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IN-VITRO HEPATOPROTECTIVE ACTIVITY OF METHANOLIC LEAF EXTRACT OF *MURRAYA KOENIGII* AGAINST CCL4 INDUCED HEPATOTOXICITY IN GOAT LIVER SLICE CULTURE

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ABSTRACT: The purpose of the current investigation was to evaluate the hepatoprotective activity of methanolic leaf extract of *Murraya koenigii* in goat liver slice culture against carbon tetrachloride (CCl_4)-induced hepatotoxicity. The amount of cell damage caused by CCl_4 was measured by the release of marker enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate dehydrogenase (LDH). Additionally, we estimated the levels of lipid peroxidation (LPO) in the membrane lipids of hepatocytes to assess the extent of hepatic injury. In comparison to untreated liver cells, those treated with CCl_4 resulted in a twofold increase in LPO, as well as the release of ALP, ALT, AST, ACP, and LDH at rates of 2.59, 3.34, 3.36, 2.85, and 6.75 times, respectively. Furthermore, liver cells treated with both CCl_4 and with methanolic leaf extract of *Murraya koenigii* exhibited significant reduction in hepatic marker enzymes. The 2,2-diphenylpicrylhydrazyl (DPPH) method was used to figure out the plant extract's free radical scavenging activity (IC_{50} 137±1.9 µg/mL). Qualitative analysis of the methanolic leaf extract revealed the presence of flavonoids (silymarin and quercetin). These findings suggest that the liver cells were shielded from CCl_4-induced oxidative/free radical-mediated injury *in-vitro* by the methanolic leaf extract of *Murraya koenigii*, due to the presence of flavonoids like silymarin and quercetin, which possess remarkable antioxidant properties providing hepatoprotective activity.

INTRODUCTION: Traditional medicinal plants may serve as a significant source of distinct biologically active compounds. Every demographic group uses plants, whether directly as indigenous remedies or indirectly in the pharmaceutical manufacturing of contemporary medications [1]. In traditional methods such as Ayurveda, Unani, and Siddha, herbal medicines are essential for the treatment and cure of various diseases and physiological conditions.

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This form of treatment, also known as conventional treatment, served as the primary source of medical care. *Murraya koenigii* is a weed plant that contains significant medicinal properties for human health applications. It is generally found in Pakistan, Thailand, Sri Lanka, and India.

The extracts of the plant's leaves, roots, and stem are employed for medicinal purposes to treat a variety of ailments, including eye infections, respiratory issues, rheumatism, and skin problems, and to reduce blood sugar levels [2]. The biochemical constituents of *Murraya koenigii* include tannins, flavonoids, acalyphamide, aurantiamide, succinimide, the pyranoquinolinone alkaloid flinders in, and the cyanogenic glucoside acalyphin. In addition, the dried methanol extract of freeze-

dried flowers and foliage of *Murraya koenigii* has yielded four identified kaempferol glycosides: biorobin, nicotiflorin, clitorin, and mauritianin [3].

Murraya koenigii, often referred to as Indian Murraya, is a member of the Rutaceae family. It is itself a common annual plant, mainly found in yards of homes and abandoned areas across the plains of India. The leaves of *Murraya koenigii* exhibit significant antibacterial efficacy against both gram-negative and gram-positive bacteria. Researchers note the herb's post-coital antifertility effect, anti-venom characteristics, wound healing capacity, antioxidant activities, anti-inflammatory effects, acaricidal characteristics, diuretic effects, and antibacterial activities [4].

Murraya koenigii has been the subject of numerous in vivo studies that evaluate its hepatoprotective effect. However, there is no evidence to support the assessment of the in vitro hepatoprotective efficacy of the methanolic leaf extract of *Murraya koenigii* against CCl_4-induced hepatotoxicity in goat liver slice culture. The intact liver is represented by the liver slice, which is a microcosm of the liver comprising highly organised cellular communities in which all kinds of cells are in mutual association. Consequently, liver slice culture is an *in-vitro* technique that provides an advantage of an *in-vivo* environment in comparison to maintained cell line cultures. This model is simple and appropriate for experimentally examining hepatotoxic conditions [8]. As a result, the current investigation was conducted to evaluate the hepatoprotective activity of methanolic leaf extract of *Murraya koenigii* in goat liver slice culture against CCl_4 in order to establish its efficacy against liver disorders.

MATERIALS AND METHODS:

Chemicals and Reagents: All chemicals utilised were of analytical grade, exhibiting excellent purity, and purchased from either Sigma Chemicals (Bangalore, India) or Hi Media Ltd. (Mumbai, India).

