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INVESTIGATION ON NOVEL ROLE OF *SOLANUM XANTHOCARPUM* AND *JUNIPERUS COMMUNIS* EXTRACT AGAINST CCl_4 INDUCED LIVER INJURY

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ABSTRACT: Background: There are very less therapeutic and reliable liver protective drugs in modern medicine to prevent and treat the liver injury caused due to drugs. Traditionally *Solanum xanthocarpum* Schradt. (SX) and Wendl. and *Juniperus communis* Linn. (JC) have been used since long for their hepatoprotective effect. The current study was carried out to explore the hepatoprotective role of SX and JC against liver toxicity in rodents induced by carbon tetrachloride (CCl_4). **Materials and Methods:** The antioxidant potential of hydroalcoholic extract of SX and JC were investigated by using established DPPH *in-vitro* assay method. The hydroalcoholic extract of SX and JC at doses (200 and 400 mg/kg b.w.) were studied for their liver protective activity against hepatic damage induced by CCl_4 in Wistar rats. An assay of the oxidative stress parameters, alkaline phosphatase, transaminase, total bilirubin, albumin and liver histopathology were performed to evaluate the hepatoprotective activity of SX and JC. The assay results were introduced as the standard error ofthe mean (SEM) for each group. **Results:** The ethanolic extract of SX and JC has shown the potent antioxidant scavenging *in-vitro* activity *viz*. DPPH IC_{50} of 69.41 ± 0.76 µg/ml, 117 ± 0.34 µg/ml respectively. The hydroalcoholic extract of SX and JC showed a significant reduction in oxidative stress parameters and improved antioxidant and liver enzymes level as compare to toxicant treated rats. Moreover, histopathological studies also revealed the similar results which supported the liver protective activity of SX and JC herbal extracts. **Conclusion:** It is concluded that SX and JC showed significant hepatoprotective activity in CCl_4 induced hepatotoxicity in rodents. The promising mechanism for their therapeutic activity is due to their antioxidant and liver protective activity which scientifically supports their traditional use.

INTRODUCTION: Hepatic diseases are among the serious and common diseases occurring worldwide even with the advancement in modern medicine, their treatment and prevention options always have a scope.

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Oxidative stress and inflammation are seen to be responsible for hepatic diseases. The liver is the important organ of the body involved in the elimination of drug and toxins. The major culprits of the liver toxicity are antibiotics, alcohol consumption, antitubercular drugs, malnutrition, infections, and other metabolic disorders.

Liver diseases progression is characterized from steatosis to chronic hepatitis, fibrosis, cirrhosis, and maybe hepatocellular carcinoma resulting in high morbidity and mortality rate [1].

The continuous researches on the management of the liver disease are required for the treatment in modern system of medicine [2]. Drugs of natural origin with antioxidant properties are widely accepted and being used in developing a world for the prevention and treatment of hepatic disorders [3]. It is considered to be inexpensive, safe and recommended for the treatment of liver disorder with very fewer side effects [4].

The experimental rodent's model of hepatotoxicity can be developed by alcohol, paracetamol, CCl_4, *etc*. For induction of liver fibrosis and hepatotoxicity experimentally, carbon tetrachloride CCl_4 is the most commonly used hepatotoxic agent [5, 6]. CCl_4 is converted into metabolite trichloromethyl radical ($^0CCl_3$) and peroxy trichloromethyl radical ($^0OOCCl_3$) by cytochrome P4502E1. A free radical derivative of CCl_4 induces and accelerates lipid peroxidation which ultimately causes liver injury [7, 8]. In this study universally accepted CCl_4 model for hepatotoxicity was selected to determine the hepatoprotective activity of SX and JC.

Solanum xanthocarpum Schrad. and Wendl. (Solanaceae) also known as Yellow Berried Nightshade (Kantkari), is a perennial herb found throughout India. This plant is reported to contain steroidal saponins in the form of sterols, glycoalkaloids, terpenoids, flavonoids, phenolic, tannins [10]. The fruits are known to have anthelmintic, laxative, urinary stone treating, aphrodisiac [9], anti-inflammatory [11], antinociceptive [12], spasmolytic [13], antioxidant [14], hepatoprotective [15] and diuretic [16] activities.

