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Comparative Evaluation of Herbal Extract in Ameliorating of Fluoride Induced Hepatotoxicity in Rats.

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Abstract- ***Fluoride exposure at toxic levels produces marked hepatotoxicity manifested by oxidative stress, inflammation, and structural liver injury. This study evaluated the comparative hepatoprotective effects of Phyllanthus niruri extract and curcumin against fluoride-induced liver damage in Wistar rats, with silymarin serving as the standard reference drug. Hepatotoxicity was induced by oral administration of sodium fluoride for 28 days, followed by concurrent treatment with Phyllanthus niruri, curcumin, or silymarin. Fluoride significantly elevated serum liver enzymes (ALT, AST, ALP) and bilirubin. Histological evaluations revealed hepatocellular necrosis, sinusoidal dilation, inflammatory infiltration, and fatty degeneration. Co-administration of the herbal treatments markedly ameliorated these biochemical and histopathological alterations in a dose-dependent manner. Curcumin demonstrated the greatest antioxidant and anti-inflammatory efficacy, closely followed by Phyllanthus niruri, both approaching the protective effects of silymarin. These findings indicate that curcumin and Phyllanthus niruri possess significant potential in mitigating fluoride-induced hepatotoxicity and may serve as promising natural alternatives for hepatoprotection.***

Index Terms ***Fluoride Toxicity, Hepatotoxicity, Oxidative Stress, Silymarin, Curcumin, Phyllanthus Niruri, Antioxidant Enzymes, Liver Histopathology, Herbal Hepatoprotective Agents.***

I. INTRODUCTION

Because of its smallest size and highest electro negativity, fluoride (F) is extremely important.[1]. Numerous nations in Asia, Africa, Australia, and South America suffer greatly from fluorosis, making it a global concern. It has been common in India for 60 years and is brought on by consuming too much fluoride[2]F is an anion that, under some circumstances, can easily pass through cell membranes through simple diffusion and almost negatively impact cell metabolism and function. The imbalance between the generation and removal of free radicals causes oxidative stress, one of the recognized mechanisms of F toxicity[3]. Under normal circumstances, numerous antioxidative defense components can scavenge reactive oxygen species (ROS), a consequence of metabolism. Oxidative stress is the result of an imbalance between ROS and antioxidants. It is well known that fluoride inhibits antioxidant enzymes, which in turn encourages the buildup of ROS. In the mouse kidney, NaF-altered ROS generation levels and antioxidative parameters have been consistently noted. According to reports, exposure to fluoride might cause oxidative stress in the liver.[4]. Fluoride impaired liver function by inhibiting several antioxidant enzymes and molecules, increasing blood indices of liver function tests, and reducing metabolic processes like glycolysis, oxidative phosphorylation, and lipid peroxidation. Additionally, it disrupts the lipid profile by raising intracellular superoxide radical levels. Hens given a feed containing sodium fluoride exhibited either mild or severe liver enlargement. According to certain research, exposure to sodium fluoride decreased the amount of protein in the liver and serum.[5]

1.1 MECHANISMS OF FLUORIDE INDUCED HEPATOTOXICITY

By interfering with the endoplasmic reticulum stress pathway, excessive fluoride can cause oxidative stress in ameloblasts, resulting in a high production (ROS) and cell death. Furthermore, via triggering IL-17A pathway, too much fluoride can induce mitochondrial autophagy and damage in mice liver cells. Clarifying the precise process of fluorosis-

induced liver damage is crucial since the liver has been identified as one of the major target organs of fluorosis due to recent in-depth research on the disease's mechanism[6]. An imbalance between the body's generation and re-neutralization of free radicals is known as oxidative stress. Mitochondria are thought to be the primary site of oxidative phosphorylation and are crucial in inducing apoptosis. It is widely known that the intrinsic apoptotic pathway can be triggered by mitochondrial malfunction brought on by excessive ROS production.[7]

1.2 EXPOSURE OF FLUORIDE

1) An imbalance between the body's generation and re-neutralization of free radicals is known as oxidative stress.
2) Mitochondria are thought to be the primary site of oxidative phosphorylation and are crucial in inducing apoptosis.
3) It is widely known that the intrinsic apoptotic pathway can be triggered by mitochondrial malfunction brought on by excessive ROS production.[8]