Preparation of Methanolic Leaf Extract of *Murraya koenigii:* The methanolic extract of *Murraya koenigii* was prepaired by using the Soxhlet extraction technique [9]. About 50g of finely powdered *Murraya koenigii* powder was subjected to Soxhlet extraction by putting it in an extraction thimble and subsequently transferring it to a Soxhlet extractor. The extraction was carried out with methanol as the solvent of extraction in 2:10 powder to solvent ratio at temperature 65 °C for 8 h. This methanolic extract was filtered using Whatman filter paper No.1, then the filtrate was concentrated using a rotary vacuum evaporator at 45 °C **Fig. 1.** The concentrated methanolic extract was preserved in a desiccator until needed for further use.

FIG. 1: (A) *MURRAYA KOENIGII* PLANT, (B) *MURRAYA KOENIGII* LEAVES (C) POWDER OF *MURRAYA KOENIGII* LEAVES

Qualitative Test for Flavonoids:

Aluminium Chloride Test: Three mL of 1% Aluminium chloride solution were added to 5 mL of methanolic extract. A yellow colouration was noted, signifying the presence of flavonoids. About 5 mL of weak ammonia solution was added to the abovementioned combination, followed by the addition of concentrated H_2SO_4. A golden colour vanished upon standing. The yellow colour that disappeared upon standing signifies a good test for flavonoids [5].

Shibat's Test: One mL of the extract is solubilised in 5 mL of 50% methanol by heating. Subsequently, introduce magnesium metal and six drops of concentrated sulphuric acid (H_2SO_4).

The presence of flavonoids is indicated by red or orange colouration [6].

Shinoda's Test: One mL of plant extract dissolved in five millilitres of methanol. Add a few drops of concentrated H_2SO_4 and fragments of magnesium ribbon. The presence of flavonoids is indicated by the presence of a pink to crimson-colored solution [7].

Pew's Test: A small amount of extract is combined with 0.1 g of metallic zinc and 8 mL of concentrated H_2SO_4. The presence of flavonoids is indicated by a red colour [8]. So, many other tests are being done to confirm the presence of flavonoids which are explained in the table below **Table 1.**

Free Radical Scavenging Activity by 2, 2-diphenylpicrylhydrazyl (DPPH) Method: We used the DPPH method to determine the extracts' free radical scavenging capacity. The DPPH solution (1 mM) was prepared in 95% methanol. The stock solution (1 mg/mL) was prepared by combining the methanol extract of the *Murraya koenigii* leaves with methanol. Each test container was filled with 500 µl of the freshly made DPPH solution (1 mM), 1 mL of the extract (5 - 100 µg/mL), and 500 µl of phosphate buffered saline (10 mM, pH 7.4). The reaction mixture was incubated in the dark at room temperature for 30 minutes, and the optical density was subsequently measured at 520 nm in comparison to the baseline. For the control, 500 µl of buffered saline was combined with 500 µl of DPPH solution in ethanol, and the solution's optical density was measured after 30 minutes. The assay was conducted in three replicates. Ascorbic acid was used as the standard. The antioxidant activity was measured by looking at how much the DPPH radical was reduced, which was done by comparing the light absorption of DPPH after adding the test samples to a control sample. We used the following equation to determine the DPPH radical scavenging capacity:

$$\text{Percentage (\%) inhibition} = [1-(AT / AC)] \times 100$$

Where, AT is the absorbance of the test sample and AC is the absorbance of the control sample.

***In-vitro* Hepatoprotective Activity:** In order to investigate the *in-vitro* effect of various concentrations of methanolic leaf extract of *Murraya koenigii* on the cytotoxicant CCl_4, the goat liver was used as the mammalian tissue.

Liver Slice Culture: Liver slice culture was conducted using the methodology reported by Chaudhari and Mahajan [10]. The fresh liver was obtained from the local slaughter house immediately following the animal's sacrifice. The liver was placed in sterilised Krebs Ringer Hepes medium (KRH 2.5 mM Hepes, pH 7.4, 118 mM NaCl, 2.85 mM KCl, 2.5 mM $CaCl_2$, 1.15 mM KH_2PO_4, 1.18 mM $MgSO_4$, and 4.0 mM glucose). The liver was sectioned into thin slices using a sharp blade, and slices weighing between 8 and 10 mg were employed for this investigation. Each experimental set had 15 tissue slices with a total weight of 100 mg. The tissue fragments were rinsed with 10 mL of KRH medium every 10 minutes for 1 hour and thereafter incubated for 60 minutes in tiny beakers containing 10 mL of KRH on a shaking water bath at 37 °C.