Juniperus communis Linn. (Cupressaceae) is a coniferous shrub which is widely distributed across the Himalayas from Kumaon at an altitude of 1700-4200 m [17, 18]. The plant is reported to contain various phytoconstituents like volatile oil, flavonoids, and coumarins [21]. JC has been reported to be used traditionally for the cure of bronchitis [19] and tuberculosis [20] which is common lung disorders. JC is reported to have anti-inflammatory, antipyretic, analgesic [21] and antimicrobial [22] activities.

However, there is no any report yet demonstrated on comparative studies of *Solanum xanthocarpum* and *Juniperus communis* for effective liver protective potential. So, the present study was designed to evaluate the hepatoprotective potential of two medicinal plants against CCl_4 induced hepatotoxicity in rats.

MATERIALS AND METHODS:

Chemicals and Reagents: L-ascorbic acid, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was procured from (Sigma-Aldrich Co., Mumbai). Carbon tetrachloride (CCl_4), Silymarin, Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), Ethylenediaminetetraacetic acid (EDTA), was purchased from Sigma Aldrich, Co., Mumbai. The diagnostic kits for Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total protein (TP) and total bilirubin (TB) were purchased from Calkine and coral private Limited.

Plant Material: *Solanum xanthocarpum* Schrad. and Wendl. (whole plant) was collected from (Dehradun) Uttarakhand, India. *Juniperus communis* Linn. (whole plant) was also collected from the local area of (Ranikhet) Uttarakhand, India in August to November 2016 and authenticated from Botanical Survey of India, Dehradun. A voucher specimen of the plants was deposited in the herbarium (115218A, 11521B).

Preparation of Plant Extract: The whole plant of SX and JC were dried and powdered. Powdered material (800g) was macerated with petroleum ether; the marc was extracted by a continuous hot extraction process using soxhlet apparatus using 80% v/v ethanol. The extract was separated by filtration and concentrated under reduced pressure and then dried in a lyophilizer (Labconco, USA). The yields obtained were 178.20 g and 198.10 g of solid residue (yield 22.27% w/w and 24.76% w/w respectively).

Phytochemical Screening: The hydroalcoholic extract of SX and JC were qualitatively tested for the presence of phytochemicals as per described standard methods [23-25].

In-vitro Free Radical Scavenging Activity:

DPPH Radical Scavenging Activity: The antioxidant activity of hydroalcoholic extract of SX and JC were assessed by determining its ability to scavenge free radicals. 1, 1-Diphenyl-2-

picrylhydrazyl (DPPH) is a stable free radical [26]. The 0.1mM solution of DPPH was prepared in methanol. Then, 1ml of this solution was added to 2ml of test drug solution at different concentration (50-250 µg/ml). The mixture was agitated continuously further allowed to stand at room temperature for 30 min. Then, its absorbance was measured at 517 nm as standard Ascorbic acid was used. The percentage of scavenging activity was determined using the following formula:

$$\text{Percentage of inhibition (\%)} = [(A_{control} - A_{sample} / A_{control})] \times 100$$

Where, $A_{control}$ - absorbance of DPPH, A_{sample} - absorbance of DPPH with test sample [27].

***In - vivo* CCl_4 Induced Hepatotoxicity in Rats:**

Experimental Animals: Young Wistar rat (180-200g) breed in the Central Animal House, SBSPGI, Balawala, Dehradun, (India) was used in the study. Animals were acclimatized to laboratory conditions at room temperature before experimentation and kept under standard conditions of a 12 h light/dark cycle with food and water *ad libitum* in polyacrylic cages. All the experiments were performed between 09.00 and 16.00 h. The experimental protocol has been approved by the Institutional Animal Ethics Committee (IAEC) of college (IAEC/ CPCSEA/2016/101) and carried out as per the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India on animal experimentation.

Experimental Protocol and Procedure: The Rats were divided into seven experimental groups consisting of six animals (n=6) in each group.

Group I received distilled water containing 0.5% Sodium Carboxymethyl cellulose (CMC-Na) (1ml/kg body weight, p.o.) for 7 days, and olive oil (1ml/kg body weight, s.c.) on days 2nd and 3rd.

