***Original Research Article***

**Ameliorative potential of S*olanum nigrum* leaf extract on some biochemical markers in CCl4-induced hepatotoxicity in rats.**

**Abstract**

Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics. This study was designed to evaluate the hepatoprotective effects of S*olanum nigrum* aqueous leaf extract on carbon tetrachloride (CCl4)-induced hepatotoxicity in rats. Thirty male albino rats divided into six groups of five animals per group namely: non hepatotoxic control, hepatotoxic control (untreated hepatotoxic group) and hepatotoxic treated groups. Animals in group two received 1.5ml/kg bw of CCl4 intraperitoneally alone. Groups three, four, five and six received 1.5ml/kg bw of CCl4 and in addition 200mg/kg of silymarin (standard drug), 50mg/kg S*olanum. nigrum* aqueous leaf extract, 100mg/kg ofS*olanum. nigrum* aqueous leaf extract and 200mg/kg S*olanum. nigrum* aqueous leaf extract by oral gavage respectively for 14 days. Activities of liver function indices were determined. Tissue antioxidant level of reduced glutathione (GSH), activities of the antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) were also assessed. Induction by CCl4 caused significant increase in the activities of biomarkers and decrease in the activities of the tissue antioxidant enzymes when compared to the control. Significant (P < 0.05) decrease in activity/level of liver function indices and improved antioxidant status in the treatment groups were observed by the intervention of the extracts when compared to the induced group without treatment. Histological examinations of the liver showed CCl4-induced hepatotoxicity. Histology of the liver revealed altered cellular architecture (poor architecture), moderate to severe congesteion of the portal vein as well as severe peri portal infiltration of inflammatory cells. The liver parenchyma also showd area with destroyed liver plates coupled with severe hemorrhage and necrosis. Normal histological features were restored after treatment with aqueous leaf extract of S*olanum nigrum* aqueous leaf extract. The study has demonstrated that aqueous leaf extract of S*olanum nigrum* ameliorates liver of albino rats against CCl4-induced toxicity and modulates the adverse effects of CCl4 on the liver.

**Keywords:** **S*olanum nigrum,* Carbontetra chloride, Hepatoprotective, Biochemical Markers**

**Introduction**

Liver is an organ in the upper abdomen that aids in digestion and removes waste products and worn-out cells from the blood. It is a vital organ present in vertebrate and some other animals, which has a wide range of functions including detoxification and protein synthesis. The liver is our greatest chemical factory, it builds complex molecules from simple substances absorbed from the digestive tract, it neutralizes toxins, it manufactures bile which aids fat digestion and removes toxins through the bowels (Maton *et al.,*1993). But the ability of the liver to perform these functions is of then compromised by numerous substances we are exposed to on a daily basis; these substances include certain medicinal agents which when taken in over doses and sometimes when introduced within therapeutic ranges injures the organ (Gagliano *et al.,* 2007).

Liver disease is worldwide problem. Conventional, drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Therefore, it is necessary to search for alternative drugs for the treatment of liver disease in order to replace currently used drugs of doubtful efficacy and safety (Ozbek *et al*., 2004). In the absence of reliable liver-protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders. This however has drawn a lot of interest and attention to the curative claims and norms of medicinal plants and other sources all over the world, especially in under developed countries in Africa and some parts of Asia (Gagliano *et al*., 2007).

Medicinal plants have been used by humans for centuries in folklore medicine (Sermakkani, 2012) *Solanum nigrum* is an important plant in traditional medicines which belong to the family of solanaceae. It is used in hepatitis, fever, dysentery, and stomach complaint (Jain *et al*., 2011). The juice of the plant is used on ulcers and other skin diseases. The fruits are used as a laxative, appetite stimulant, and for treating asthma and "excessive thirst". Traditionally the plant is used to treat tuberculosis (Zakaria *et al.,* 2006). This study was designed to investigate the hepatoprotective effect of the aqueous bark extract of *Solanum nigrum* on the Carbon tetra chloride induced liver damage.

**Materials and Methods**

**Collection of sample**

Plant materials, *Solanum nigrum*, leaves were collected from the nearby farm around the hostels at Federal Polytechnic Ede, Nigeria in the month of May, 2022, air- dried in the laboratory, pulverized and then stored in an airtight container.

**Reagents and Chemicals**

All chemicals and all other reagents were all of analytical grade.