EXPERIMENT DESIGN:

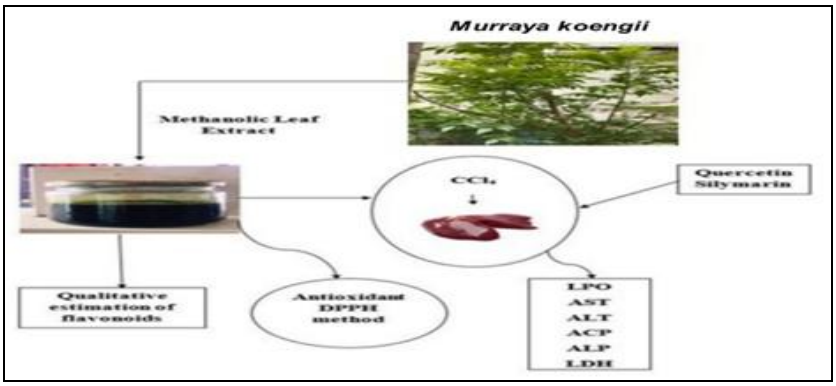

FIG. 2: THE DESIGN FOR PHYTOCHEMICAL ANALYSIS AND *IN-VITRO* CCL_4-INDUCED HEPATOTOXICITY STUDY BY USING GOAT LIVER TISSUE

The experimental designs were developed based on the methodology of Chaudhari and Mahajan[8] with minor modifications. The cleaned liver segments were subsequently partitioned into 10 distinct culture groups for specific treatment. 1 mL of 15 mM cytotoxic CCl_4 and 1 mL of various quantities of the plant extract *Murraya koenigii*, quercetin, and the standard medication Silymarin were utilised for the experiment. Group 1 served as the control group devoid of toxicants. Group 2 was administered 1 mL of 15 mM CCl_4. Groups 3 to 5 were administered 1 mL of CCl_4 and 1 mL of plant extract at varying concentrations (10, 15, and 25 µg/mL). Groups 6 and 7 were administered 1 mL of CCl_4 and 1 mL of quercetin (10 µg/mL) and silymarin (10 µg/mL), respectively. Groups 8, 9, and 10 were administered 1 mL of plant extract (25 µg/mL), quercetin (10 µg/mL), and silymarin (10 µg/mL), respectively. All groups, 1, 8, 9, and 10, were kept with CCl_4 for 1 hour at 4 °C, and then the treated groups were given different amounts of plant extract, quercetin, and silymarin. After completion of the treatment, all cultures were incubated in a water bath at 37 °C for 2 hours. After completion of incubation, the culture medium was homogenised with ice-cold normal saline utilising a glass Teflon homogeniser at 4 °C. Subsequent to homogenisation, each sample was subjected to centrifugation at 10,000 rpm for 20 minutes at 4 °C to eliminate cellular debris and other suspended particulates. The resulting clear supernatants were collected and analysed for the release of biochemical markers, including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), acid phosphatase (ACP), lactate dehydrogenase (LDH), and lipid peroxidation **Fig. 2**.

Determination of Lipid Peroxidation: The extent of lipid peroxidation was evaluated by measuring Thiobarbituric acid reactive substances (TBARS) according to the standard technique established by Fraga *et al* [11]. Utilising this procedure, 1.0 mL of the tissue homogenate was subjected to treatment with a reagent comprising Thiobarbituric acid, Trichloroacetic acid, and Hydrochloric acid (TBA-TCA-HCl) in a 1:1:1 ratio and mixed thoroughly. The mixture was maintained in a hot water bath for 15 minutes. Subsequent to chilling, the tubes were centrifuged at 3,000 rpm for 10 minutes, and the supernatant was collected for analysis. A series of conventional malondialdehyde solutions with concentrations ranging from 2 to 10 µmoles were handled in an analogous manner. The absorbance of the pink complex was measured at 535 nm relative to the reagent blank. The outcome was quantified as µmoles of malondialdehyde (MDA) generated per minute per mg of protein.

Estimation of Acid Phosphatase Activity: The activity of Acid Phosphatase was evaluated using the procedures outlined by Anon[12]. The reaction mixture for ACP analysis consisted 0.1 mL of tissue homogenate and 1 mL of 1% p-Nitrophenyl Phosphate substrate in 0.1 M citrate buffer at pH 4, incubated at 37 °C for 30 minutes. Subsequently, 1.5 mL of 0.1 N sodium hydroxide was introduced to terminate the reaction. The hydrolytic product, yellow p-nitrophenol, was quantified at 405 nm utilising a Shimadzu UV-1900i UV-VIS spectrophotometer from Japan. The enzymatic activity was assessed with a standard graph of p-nitrophenol. The ACP activity was quantified as the dephosphorylation of p-Nitrophenyl Phosphate (PNPP) to p-nitrophenol (PNP) in µMoles/min/mg protein.

Activities of Aspartate Transaminase (AST) and Alanine Transaminase (ALT): The activities of AST and ALT were evaluated using the Reitmann and Frankel method [13]. One mL of substrate, aspartate transaminase for AST and alanine transaminase for ALT, was combined with 0.2 mL of homogenate and incubated for 1 hour for aspartate transaminase and 30 minutes for alanine transaminase, followed by the addition of 2 drops of aniline-citrate reagent for testing. The reaction stopped using 1.0 mL of 1 mM 2,4-Dinitrophenyl Hydrazine (2,4-DNPH) solution, and the tubes were maintained at room temperature for 20 minutes. 1.0 mL of 0.4 N sodium hydroxide was administered to each tube. Sets of sodium pyruvate standards were processed in an analogous fashion. The optical density was detected at 540 nm. The activities of aspartate and alanine transaminase were quantified as µmoles per minute per milligramme of protein.