Group II (CCl_4) received 0.5% CMC-Na (1ml/kg body weight, p.o.) for 7 days, and a 1:1 mixture of CCl_4 and olive oil (2mL/kg body weight, s.c.) on days 2nd and 3rd.

Group III was treated with the standard drug silymarin (50mg/kg body weight, p.o.) daily for 7 days, and also received the CCl_4–olive oil mixture (1:1, 2ml/kg body weight, s.c.) on days 2nd and 3rd, 30 min after administration of silymarin.

Groups IV-VII (test group animals) was administered orally hydroalcoholic extract of SX and JC at the dose of (200 and 400 mg/kg body weight, p.o.) for 7 days respectively. Additionally, 30 min after administration of test drug, they received a dose of CCl_4-olive oil mixture (1:1, 2 ml/kg, s.c.) on 2nd and 3rd day.

On the 7th day, animals were anesthetized by thiopentone sodium (45-50 mg/kg, i.p.), blood was collected, allowed to clot, and serum was separated for assessment of enzyme activity. The rats were sacrificed by bleeding; the livers were carefully dissected then removed and rinsed with ice-cold isotonic saline then kept on ice. The liver was separated and weighed. A 10% (w/v) tissue homogenates were prepared in 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged at 10,000 × g for 15 min, and aliquots of the supernatants were separated and used for biochemical tissue estimation. Some parts of the liver tissue were immediately transferred into 10% formalin for histopathological investigation [28, 29].

Estimation of Serum Biochemical Parameters: Biochemical parameters were assayed according to standard methods. Estimation of the serum biochemical parameters like Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Alkaline phosphatase (ALP) [30], and Total bilirubin (TB) [31] was measured using commercial enzymatic biochemical diagnostic kits.

Estimation of Tissue Biochemical Parameters:

Measurement of Lipid Peroxidation: The extent of lipid peroxidation in the liver was determined quantitatively by performing the method as developed by Ohkawa *et al*., 1979. The amount of malondialdehyde (MDA) was measured by reaction with thiobarbituric acid at 532 nm using Shimadzu spectrophotometer (Japan). The values were calculated using the molar extinction coefficient of the chromophore (1.56×10^5 M^{-1} cm^{-1}) and expressed as a percentage of control [32].

Measurement of Nitrite: The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide was determined by a colorimetric assay with Greiss reagent (0.1% N-(1-Napththyl) ethylenediamine dihydrochloride, 1%

sulphanilamide and 5% phosphoric acid). Equal volumes of the supernatant and Greiss reagent were mixed, and the mixture was incubated for 10 min at room temperature in the dark. The absorbance was measured at 540 nm using Shimadzu spectrophotometer (Japan). The nitrite concentration in the supernatant was determined from a sodium nitrite standard curve and expressed as a percentage of control [33].

Measurement of Reduced Glutathione: Reduced glutathione was estimated according to the method by Ellman 1959. 1ml supernatant was precipitated with 1 ml of 4% sulphosalicylic acid and cold digested for 1 h at 48 °C. The samples were then centrifuged at 1200 × g for 15 min at 4 °C. To 1 ml of the supernatant obtained, 2.7ml of phosphate buffer (0.1 mmol/l, pH 8) and 0.2 ml of 5, 5'dithio-bis (2-nitrobenzoic acid) (DTNB) was added. The yellow color developed was measured at 412 nm using Shimadzu spectrophotometer (Japan). Results were calculated using molar extinction coefficient of the chromophore (1.36×10^4 $(mol/l)^{-1}cm^{-1}$) and expressed as a percentage of control [34].

Measurement of Catalase: Briefly, the assay mixture consisted of 12.5mM H_2O_2 in phosphate buffer (50mM of pH7.0) and 0.05 ml of supernatant from the tissue homogenate (10%) and the change in absorbance was recorded at 240 nm. Results were expressed as mM of H_2O_2 decomposed per milligram of protein/min [35].

Measurement of Protein Content: The protein content was estimated by the Biuret method using bovine serum albumin as a standard [36].

Histopathological Studies: Liver tissues were fixed in 10% formalin for at least 24 h, embedded in paraffin, and cut into 5μm-thick sections using a rotary microtome. The sections were stained with eosin methylene blue dye and observed under a microscope (Olympus, Japan) to observe histopathological changes in the liver.