**Preparation Extracts**

*Solanum nigrum* leaves were washed with sterile water and allowed to drain and air-dried for 23 days at room temperature. The air-dried samples were ground to fine powder using a blender. 500g of sample was soaked in 1.5L of ethanol for three days. This was filtered and later air-dried to obtain the extracts powder. The extract was kept in the freezer at 4 0C for further studies.

**Animals protocol**

25 wistar albino rats weighing 150 kg – 170 kg were obtained from the animal house at The Federal Polytechnic, Ado Ekiti, Ekiti State, housed in clean wire meshed cages under standard conditions temperature (24 ± 1oC), relative humidity, and 12 / 12-hour light and dark cycle. They were allowed to have free access to food (commercial pelletized diet from Vital Feed Mill) and drinking water *ad libitum* daily. The rat beddings were changed and replaced every day throughout the experimental period.

**Experimental Design**

30 male wistar albino rats were randomly divided into seven groups (I-VI) of five animals in each group.

**Animal treatment**

The animal treatment is shown in the table below

**Table 1:** Animal treatment

|  |  |
| --- | --- |
| **Groups** | **Treatment** |
| Group 1: Normal control (NC) | Distilled water only for 14 days |
| Group 2: Induced control (IC) | 1.5ml/kg bw CCl4 alone for a single administration |
| Group 3 | 3ml/kg bw CCl4+ 200mg/kg Silymarin for 14 days |
| Group 4 | 3ml/kg bw CCl4+ 50 mg/kg *Solanum nigrum* leaf extract for 14 days |
| Group 5 | 3ml/kg bw CCl4+ 100mg/kg *Solanum nigrum* leaf extract for 14 days |
| Group 6 | 3ml/kg bw CCl4+ 200 mg/kg *Solanum nigrum* leaf extract for 14 days | Group 5 | 3ml/kg bw + 100 ml/kg *Morinda lucida* leaf extract for 14 days |

**Dissection of Rats**

The rats were dissected and portion of blood was collected into plain bottles for determination of biochemical parameters.

**Preparation of Serum**

Serum was prepared by centrifugation at 3000 rpm for 15 min at 25⁰C. The clear supernatant was collected and used for the estimation of serum biochemical parameters.

**Preparation of Homogenates**

The liver was excised using scissors and forceps. It was washed in buffer solution, blotted with filter paper and weighed. They were then chopped into bits and homogenized in ten volumes of the homogenizing phosphate buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenates were centrifuged at 3000 rpm at 4°C for 30 mins. The supernatant obtained was collected and stored under 40C and then used for biochemical analyses.

**Antioxidant assay**

**Determination of Catalase Activity**

This experiment was carried out using the method described by Sinha (1972).

**Determination of Superoxide Dismutase (SOD) Activity**

The level of SOD activity was determined by the method of Misra and Fridovich (1972).

**Determination of Reduced Glutathione (GSH) Level**

The method of Beutler *et al.,* (1963) was followed in estimating the level of reduced glutathione (GSH).

**Determination of Total Protein (TP) in Serum**

The Biuret method described by Weichselbaum (1995) was employed in the determination of total protein in the serum using commercially available kits (Randox laboratories, UK).

**Liver Function Indices**

**Assay of Aspartate Aminotransferase (AST) Activity**

AST activity was determined following the principle described by Reitman and Frankel (1957).

**Assay of Alanine Amino transferase (ALT) Activity**

The principle described by Reitman and Frankel (1957) was followed in the assay of ALT using commercially available assay kit (Randox laboratories, UK) according to the instructions of the manufacturer.

**Assay of Alkaline Phosphatase (ALP) Activity**

Assay of serum ALP was based on the method of (Englehardt *et al*., 1970) using commercial assay kits (Randox laboratories, UK) according to the instructions of the manufacturer.

**Assay of Albumin (ALB) Activity**

Albumin activity was determined following the principle described by Grant *et al* 1987.

**Assay of Bilirubin (BIL)**

Bilirubin activity was determined following the principle described by Jendrassik and Grof 1938 and modified by Sherlock 1951.

**Principle**

Colorimetric method based on that described by Jendrassik and Grof (1938). Direct (conjugated) bilirubin reacts with diazotised sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin by the reaction with diazotized sulphalinic acid.