Activity of Alkaline Phosphatase (ALP): Alkaline phosphatase was evaluated using the King and Armstrong technique [14], with disodium phenyl phosphate as the substrate. A pre-incubation

mixture of bicarbonate buffer (0.1 M, pH 10) and substrate (0.01 M) in 2.9 mL of distilled water was maintained at 37 °C for 10 minutes. 0.2 mL of tissue homogenate was introduced and incubated at 37 °C for 15 minutes. The reaction stopped by the addition of 1.0 mL of Folin-phenol reagent. The suspension was centrifuged at 3,000 rpm for 5 minutes, after which 10% sodium carbonate was added to the supernatant. The solution was incubated at 37 °C for 10 minutes. Standard phenol solutions (2.5 - 10.0 µg) were treated with Folin-phenol reagent and sodium carbonate. The blue colour observed was measured at 680 nm. The enzyme activity was quantified in micromoles per minute per milligramme of protein.

Estimation of Lactate Dehydrogenase (LDH): The activity of lactate dehydrogenase was evaluated using the King technique [15]. About 0.1 mL of homogenate and 0.25% NAD+ was added to 1 mL of buffered lithium lactate substrate (0.1 M, pH 7.4) and incubated at 37 °C for 5 minutes. Subsequently, DNPH (0.02%) was introduced and incubated for 15 minutes at 37 °C. Subsequently, 0.4 N NaOH was introduced, and the resulting colour intensity was quantified at 420 nm relative to a reagent blank. Standard sodium pyruvate sets were subjected to analogous treatment. The activity of LDH was quantified as IU/L or µmols/min/mg protein.

Statistical Analysis: Statistical analyses were conducted utilising one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test, employing the SPSS 25.0 statistical program (IBM, USA). Differences were deemed significant at $p < 0.05$ in comparison to the CCl_4 treatment group. Data are expressed as mean ± standard deviation for n = 3. All biochemical assessments were conducted in triplicate.

RESULTS AND DISCUSSION: Herbal drugs are considered safe and provide the ability to treat liver problems, resulting in their increased popularity in recent years. Furthermore, plant-derived pharmaceuticals are economically advantageous as well. A wide range of medicinal plants found across India are recognised as hepatoprotective agents and are widely utilised for treating liver ailments. Several plants and polyherbal compositions have hepatoprotective properties. Approximately 160 phytoconstituents and other phytochemicals are purported to exhibit hepatoprotective action. In India, around 87 plants are utilised, of which 33 are patented and possess exclusive multi-ingredient compositions.

This study examined the *in-vitro* effects of varying concentrations of methanolic leaf extract of *Murraya koenigii*, quercetin, and silymarin on CCl_4-induced hepatotoxicity in goat liver slice cultures. The freshly sliced liver was rinsed with 10 mL of Krebs Ringer Hepes medium (KRH) for varying time intervals, and the washed liver slices were utilised to assess the in vitro hepatoprotective efficacy of different concentrations of methanolic leaf extract of *Murraya koenigii*. Hepatotoxicity was generated by administering CCl_4, and all treated groups received varying concentrations of methanolic leaf extract of *Murraya koenigii*, quercetin, and silymarin. Furthermore, all previously mentioned liver markers, together with lipid peroxidation, were assessed. One hour post-administration of CCl_4, levels of AST, ALT, LDH, ACP, ALP, and lipid peroxidation markedly elevated in comparison to the control group (Table 1; $p < 0.001$), indicating CCl_4-induced hepatic damage in goat liver slice culture.

The elevation of liver enzymes generated by CCl_4 was reduced by the addition of *Murraya koenigii* extract. AST, ALT, LDH, ACP, and ALP are sensitive biomarkers of CCl_4-induced hepatic damage and constitute the most critical values in standard clinical liver function tests. The findings demonstrated an elevation in liver enzymes in CCl_4-induced goat liver slices, while the extracts of quercetin and silymarin significantly improved enzymatic activity at dosages of 25, 10, and 10 µg/mL ($p < 0.05$).The specified dosages of the plant extracts, quercetin and silymarin, recovered the liver enzymes to normal levels.

Preliminary Flavonoid Screening: Flavonoids are polyphenolic chemicals regarded as essential nutrients. Their fundamental chemical structure comprises two benzene rings connected by a three-atom heterocyclic carbon chain. The oxidation of the structure yields many families of flavonoids, including flavones, flavonols, flavanones, anthocyanins, flavanols, and isoflavones. Flavonoids has the capacity to neutralise free

radicals and inhibit lipid peroxidation. The biological utility of medicinal plants is frequently linked with the presence of flavonoids. Numerous forms of flavonoids are recognised for their diverse impacts on health. Consequently, flavonoid concentration may serve as a criterion for assessing the quality of therapeutic plants utilised [16]. The initial flavonoid screening of the extract confirmed the presence of flavonoids, and the findings of the phytochemical analysis are summarised in **Table 1**.