Statistical Analysis: All experiments were done in triplicate and results were reported as mean ± S.E.M. (n = 6). The data analyzed was done by one-way ANOVA, and statistically significant effects were further analyzed by means comparison using Tukey's multiple comparison analysis. The $p<0.05$ was considered to be statistically significant.

RESULTS:

Phytochemical Screening: Preliminary phytochemical screening of hydroalcoholic extract of SX and JC are shown in **Table 1**.

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF HYDROALCOHOLIC EXTRACTS OF *SOLANUM XANTHOCARPUM* AND *JUNIPERUS COMMUNIS*

Class of compound	Hydroalcoholic extract of plants	
	Solanum xanthocarpum	***Juniperus communis***
Carbohydrates	+	-
Glycosides	+	+
Amino acids	+	-
Proteins	+	-
Steroids and triterpenoids	++	++
Alkaloids	+	+
Phenolic compound and Tannins	++	++
Flavonoids	+	+
Saponins	+	-

(+) = Positive, (-) = Negative

In-vitro Free Radical Scavenging Activity:

DPPH Radical Scavenging Activity: The antioxidant activity of *Solanum xanthocarpum* and *Juniperus communis* were determined by its capacity to scavenge DPPH radical. The hydroalcoholic extract of SX and JC showed DPPH radical scavenging activity with an IC_{50} of 69.41 ± 0.76 μg/ml, 117 ± 0.34 μg/ml respectively. Ascorbic acid (IC_{50} 24.14 ± 0.16 μg/ml) showed an excellent activity. The activity of hydroalcoholic extract of SX has significantly higher free radical quenching capacity when compared to the hydroalcoholic extract of JC are shown in **Fig. 1**.

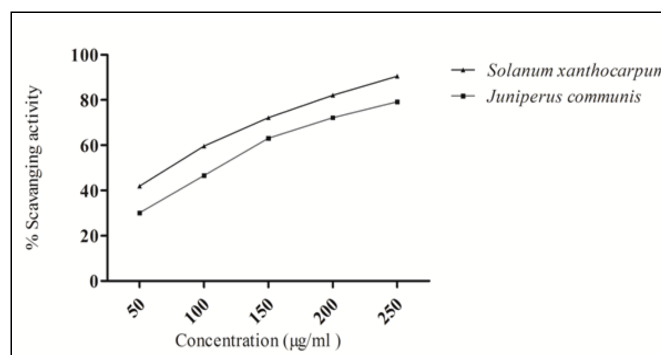

FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY OF DIFFERENT CONCENTRATION OF *SOLANUM XANTHOCARPUM* AND *JUNIPERUS COMMUNIS*
Values expressed as means ± SEM

***In-vivo* CCl_4 Induced Hepatotoxicity in Rats: Effect of Hydroalcoholic Extract of *Solanum xanthocarpum* and *Juniperus communis* on Hepatic Markers:** The hepatoprotective effect of hydroalcoholic extract of *Solanum xanthocarpum* and *Juniperus communis* were assessed by measuring liver enzymes biochemically. The biochemical parameter like SGOT, SGPT, ALP, and serum bilirubin were significantly (P<0.05) elevated and as compared to control group indicating liver damage. However, rats treated with hydroalcoholic extract of SX and JC at the dose of 200, 400 mg/kg shown significant (p<0.05) decrease in the levels of liver enzymes SGOT, SGPT and ALP in the CCl_4-treated rats suggesting their hepatoprotective potential. A hydroalcoholic extract of SX showed a more significant effect to reduce the SGOT, SGPT, ALP and bilirubin levels **Table 2**.