**Procedure**

200µl of diluted sample , Reagent 1(Sulphalinic acid), R2 (Nitrite) and R3 (Caffeine) were mixed and allowed to stand 10min at 20oC-25 oC. Then 200 µl Reagent R4 (tartarate) was mixed and allowed to stand for 5-30 min at 20oC-25 oC . The absorbance of the sample against the sample blank was read at 578nm.

Total Bilirubin (mg/dl) = 10.8 x ATB

**Statistical Analysis**

All values are expressed as mean ± SD. Statistical evaluation was done using One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using SPSS 11.09 for windows (Anthony and Richard, 2006). The significance level was set at p < 0.05.

**Results and Discussion**

**Table 2. Serum Biomarkers**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Conc./ Parameters** | **ALP(mg/dl)** | **ALT(U/L)** | **AST(U/L)** | **CK(U/I)** | **ALB(mg/dl)** | **BIL(mg/dl)** |
| A | 38.10±3.05d | 52.39±2.17e | 20.19±1.06 e | 18.30±1.03e | 42.60±1.04a | 65.92 ±1.29e |
| B | 65.96±1.68a | 85.72±2.02a | 44.60±1.33a | 32.06±0.64a | 24.66±0.59e | 106.21±2.06a |
| C | 37.02±1.19e | 54.33±1.28d | 22.31±0.72d | 19.63+0.77d | 40.53+0.60b | 71.53±1.60d |
| D | 54.35±2.14b | 65.32±1.14b | 36.27±1.30b | 25.52±2.18b | 29.08±1.31d | 90.46±1.44b |
| E | 45.41±1.87c | 58.63 ± 2.09c | 28.50±1.12c | 23.75±0.78c | 34.22±0.58c | 81.27±2.11c |
| F | 45.41±1.87c | 58.63 ± 2.09c | 28.50±1.12c | 23.75±0.78c | 34.22±0.58c | 81.27±2.11c |

Values are expressed as mean ± standard deviation (n=5). Values with the different superscript(s) in a column are significantly different (P<0.05).

**Key:**

ALP = Alkaline phosphatase

ALT = Alanine amino transferase

AST = Aspartate amino transferase

CK = Creatinine kinase

ALB = Albumin

BIL = Bilirubin

A=control, B=3ml/kg bw CCl4 alone, C= 3ml/kg bw CCl4 + 200mg/kg silymarin, D=3ml/kg bw CCl4 + 50mg/kg *S. nig*, E=3ml/kg bw CCl4 + 100mg/kg, F=3ml/kg bw CCl4 + 200mg/kg

**Table 3. Liver biomarkers**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Conc.** | **ALP(mg/dl)** | **ALT(U/L)** | **AST(U/L)** | **GGT(mg/dl)** | **ALB(mg/dl)** | **BIL(mg/dl)** |
| A | 56.73 ±1.55f | 87.66 ± 2.13f | 65.54±1.29f | 7.22±0.20e | 51.35±2.06a | 28.32 ± 1.67f |
| B | 91.60±1.69a | 130.94±3.43a | 102.63±3.08a | 10.09±0.42a | 24.75±0.83f | 52.14± 1.80a |
| C | 58.87+2.04e | 90.20+2.10d | 68.40+1.39e | 7.45+0.38d | 47.28±1.48b | 30.73+1.23e |
| D | 78.08±3.19b | 113.27±3.00b | 85.10±2.27b | 9.80±0.52b | 28.68±0.87e | 43.29±2.07b |
| E | 67.24±2.40c | 95.23±2.17c | 79.68±2.05c | 8.34±0.63c | 36.73±1.20d | 37.20±1.36c |
| F | 61.31±1.31d | 88.52±2.04e | 71.29±1.55d | 7.75±0.56d | 46.84±1.43c | 31.49±1.27d |

Values are expressed as mean ± standard deviation (n=5). Values with the different superscript(s) in a column are significantly different (P<0.05).

**Key:**

ALP = Alkaline phosphatase

ALT = Alanine amino transferase

AST = Aspartate amino transferase

GGT = Gamma glutamyl transferase

ALB = Albumin

BIL = Bilirubin

A=control, B=3ml/kg bw CCl4 alone, C= 3ml/kg bw CCl4 + 200mg/kg silymarin, D=3ml/kg bw CCl4 + 50mg/kg *S. nig*, E=3ml/kg bw CCl4 + 100mg/kg, F=3ml/kg bw CCl4 + 200mg/kg

Carbon tetrachloride-induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of medicinal plants and drugs (Ugwu and Suru, 2021). Drug-induced liver injury (DILI) is a common condition that can be triggered by nearly all categories of medications. Most instances of mild DILI improve following the cessation of the drug (Roy *et al.,* 2024).