TABLE 1: PRELIMINARY FLAVONOID SCREENING FROM METHANOLIC LEAF EXTRACT OF *MURRAYA KOENIGII*

Test	Pocedure	Observation	Inference
Aluminium chloride test	3 mL of 1 % Aluminium chloride + 5 mL of extract + 5 mL of dilute ammonia solution + concentrated H_2SO_4	A yellow coloration was observed	Presence of flavonoid
Shibat's test	1mL extract + 5 mL 50 % methanol+metalmagnesium +6dropsconcentratedH_2SO_4	Orange colour	Presence of flavonoid
Shinoda's test	1mLextract+5mL ethanol+magnesiumribbon + few drops concentrated H_2SO_4	A pink to crimson coloured solution	Presence of flavonoid
Pew's test	Few mL extract + 0.1 g metal Zinc + 8 mL concentrated H_2SO_4	A red colour	Presence of flavonoid
LeadAcetate Test	Add a few drops of 10% lead acetate solution to the extract	Yellow precipitate observed	Presence of flavonoids
Alkaline Reagent Test	Add a few drops of 2% NaOH solution to the extract, followed by dilute HCl	Yellow color observed	Presence of flavonoids
Ferric Chloride Test	Add a few drops of 1% $FeCl_3$ solution to the extract.	Greenish-black coloration	Presence of flavonoids
Sodium Hydroxide Test	Add a few drops of 10% NaOH to the extract, observe color, then add dilute HCl to see if the color disappears.	Intense yellow color observed	Presence of flavonoids
Ammonia Test	Expose the extract on filter paper to ammonia vapor or add a few drops of ammonium hydroxide to the extract	Yellow fluorescence under UV light	Presence of flavonoids
Magnesium-HCl Test	To the extract, add magnesium ribbon followed by a few drops of concentrated HCl.	Yellow fluorescence under UV light	Presence of flavonoids

The phytochemical analysis is very much important to evaluate the possible medicinal utilities of a plant and also to determine the active principles responsible for the known biological activities exhibited by the plants. Further, it provides the base for targeted isolation of compounds and to perform more precise investigations. The phytochemical analysis is necessary for the assessment of the potential medicinal properties of a plant and for the identification of the active principles responsible for the known biological activities of the plant. Additionally, it serves as a framework for conducting more precise investigations and the targeted isolation of compounds.

Free Radical Scavenging Activity by DPPH Method: The free radical scavenging activity of the methanol extract was evaluated with an IC_{50} value of 137±1.9 µg/mL, while that of standard ascorbic acid was having an IC_{50} of 4.1±0.04 µg/mL. The DPPH radical scavenging assay quantifies the essential characteristic of antioxidants: their ability to scavenge proton radicals. The hydrogen-donating capacity of antioxidant molecules influences their free radical scavenging nature. It is advisable to subject a plant extract to assays that assess a variety of activities, including the inhibition of membrane lipid peroxidation, the scavenging of reactive oxygen species, and metal ion chelation, to characterise its antioxidant activity. Antioxidant-rich plant extracts are sources of nutraceuticals that alleviate oxidative stress, thereby preventing or slowing down the progression of degenerative diseases [17]. The finding suggests that the methanol leaf extract of *Murraya koenigii* has the potential to serve as a source of natural antioxidants or nutraceuticals, which could be used to counteract oxidative stress and provide health benefits.

***In-vitro* Hepatoprotective Activity:** CCl_4 is a typical lipid peroxidative agent used to induce

hepatotoxicity in *in-vitro* models. The Thiobarbituric acid reactive substances (TBARS) assay was employed to assess the extent of lipid peroxidation in the liver slice. TBARS were used to calculate lipid peroxidation, which was expressed as µmoles of malondialdehyde formed/min/mg protein [18]. The results of lipid peroxidation in various regimens were presented in **Table 2**. The lipid peroxidation level in the liver tissue homogenates treated with CCl_4 was significantly elevated by 2.25 fold ($p < 0.05$) in comparison to the control treatment [19].

This elevated level of lipid peroxidation has been previously reported in the liver of rats following oral administration of CCl_4 and in liver cells that were treated with CCl_4 *in-vitro* [20, 8]. The level of lipid peroxidation decreased significantly ($p < 0.05$) in comparison to the CCl_4-treated group after treatment with varying concentrations of methanolic leaf extract (10, 15, and 25 µg/mL). In the presence of methanolic leaf extract of *Murraya koenigii*, Quercetin, and Silymarin, the level of lipid peroxidation in liver slice homogenate treated with CCl_4 was significantly reduced ($p < 0.05$).