TABLE 2: EFFECT OF *SOLANUM XANTHOCARPUM* AND *JUNIPERUS CUMMINUS* ON BIOCHEMICAL PARAMETERS OF CCl_4 DAMAGED LIVERS IN RATS

Groups	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	TB (mg/dl)	Protein (gm/dl)	Albumin (gm/dl)
Normal-control	30.5±1.5	32±1.2	145.25±1.49	0.7±0.09	6.425±0.08	3.975±0.1
CCl_4-control	106.75±6.9^{a}	195±7.5^{a}	428.5±60.0^{a}	1.0475±0.007^{a}	3.95±0.3^{a}	3.725±0.2^{a}
Silymarin (50mg/kg)	43.75±3.7	57.25±3.7	147.75±2.2	0.615±0.06	6.725±0.2	3.95±0.2
SXE(200mg/kg)	61.25±2.3^{b}	142.25±10.3^{b}	155.5±3.6^{b}	0.7125±0.01^{b}	5.675±0.1^{b}	3.775±0.3^{b}
SXE (400mg/kg)	58.5±4.2^{b,c}	70.75±4.8^{b,c}	146.25±1.6^{b,c}	0.4±0.04^{b,c}	6.6±0.2^{b,c}	4.375±0.08^{b,c}
JCE (20mg/kg)	89.5±7.7^{b}	181.25±9.03^{b}	214±29.7^{b}	0.7625±0.02^{b}	6.15±0.3^{b}	3.45±0.1^{b}
JCE (400mg/kg)	72.9±1.4^{b,c}	127.75±11.3^{b,c}	149.5±3.4^{b,c}	0.4975±0.03^{b,c}	5.675±0.2^{b,c}	4.12±0.1^{b,c}

