPT, and ALP and the hepatoprotective value of flavonoids and phenolics are characteristics of their antioxidant potential, which indicates oxidative stress and other inflammatory replication that are affected by phytocompounds[49].

The histopathological examination was supported by this study because ARGS and ARGSC extracts (methanolic) were found to gradually restore normal liver tissue architecture at a 180mg/kg dose (Figure 4). CCl_4 caused toxicity in liver tissue, as proved by extensive destruction of hepatocytes and their integrity, but ARGS and ARGSC methanolic extracts managed well the CCl_4-induced toxicity as seen in histopathological results. Our study revealed that extracts of the stem and stem callus significantly regenerate liver injury with hepatocytes, which are similar to the normal cell architecture. Silymarin protects the liver cells and shows normal cell architecture in the histopathological results of CCl_4-induced rats. Silymarin is a potent antioxidant bio-compound that is commonly used in the treatment of liver diseases and is chemically a flavonolignan[50]. Our investigations show that ARGSC has higher potential than the ARGS dose for hepato-restorative activity (Figure 4).

5. Conclusion

The present study revealed the antioxidant and hepato-restorative potential of methanolic extracts of the stem and stem callus of *A. mexicana* L. against CCl_4-induced toxicity in albino Wistar rats. The current study confirms

its antioxidant and hepato-restorative potential against liver damage. GC-MS analysis revealed that *A. mexicana* L. methanolic extract is a rich source of antioxidant and hepatoprotective metabolites. The methanolic extract of the stem callus showed higher efficacy than the stem of *A. mexicana* against hepatic injury. These findings indicate a possible alternative cost-effective drug formulation to be developed after further experiments to identify specific compounds responsible for the restoration of the liver from potential damage.

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7. Ethical Statement

All the researchwork was started after seeking consent from the Institutional Animal Ethical Committee (IAEC), Department of Zoology, Chaudhary Charan Singh University, Meerut, Uttar Pradesh.

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Author Contribution

Vimala Y: Conceptualisation, writing manuscript; Mahendra S: Experimental study; Ishwar Singh and Laxman Nagar: Analysed data and statistical analysis. All authors read and agreed to the publication of menuscript.