CCl_4 significantly reduced the lipid peroxidation in liver tissue homogenate by 25, 36, and 48% ($p < 0.05$) when treated with a 25 µg/mL concentration of methanolic leaf extract and a 10 µg/mL concentration of Quercetin and Silymarin. Biological materials, especially membranes, possess elevated levels of unsaturated lipids. In the presence of a free radical activator and oxygen, they may undergo oxidation. Lipid peroxidation is recognised as a widespread biological degenerative event and may represent a significant *in-vivo* process [21]. Carbon tetrachloride-induced hepatic injury is a prevalent experimental approach to assess the hepatoprotective efficacy of medicinal plants and pharmaceuticals [22]. The cytochrome P-450 dependent monooxygenase enzyme system metabolises CCl_4 to the trichloromethyl radical ($CCl_3^{\bullet}$). The $CCl_3^{\bullet}$ interacted with molecular oxygen, resulting in the formation of a highly reactive trichloromethyl peroxy radical. Functional and morphological alterations transpire in the cell membrane when fatty acids in the cytoplasmic membrane phospholipids are oxidised by free radicals generated during the metabolism of CCl_4 in the liver [23].

Furthermore, these radical forms establish covalent connections with sulfhydryl groups of different membrane components such as GSH, resulting in depletion and an elevation in malondialdehyde levels, so inducing lipid peroxidation [24] **Fig. 3.** Reactive free radicals induce cellular damage via two primary mechanisms: covalent attachment to cell membrane lipids and lipid peroxidation. Moreover, oxidative stress can induce reversible or permanent modifications of susceptible proteins, resulting in increased reactivity to proteolytic degradation [25].

Lipid peroxidation and free radicals are integral to the primary processes by which hepatotoxins damage hepatocytes. The hepatoprotective properties of *Murraya koenigii* may be ascribed to its antioxidant potential. The antioxidant efficacy of *Murraya koenigii* leaf extracts was evaluated by quantifying their DPPH scavenging activity. The extracts exhibited significant DPPH radical scavenging activity equivalent to that of ascorbic acid, the positive control. This impact may result from a tendency to donate hydrogen. The ability of the extracts to scavenge DPPH radicals can facilitate the suppression of lipid peroxidation [26].

TABLE 2: EFFECT OF METHANOLIC LEAF EXTRACT OF *MURRAYA KOENIGII*, QUERCETIN AND SILYMARIN TREATMENT ON RELEASE OF BIOCHEMICAL MARKERS OF HEPATOTOXICITY INDUCED BY CCL_4 IN THE GOAT LIVER TISSUE *IN-VITRO*

Set No	Lipid peroxidation	Lactate dehydrogenase	Alkaline phosphatase	Alanine transaminase	Aspartate transaminase	Acid phosphatase
Control	1.34 ± 0.05	10.18 ± 1.36	15.17± 1.51	72.92± 2.79	55.60 ± 1.90	23.92 ± 2.50
CCl_4	3.02 ± 0.34a	68.80 ± 1.55a	39.30± 2.61a	244.22 ± 2.10a	187.13±4.81a	68.24 ± 4.41a
CCl_4+LE10 µg/Ml	3.35 ± 0.11*	66.19 ± 0.93*	38.72 ± 1.8*	219.10 ± 3.55*	146.16±2.85*	63.24 ± 0.94*
CCl_4+LE15 µg/mL	2.47 ± 0.08*	52.32 ± 2.31*	30.17 ± 0.7*	218.57 ± 2.6*	152.73 ± 2.5*	52.95 ± 1.4*
CCl_4+LE25 µg/mL	2.71 ± 0.08*	19.85 ± 1.49*	23.47 ± 1.7*	187.33±2.2*	107.47 ± 2.3*	45.15 ± 1.0*
CCl_4+Q 10 µg/mL	2.24 ± 0.05*	45.37 ± 2.69*	21.16 ± 1.2*	142.11 ± 2.3*	82.13 ± 1.22*	37.35 ± 0.5*
CCl_4+ S10 µg/mL	1.12 ± 0.03*	31.18 ± 2.11*	18.25 ± 0.8*	116.26 ± 1.8*	62.23 ± 1.63*	31.24 ± 1.3*
LE 25 µg/mL	1.41 ± 0.03*	16.21 ± 1.29*	32.96 ± 2.2*	86.38 ± 1.97*	58.29 ± 0.95*	27.19 ± 1.2*

Q 10 µg/mL	1.17 ± 0.05*	12.36 ± 2.46*	16.19 ± 1.2*	72.24 ± 2.14*	51.35 ± 1.07*	21.18 ± 0.5*
S 10 µg/mL	0.87 ± 0.04*	9.26 ± 1.20*	15.61 ± 1.3*	46.92 ± 1.68*	42.73 ± 2.67*	85.89 ± 0.7*

LE- Leaf Extract (methanolic), Q- Quercetin, S- Silymarin. (All values are expressed as Mean ± S.D. (n = 3); Superscript (a) in columns indicates value differ significantly ($p < 0.001$) from control mean; Superscript asterisk in columns indicate value differ significantly (*$p < 0.05$) from CCl_4 mean).