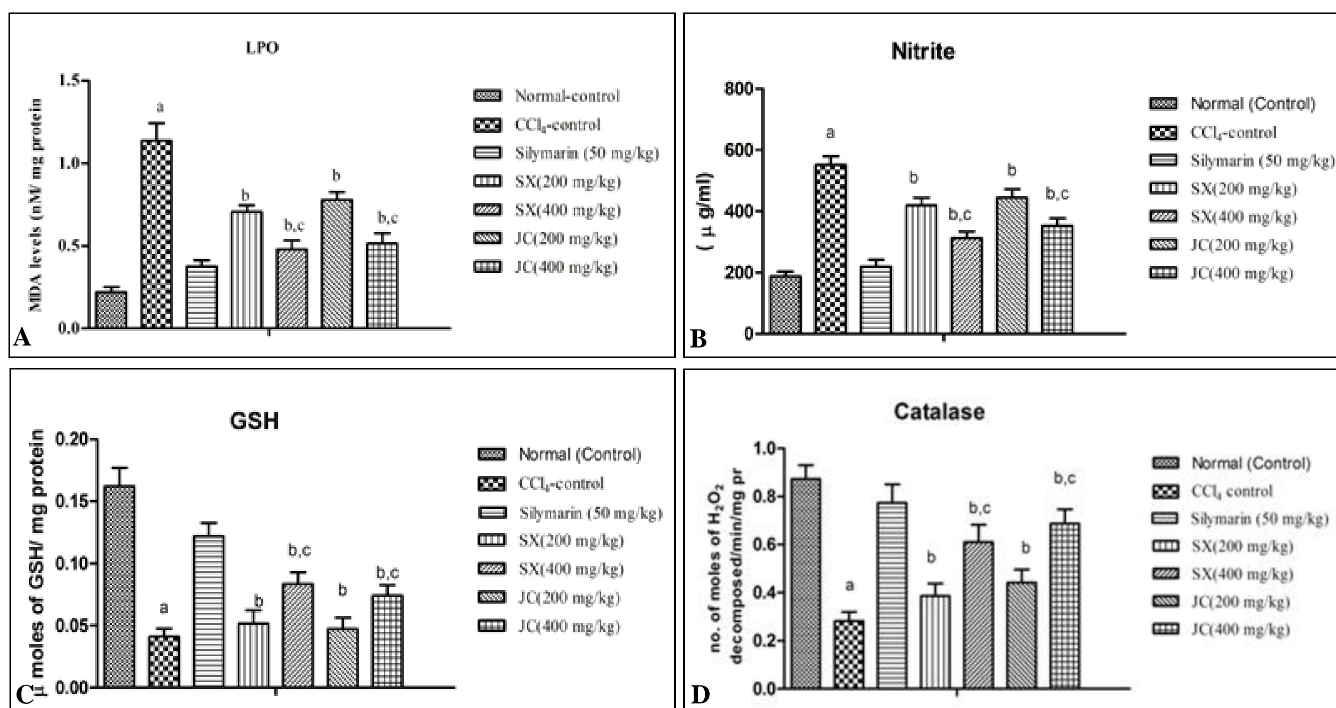

FIG. 2: EFFECT OF HYDROALCOHOLIC EXTRACTS OF *SOLANUM XANTHOCARPUM* AND *JUNIPERUS COMMUNIS* ON BIOCHEMICAL ALTERATION IN CCl_4 TREATED RATS. A. MDA LEVEL B. NITRITE CONCENTRATION C. REDUCED GLUTATHIONE (GSH) D. CATALASE. Results were expressed as mean ± S.D; $^{a}p < 0.05$ vs. Normal control. $^{b}p < 0.05$ vs. CCl4 control group, $^{c}p < 0.05$ *vs*. Silymarin (50 mg/kg).

Effect of Hydroalcoholic Extract of *Solanum xanthocarpum* and *Juniperus communis* on Oxidative Stress Parameters (Lipid Peroxidation, Nitrite, Catalase and Reduced Glutathione) in CCl_4 Induced Hepatotoxicity in Rats: Chronic administration of CCl_4 significantly caused oxidative stress (increased MDA level, nitrite concentration, depleted catalase (CAT) and reduced glutathione (GSH) enzyme activity) as compared to normal control group. The hydroalcoholic extract of SX and JC (200, 400mg/kg b.w.) treated rats significantly (p<0.05)

decrease oxidative stress (MDA levels, nitrite concentration and restored the level of endogenous antioxidant enzyme *viz*. catalase CAT and reduced GSH) dose-dependently as compared to CCl_4 treated rats indicating antioxidant effect. Moreover, the administration of standard silymarin (50mg/kg) significantly ($p < 0.05$) attenuated the oxidative damage in CCl_4 induced liver injury as shown in **Fig. 2A-D**.

Histopathological Studies: The different groups of rats were studied for the cellular architecture of the liver tissue by a histopathological analysis which is presented in **Fig. 3A-G**.