Plants synthesize polyphenols as a defense mechanism against stress ([Tuladhar *et al.*, 2021](https://pmc.ncbi.nlm.nih.gov/articles/PMC12124805/#ref30)). Hence, the present research was designed to evaluate the hepatoprotective effects of S*olanum nigrum* leaf extract extract in CCl4-induced liver damage.

Assessment of liver function can be made by estimating the activities of serum ALT, AST, ALP and bilirubin which are enzymes originally present in high concentration in cytoplasm (Lala *et al*., 2023). When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage (Kalas *et al*., 2021). When rats were treated with carbon tetrachloride, it induced hepatotoxicity by metabolic activation, therefore, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. Carbon tetrachloride is metabolically activated by the cytochrome P-450 dependent mixed oxidase in the endoplasmic reticulum to form trichloromethyl free radical (CCl3) which combines with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation. These result in changes of structures of the endoplasmic reticulum and other membranes, loss of metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation, leading to liver injury (Kalas *et al*., 2021). These may explain what happened in the CCl4-treated groups in the present study.

Oxidative stress is defined as an imbalance in the oxidant-to-antioxidant ratio, causing the generation of free radicals (Palsamy *et al*., 2010). The production of oxidants such as ROS-like superoxide anions, hydrogen peroxide and hydroxyl radicals by activated Kupffer cells has been identified as central to hepatic injuries (Wei *et al*., 2010). Oxidative stress is linked with numerous health problems, including atherosclerosis, ischemic reperfusion injury, inflammation, cancer, aging, and neurological disorders. Although these conditions have different causes, research indicates that ROS can cause biological damage, that can initiate or worsening these diseases (Checa and Aran, 2020). Aerobic organisms encounter challenges from ROS, which are integral to plant communication ([Mansoor *et al.*, 2022](https://pmc.ncbi.nlm.nih.gov/articles/PMC12124805/#ref20)).

Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of a known disease and determine the effects of potentially hepatotoxic drugs (Harris, 2005). The antioxidant properties of *S. nigrum* Ethanolic Leaf Extracts were assessed using the DPPH assay, which revealed a more potent free radical scavenging ability of the plant. This aligns with previous studies that identified compounds such as carotenoids, phenolics, flavonoids, and tannins as contributors to the antioxidant activity of *S. nigrum* ([Uka *et al.*, 2020](https://pmc.ncbi.nlm.nih.gov/articles/PMC12124805/#ref31); [Mani *et al.*, 2022](https://pmc.ncbi.nlm.nih.gov/articles/PMC12124805/#ref19)). Plant-based products have the ability to protect against and reduce damage from chemicals in the liver and kidneys ([Guan and He, 2020](https://pmc.ncbi.nlm.nih.gov/articles/PMC12124805/#ref10)). A previous study showed excellent antioxidant performance when *S. nigrum* leaf was administered after stress ([Mukhopadhyay *et al.*, 2020](https://pmc.ncbi.nlm.nih.gov/articles/PMC12124805/#ref22)). Another research showed that 50% ethanol extract of the whole *S. nigrum* plant demonstrated hydroxyl radical scavenging potential, suggesting a cytoprotective mechanism ([Zaghlool](https://pmc.ncbi.nlm.nih.gov/articles/PMC12124805/" \l "ref33)*[et al.](https://pmc.ncbi.nlm.nih.gov/articles/PMC12124805/" \l "ref33)*[, 2022](https://pmc.ncbi.nlm.nih.gov/articles/PMC12124805/" \l "ref33)).

Induction by carbon tetrachloride (CCl4)significantly increase the activity of biomarker-enzymes alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and the level of bilirubin, while the level of albumin was decreased in an untreated group compared to the control. The observed decrease in the activity of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and the level of bilirubin, as well as increase in the level of albumin in S*olanum nigrum* leaf extract treated rats compared to the untreated groups reflects the antioxidant potential of ethanolic extracts of S*olanum nigrum* leaf. Furthermore, bilirubin which is one of the most useful tools for diagnosing the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of the hypatocytes (Guerra Ruiz *et al*., 2021; Ramírez-Mejía *et al*., 2024). Decrease in serum bilirubin after treatment with *S.nigrum* in liver damage induced by CCl4, indicated the effectiveness of the extract in normal functional status of the liver. However, reduction of ALT and bilirubin levels points towards an early improvement in the secretary mechanism of the hepatic cells. The therapeutic effect of any hepatoprotective drug depends on its ability to either reduce the harmful effect or restore the normal hepatic physiology that has been disturbed by a hepatotoxin. The plant extract decreased CCl4-induced elevated enzyme levels in tested groups, this indicates the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells. The high enzyme activity in the CCl4-treated group than in the control group, indicates possible liver and kidney damage. However, *S. nigrum* administration at all doses significantly reduced the enzyme activities showing its hepatoprotective nature.