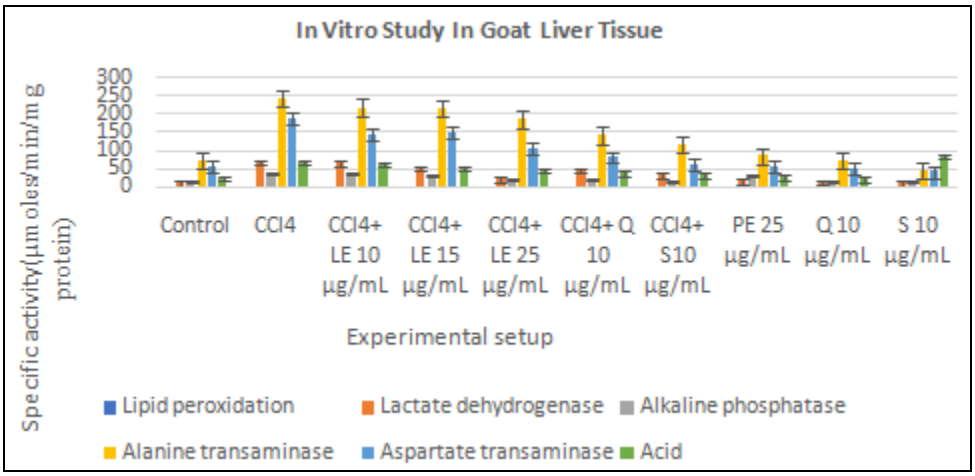

FIG. 3: EFFECT OF METHANOLIC LEAF EXTRACT OF *MURRAYA KOENIGII* QUERCETIN AND SILYMARIN TREATMENT ON RELEASE OF BIOCHEMICAL MARKERS OF HEPATOTOXICITY INDUCED BY CCL_4 IN THE GOAT LIVER TISSUE *IN-VITRO*

A significant number of medicinal plants with well-established traditional uses have been recommended for the treatment of liver disorders in the absence of reliable hepatoprotective medications in modern medicine. The hepatoprotective efficacy of *Murraya koenigii* leaf extracts was demonstrated in an *in-vitro* hepatotoxicity model in the present study. The results of our study are in agreement with those of Chaudhari and Mahajan [8]. They established that the methanolic stem bark extract of *Terminalia arjuna* inhibits lipid peroxidation caused by CCl_4 by 55% at a concentration of 100 µg/mL, while quercetin inhibits lipid peroxidation by 51% at a concentration of 10 µg/mL.

Lipid peroxidation in liver cells can be initiated by reactive oxygen species (ROS) or reactive nitrogen species (RNS), which in turn initiates a sequence of reactions that ultimately results in liver injury [27]. The liver produces a variety of enzymes, which are typically distributed within the cells of the liver. The sensitive biomarkers of liver injury are the changes in the activities of these enzymes. The functional status of the liver and the detection of liver injury are determined by the estimation of a variety of liver enzymes in serum, including AST, ALT, ALP, ACP, and LDH [28]. In comparison to the control group, the administration of CCl_4 significantly increased ($p < 0.05$) the activity of liver serum marker enzymes, including AST, ALT, ALP, ACP, and LDH. In comparison to the CCl_4-induced set, the ACP, LDH, AST, ALT, and ALP levels of the liver tissue treated with CCl_4 in the presence of methanolic leaf extract of *Murraya koenigii* (10, 15, and 25 µg/mL), Quercetin, and Silymarin (10 µg/mL) were significantly reduced ($p < 0.05$). In general, the elevated levels of ACP, LDH, AST, ALT, and ALP are reduced by the methanolic leaf extract of *Murraya koenigii*, Quercetin and Silymarin, which are based upon the concentration of the test samples.

Previous study on the mechanism of hepatic injury has suggested that cytochrome P450 in the liver endoplasmic reticulum initially metabolises CCl_4 to the highly reactive trichloromethyl (CCl_3•) radical. This radical readily reacts with O_2 to produce the peroxy trichloromethyl ($CCl_3OO^{\bullet}$) radical. The structure and function of the cell membrane are disrupted as a result of the interaction between these free radicals and cellular macromolecules, particularly unsaturated fatty acids, which is known to initiate lipid peroxidation. The present study established that the CCl_4 challenge resulted in hepatocellular injury, as evidenced by the significant increase in enzyme activities (AST, ALT, ACP, LDH, and ALP). The most sensitive markers for the diagnosis of hepatic injury are ALT, AST, ACP, LDH, and ALP, as they are found