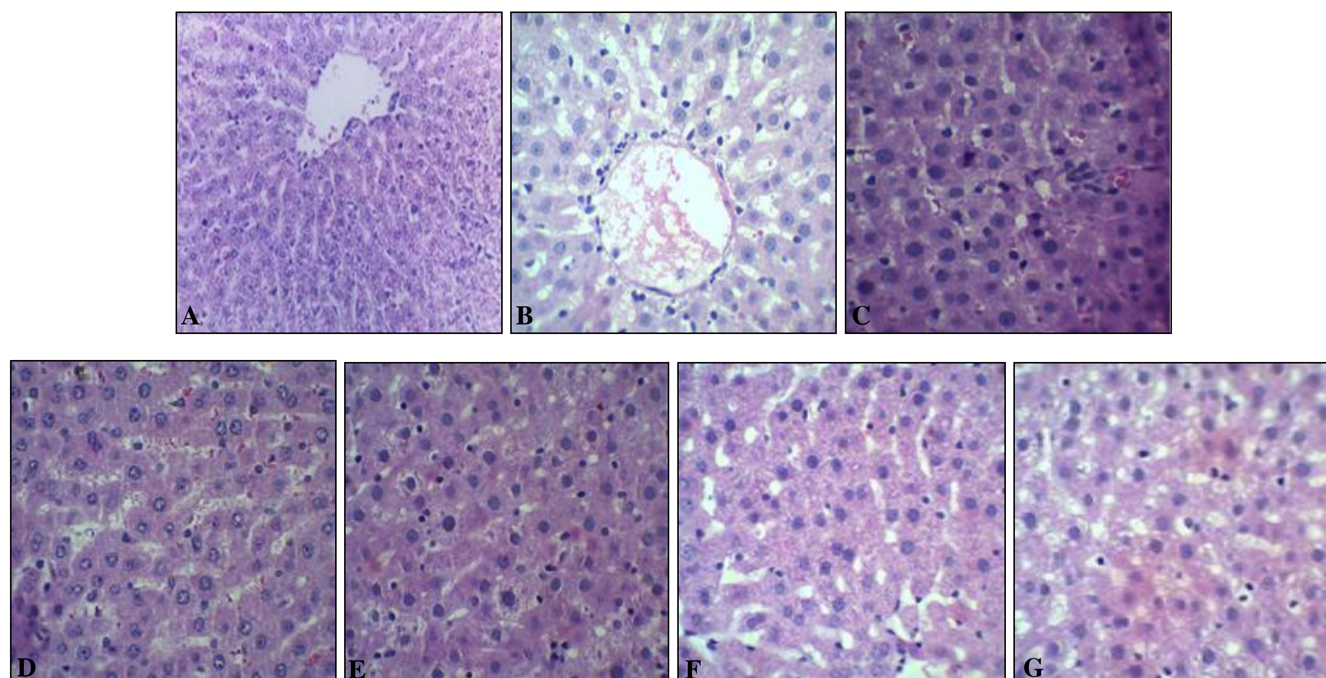

FIG. 3: HISTOPATHOLOGY OF LIVER TISSUES. PHOTOMICROGRAPHS WERE TAKEN AT 100X

A: Liver section of normal control rats showing central vein surrounded by a hepatic cord of cells (normal architecture)

B: Liver section of CCl_4 treated rats showing massive fatty changes along with congestion in a central vein, necrosis, ballooning degeneration and the loss of cellular boundaries.

C: Liver section of rats treated CCl_4 and 50 mg/kg b.w. of Silymarin showing normal liver architecture.

D: Liver section of rats treated CCl_4 and 200 mg/kg b.w. of hydroalcoholic extract of SX showing inflammatory collections and focal necrosis with sinusoidal dilatation.

E: Liver section of rats treated CCl_4 and 400 mg/kg b.w. of hydroalcoholic extract of SX showing regeneration of hepatocytes toward near normal liver architecture.

F: Liver section of rats treated CCl_4 and 200 mg/kg b.w. of hydroalcoholic extract of JC showing inflammatory collections around the central vein and focal necrosis.

G: Liver section of rats treated $CCl_4$and 400 mg/kg b.w. of hydroalcoholic extract of JC showing less inflammatory cells and, absence of necrosis.

DISCUSSION: Oxidative stress is a process where the physiological balance between pro-oxidants and antioxidants is disrupted, resulting in potential damage to the body organs [37]. Oxidative stress is responsible for the liver diseases resulting in one of the serious health issues worldwide [38]. Antioxidants derived from natural source help in counteracting the oxidative stress induced by the number of hepatotoxins [39]. Therefore, in the present study the comparative liver protective activity of ethanolic extract of *Solanum xanthocarpum* Schradt. and Wendl. and *Juniperus communis* Linn. (whole plant) was demonstrated against CCl_4 induced liver toxicity. Preliminary phytochemical screening of SX and JC showed the presence of steroids, triterpenoids, glycosides, flavonoids and phenolic compounds. These phytoconstituents have been previously reported to have antioxidant as well as hepatoprotective potential [40, 41]. Hydroalcoholic extract of SX and JC showed remarkable antioxidant activity in DPPH radical scavenging assay. The radical scavenging activity of SX was more significant as compared to JC. Antioxidant activity of both plants extracts on DPPH radicals may be attributed to a direct role in trapping free radicals by donating hydrogen atom or electron.

The antioxidant activity of both plants may be due to the high flavonoids and phenolic contents as phenolic compounds received attention for their potential antioxidant activity [42].

CCl_4 is conventionally used to induce liver toxicity in rats. CCl_4 is actively metabolized in the liver tissues to its highly reactive trichloromethyl free radical CCl_3. Trichloromethyl free radical reacts with cellular macromolecular protein and polyunsaturated fatty acids in the presence of molecular oxygen to form more toxic trichloromethyl peroxyl radicals along with H_2O_2, O_2, OH that leads to liver damage [37]. The liver toxicity induced by CCl_4 elevates the liver marker enzymes as seen in the blood [43]. The rise in serum levels of SGOT and SGPT indicate the damaged structural integrity of the liver [44]. The level of enzyme SGOT and SGPT were increased with the toxicant CCl_4 treatment but after treatment with hydroalcoholic extract of SX and JC the elevated level were changed which indicates the protective action of plant extract.