**Conclusion**

In conclusion, the results demonstrated that *S. nigrum* exhibits antioxidant activity and protects against CCl4-induced hepatotoxicity. S*.nigrum* leaf extract significantly reduces ALT, ALP and AST activities, and mitigates hepatocyte damage induced by CCl4 exposure. These findings underscore the potential of *S. nigrum* as a safe, effective, and promising natural herbal medicine; therefore, its potential therapeutic properties can contribute to medical and pharmaceutical practices in the treatment of liver diseases. Further research is needed to fully understand the mechanisms by which *S. nigrum* protects against cellular damage and also assess toxicity and appropriateness of dosages that are safe when used.

**References**

Beutler, E., Duran, O. and Kelly, M.B. (1963). Improved method for the determination of blood glutathione. *Journal of Laboratory Clinical Medcine*; **61**: 882-890.

Englehardt A.*.,* Aerztl Labor. (1970): 16 42.

Gagliano, N., F. Grizzi and G. Annoni, 2007. Mechanism of aging and liver functions. *Digest. Dis. Sci.,* 25:118-123

Grant G. H (1987: Amino acids and proteins; fundamentals of clinical chemistry. Tietz N.W. editor, third Edition, W. B Saunders company Philadelphia USA pg 382-329.

Guerra Ruiz, A. R., Crespo, J., López Martínez, R. M., Iruzubieta, P., Casals Mercadal, G., Lalana Garcés, M., and Morales Ruiz, M. (2021). Measurement and clinical usefulness of bilirubin in liver disease. *Advances in Laboratory Medicine/Avances en Medicina de Laboratorio*, *2*(3), 352-361.

Hansen, T. W., Wong, R. J., & Stevenson, D. K. (2020). Molecular physiology and pathophysiology of bilirubin handling by the blood, liver, intestine, and brain in the newborn. *Physiological reviews*, *100*(3), 1291-1346.

Harris, E. H. (2005): Elevated liver function tests in type 2 diabetes. Clinical Diabetes, 23(3), 115–119.

Jain R, Sharma A, Gupta S, Sarethy I, P, and Gabrani R. (2011): *Solanum nigrum*: Current Perspectives on Therapeutic Properties. *Alternative Medicine Review, LLC*.; 16(1):78-85.

Jendrassik*, L. and* Grof*, P.* (1938): Simplified Photometric Methods for the Determination of Bilirubin. *Biochemical Journal*, 297; 81-89.

Jomova K., Raptova R., Alomar S.Y., Alwasel S.H., Nepovimova E., Kuca K., Valko M. (2023) Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Arch. Toxicol.;97(10):2499–2574.

Kabbashi A.S., Eltawaty S.A., Ismail A.M., Elshikh A.A., Alrasheid A.A., Elmahi R.A., Koko W.S., Osman E.E. (2024) Ethanolic extract of Mangifera indica protects against CCl4-induced hepatotoxicity via antioxidant capabilities in Albino rats. J. Toxicol.;2024:5539386.

Kalas, M. A., Chavez, L., Leon, M., Taweesedt, P. T., and Surani, S. (2021). Abnormal liver enzymes: A review for clinicians. *World journal of hepatology*, *13*(11), 1688.

Kumari, K., Pahuja, S. K., & Kumar, S. (2023). An evolution of bilirubin physiology and analysis. *Current Signal Transduction Therapy*, *18*(2), 1-13.

Lala, V., Zubair, M., and Minter, D. (2023). Liver function tests. *StatPearls*.

Mani R.K., Paramashree J., Bharathi D., Ahmed S. (2022) The traditional and pharmacological properties of Solanum nigrum: a review. Int. J. Toxicol.