in the cytoplasm and their deficiency occurs swiftly following cellular damage [29]. The results of this study are consistent with those of Chaudhari and Mahajan [8] and Abdel-Ghany *et al.*[26], which demonstrated extensive liver injury (as indicated by a substantial increase in ALT, AST, ACP, LDH, and ALP levels) following the administration of CCl_4. ALT is regarded as a more specific indicator of hepatic disorders due to its predominant presence in the cytosol of hepatocytes, despite its modest concentration in other tissues.AST is found in the liver, heart, skeletal muscles, brain, and pancreas, and it is present in both cytosolic and mitochondrial forms. CCl_4 administration may elicit an increase in ALT and AST levels, which may be attributed to hepatocellular injury. Furthermore, the oxidative stress induced by CCl_4 has been demonstrated to result in the depletion of adenosine triphosphate and mitochondrial dysfunction. CCl_4 was found to decrease glutathione content by enhancing the production of lipid peroxide, as reported by Abdel-Ghany *et al.*[26]. Additionally, the induction of cirrhosis results in a reduction in the number of enzymes that scavenge free radicals, including glutathione reductase, peroxidase and catalase.

The formation of free radicals, which may be more toxic than the parent compound, is a result of the metabolism of a variety of metabolites and exogenous toxic compounds (pesticides, pharmaceuticals, metals) within the hepatic tissue. CCl_4, a hepatotoxin that has been extensively studied, is converted into its metabolites, including $CCl_3^{\bullet}$ radicals, which are implicated in the pathophysiology of the liver, including cirrhosis, genotoxicity of hepatic tissue, and hepatic carcinoma [30].

The results of our current study indicate that the secretion of hepatic biomarker profiles was significantly increased as a result of hepatic lesions caused by free radicals in liver slice cultures that were exposed to CCl_4. CCl_4 induces hepatotoxicity, which results in a 6.75, 3.34, 3.36, 2.85, and 2.59-fold increase in the levels of biochemical markers LDH, ALT, AST, ACP, and ALP in the culture medium compared to the control. In terms of biochemical markers ALT, AST, ACP, and ALP, the hepatotoxicity of CCl_4 is significantly diminished by silymarin, quercetin, and varying concentrations of methanolic leaf extract of *Murraya koenigii*. Nevertheless, the leaf extract (25 µg/mL) significantly reduces the level of LDH in the culture medium that is elevated by CCl_4 toxicity, in comparison to silymarin and quercetin, thereby demonstrating its function in cardio protection. The medium's elevated levels of LDH and other hepatic biomarkers, such as AST, ALT, ACP, and ALP, were reduced by the treatment of liver slice culture with CCl_4 in conjunction with methanolic leaf extract of *Murraya koenigii*, Quercetin, and Silymarin. This suggests that the hepatoprotective action of the compounds was demonstrated. Quercetin, the most prevalent of the flavonoids, is composed of three rings and five hydroxyl groups. Quercetin is a member of the flavanones class of flavonoids and serves as the structural foundation for numerous other flavonoids, such as the citrus flavonoids rutin, hesperidins, Naringenin, and tangeritin. It is present in a wide variety of plant species, including rinds, foliage, and barks. Quercetin was demonstrated to have a protective effect on reperfusion-induced ischaemic tissue injury by functioning as a free radical scavenger.

Quercetin mitigates tissue injury caused by free radicals through a variety of mechanisms. The direct scavenging of free radicals is one method. Flavonoid, specifically Quercetin, can inhibit LDL oxidation *in-vitro* by scavenging free radicals. Quercetin has been reported to possess hepatoprotective properties against ethanol-induced liver injury by means of antioxidant activity, anti-inflammatory effects, down-regulation of CYP2E1 and CYP3A, and an increase in glutathione levels [31].

The metabolism of this xenobiotic results in the generation of free radicals, which is one of the mechanisms by which alcohol causes liver injury. Silymarin is an antioxidant that safeguards the liver from the free radical damage generated by alcohol metabolism. Silymarin is the most frequently used natural compound for the treatment of hepatic disorders worldwide as a result of its antioxidant, anti-inflammatory, and anti-fibrotic properties. Silymarin enhances protein synthesis and stabilises biological membranes [32]. In addition to these medications, the methanolic leaf extract of *Murraya koenigii*, which was concentrated at 25

μg/mL, demonstrated potential *in-vitro*. Quercetin and silymarin are natural hepatoprotective agents. The presence of flavonoids, as well as its antioxidant and anti-inflammatory properties, is likely responsible for the hepatoprotective effects observed in 9 of 10 goat liver slices that were affected by CCl_4-induced liver injury.

CONCLUSIONS: The results of the current study suggest that the methanolic leaf extract of *Murraya koenigii* has a hepatoprotective effect against hepatic injury induced by CCl_4. The flavonoids and antioxidant properties of *Murraya koenigii* leaf may be responsible for its hepatoprotective effects. It is evident that flavonoids generate antioxidant activity; consequently, this mechanism suggests that the plant extract may mitigate liver injury caused by oxidative stress. Our observations of *in-vitro* hepatoprotection substantiate and enhance the discoveries of previous researchers. As a result, it is necessary that this plant be documented for its wide popularity as a natural medicine.

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