The enzyme alkaline phosphate (ALP) is a membrane-bound glycoprotein enzyme with a high concentration in sinusoid and endothelium. It is excreted into the bile, but on treatment with toxicant CCl_4, there is the elevation of serum ALP level due to hepatobiliary disorder [45]. In the present study, the treatment with hydroalcoholic extract of plants reduced the level of ALP in treated animals. CCl_4 induced elevation of ALP is in line with high levels of serum bilirubin. The decrease in the raised ALP enzyme activity along with the fall of higher bilirubin level indicated some benefits in biliary functions in rats with hepatic injury. The significant control of ALP and bilirubin levels in treated groups points toward an early improvement in the secretary mechanism of hepatocytes [28].

Treatment with SX and JC hydroalcoholic extract reduces the biochemical enzyme level, which indicates the preservation of structural and functional integrity of the hepatocellular membrane in rats. The decrease in the total protein (TP) is attributed to the initial damage produced and localizes in the endoplasmic reticulum which results in the loss of cytochrome - 450 enzymes indicating the functional failure of protein synthesis and accumulation of triglycerides leading to fatty liver [46]. Treatment with both plant extract enhances the total protein level accelerate the regeneration and protection of liver cells indicating the hepatoprotective activity of plants.

GSH is a non-enzymatic antioxidant bio-molecules present in the tissue. It removes the free oxygen species, such as H_2O_2, superoxide anions and alkoxy radicals, maintains the membrane protein thiols, and act as a substrate for GPx and glutathione S-transferase (GST) [47]. Thus GSH maintains the body's endogenous antioxidant defense mechanism and conjugates with free radicals directly to protect the integrity of cell membranes [48]. Increase in MDA levels; intoxicant CCl_4 treated rats; indicate an increase in lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms [49]. The level of GSH decrease and the LPO increase in treatment with toxicant CCl_4 treatment. Animals treated with plant extract significantly restore the hepatic GSH and LPO content toward normal level shown in **Fig. 2A, C**.

Catalase plays an essential role in protection against the harmful effects of hydrogen peroxide and lipid peroxidation in diseases related to oxidative stress [50]. The suppression of CAT level is an indication of liver damage in CCl_4 treated animal group. On the administration of both plant extracts there is the significant restoration of the reduced catalase (CAT) level as shown in **Fig. 2D**. Nitrite is a stable metabolite produced from the metabolism of NO. The increased NOS activity has been observed in liver homogenate of rats exposed to CCl_4, that led to increased nitrite levels indicates the oxidative and nitrosative stress in animals. The exposure to reactive and nitrogen species RNOS may cause the lipid peroxidation in cell membranes, which generates reactive species that damage the cell proteins and promote their degradation [51]. On treatment with SX and JC hydroalcoholic extract there was a significant reduction in the elevated nitrite concentration as shown in **Fig. 2B**. The hepatoprotective potential of hydroalcoholic extract SX and JC is dose-dependent. As the results have shown the hydroalcoholic extract, SX (400 mg/kg) showed a maximum reduction in MDA level, nitrite concentration and resorted the catalase (CAT), reduced GSH level.

Histopathological examinations of treated CCl_4 animal liver showed hepatic toxicity which was evidenced by cellular necrosis, nodal formation, profound steatosis and fibrosis as compared to normal hepatic architecture of normal animal liver section, which is clearly shown in **Fig. 3A, B**. On treatment with SX and JC hydroalcoholic extract the animal showed recovery of damaged parenchyma, which was comparable to that of the standard drug Silymarin treated animal liver section shown in **Fig. 3C-G**.

The *in-vitro* and *in-vivo* antioxidant activities of SX and JC may be associated with the flavonoids, phenolic, and terpenoid compounds present in the extract which has been known for their antioxidant and hepatoprotective activities [52].

CONCLUSION: In a nutshell it is concluded that both the plants *Solanum xanthocarpum* and *Juniperus communis* extracts may have promising hepatoprotective properties due to their antioxidant potential. The results suggested that the plants exhibited hepatoprotective effect that may be due to the presence of phenolic compounds which acts as antioxidants. It is also observed that *Solanum xanthocarpum* plant has more significant hepatoprotective activity compared to *Juniperus communis* plant.

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