Mansoor S., Ali Wani O., Lone J.K., Manhas S., Kour N., Alam P., Ahmad A., Ahmad P. (2022) Reactive oxygen species in plants: from source to sink. Antioxidants. 2022;11:225.

Marzinke, M. A., & Dufour, D. R. (2020). Laboratory diagnosis of liver disease. In *Contemporary Practice in Clinical Chemistry* (pp. 545-559). Academic Press.

Maton, A., H. Jean, C. W. McLaughlin, M.Q. Warner, D. Lattart and J. D. Wright, (1993): Human Biology and Health. Eaglewood Cliffs, New Jersey, Prentice Hall, USA.

Melaram R. (2021) Environmental risk factors implicated in liver disease: a mini-review. Front. Public Health. 9:1–7.

Ozbek, H. S., I. Ugras, I. U. Bayram and E. Erdogan, (2004): Hepatoprotective effect Foeniculum vulgare essential oil: A carbon tetrachloride induced liver fibrosis model in rats. *Scand. J. Anim. Sci*., 31: 9-17.

Palsamy P, Sivakumar S, Subramanian S. (2010): Resveratrol attenuates hyperglycemia-mediated oxidative stress, proinflammatory cytokines and protects hepatocytes ultrastructure in streptozotocin-nicotinamide-induced experimental diabetic rats. *Chem Biol Interact*. 86:200–10.

Ramírez-Mejía, M. M., Castillo-Castañeda, S. M., Pal, S. C., Qi, X., & Méndez-Sánchez, N. (2024). The multifaceted role of bilirubin in liver disease: a literature review. *Journal of Clinical and Translational Hepatology*, *12*(11), 939.

Rawal, R., Kharangarh, P. R., Dawra, S., Tomar, M., Gupta, V., & Pundir, C. S. (2020). A comprehensive review of bilirubin determination methods with special emphasis on biosensors. *Process biochemistry*, *89*, 165-174.

Reitman S. and Frankel S, (1957): *Amer. J. Clin. Path*.,28:56.

Roy, S., Shah, Z., & Chakraborthy, G. S. (2024). A brief overview of drug-induced liver damage. *The Egyptian Journal of Internal Medicine,* 36(1), 56.

Sermakkani M. V, and Thangapandian. (2012): GC-MS Analysis of *Cassia italica* Leaf

methanol extract. *Asian Journal of Pharmaceutical and Clinical Research*; 5(2). 57-72.

Sinha, A. K. (1972): Colorimetric assay of catalase. *Journal of Analytical Biochemistry*; **47**:389

-394.

Supraja G., Maheswari K.C., Pamarthy D., Saritha K.V.R. (2022) Phytochemicals for

hepatocellular carcinoma therapy: from in vitro to clinic. *Thera. Precision Medi. Manag.*

*Hepato. Carcin.3:109–132.*

Tuladhar P., Sasidharan S., Saudagar P. (2021) Role of phenols and polyphenols in plant

defense response to biotic and abiotic stresses. In: Jogaiah S., editor. Biocontrol agents and

secondary metabolites. Darya Ganj, Delhi: Woodhead Publishing. pp. 419–441.

Uka E., Etim O.E., Effiong A.O., Jacobs I.E. (2020) Phytochemicals, acute toxicity and in-vitro

antioxidant activity of ethanol extract of Sphenocentrum jollyanum leaves. Journal. Drugs

Pharm. Sci; 4:10–20.

Ugwu, C. E., and Suru, S. M. (2021). Medicinal plants with hepatoprotective potentials against

carbon tetrachloride-induced toxicity: a review. *Egyptian Liver Journal*, *11*(1), 88.

Wei Y, Chen P, de Bruyn MD, Zhang W, Bremer E, and Helfrich W. (2010): Carbon monoxide-releasing molecule-2 (CORM-2) attenuates acute hepatic ischemia reperfusion injury in rats. *BMC Gastroenterol*.;10:42.

Weichselbaum, R. R. and Hellman, S. (1995): Oligometastase. *Journal of Clinical*

Biochemistry; 13: 8-10.

Zhang, Y., Luan, H., & Song, P. (2025). Bilirubin metabolism and its application in disease prevention: mechanisms and research advances. *Inflammation Research*, *74*(1), 81.

Zakaria ZA, Gopalan HK, and Zainal H. (2006): Anti-nociceptive, anti-inflammatory and

antipyretic effects of *Solanum nigrum* chloroform extract in animal models. *Yakugaku Zasshi*.;

126:1171-1178.