1.3 FLUOIRDE ACCUMULATION IN TISSUE

Results from short-term studies utilizing radioactive fluoride in lab animals show that intracellular fluoride concentrations are 10–50% lower than those of plasma, yet they change concurrently and proportionately to those of plasma. The tissue-to-plasma ratios of radioactive fluoride support the hypothesis that HF is the condition in which fluoride migrates and establishes diffusion equilibrium across cell membranes. Because most cell membranes include a pH gradient that can be altered by changing extracellular pH, it is feasible to increase the net flux of fluoride into or out of cells. This is the justification for the suggestion that alkalinizing body fluids may help treat acute fluoride toxicity.[9]

1.4 How fluoride affect hepatic structure and functions

Long-term excessive fluoride consumption can result in fluorosis, which can have skeletal and dental symptoms. Chronic fluorosis has been shown to induce metabolic, functional, and structural damage in a variety of tissues; however, the precise pathways that fluoride modulates are still unknown. The was to assess the impact of fluoride in drinking water on rats' antioxidant defense system. Long-term excessive fluoride consumption can result in fluorosis, which can cause symptoms in the teeth and skeleton. Chronic fluorosis has been shown to induce metabolic, functional, and structural damage in a variety of tissues; however, the precise pathways that fluoride modulates are still unknown. This study's objective was to assess the impact of fluoride given [10]. Exposure Fluoride interferes with collagen synthesis. and causes it to break down in the lungs, kidney, trachea, bone, tendon, muscle, skin, and cartilage. According to a different study, fluoride promotes granule production Considering the amount of oxygen that white blood cells use,but when a foreign chemical in the blood challenges the white blood cell, these processes are inhibited. Fluoride depletes white blood cells' energy stores and their capacity to effectively eliminate foreign substances through phagocytosis. Fluoride at concentrations as low as 0.2 parts per million causes resting white blood cells to produce more superoxide, which essentially eliminates phagocytosis. White blood cells' capacity to eliminate pathogenic agents may be significantly reduced by fluoride concentrations as little as 1 ppm. Fluoride raises the growing rate of tumors in people who are susceptible to cancer and, causing it to target. Fluoride decreases the production of antibodies in the blood, according to another significant study. Fluoride can occasionally reduce thyroid function. The body's tissues are disrupted by fluorides. According to a study, fluoride encourages the growth of bone cancer. The human body ages prematurely due to fluorides. Children who consume fluoride from mouthwashes and dentifrices run a serious risk to their overall health, life expectancy, and biological development. A study found that fluoride concentrated in different tissues, including the thyroid, bone, and teeth, and rose rapidly in the blood. [11].

II. ROLE OF HERBAL EXTRACT IN HEPATIC PROTECTION

Numerous plants have been demonstrated to have antioxidant, anticholestatic, antinecrotic, antifibrotic, and anticancer qualities that are beneficial for

hepatocellular carcinoma, cirrhosis, fibrosis, steatosis, and viral hepatitis, among other liver illnesses. Curcuma longa, Silybum marianum, quercetin, naringenin, coffee, Stevia rebaudiana, resveratrol, l-theanine, hesperidin, colchicine, Rosmarinus officinalis, and glycyrrhizin are some of the most researched plants, plant-derived chemicals, and extracts.[12] Liver disorders have traditionally been treated with herbs and herbal remedies. Numerous plants include components with different modes of action and bioactivities that could be used as medicine to treat liver problems. Nonetheless, a few of them have been thoroughly investigated for their bioactive components and hepatoprotective activity mechanism.[13].

2.1 GENERAL MECHANISM OF HEPATOPROTECTION BY HERBS

The main reasons herbal medicines are utilized are that they are less costly than conventional drugs, have less adverse drug reactions, making them safer, and have fewer side effects. Immunomodulation, hepatic DNA synthesis stimulation, glutathione reductase and superoxide dismutase simulation to prevent oxidation in hepatocytes, reduction of intracellular reactive oxygen species by increasing antioxidant levels, inhibition of nucleic acid polymerases to suppress viral mRNA transcription and translation, free radical scavenging, and reduction of hepatic fibrosis by lowering transforming growth factor beta-1 levels. To discover, describe, and standardize the active substances, beneficial chemicals, and their formulations, more research is necessary.[14]. Plant-based treatments were primarily used to treat liver ailments, and modern pharmaceuticals have little to offer in terms of hepatic disease relief. However, there were few drugs available to treat liver problems (19, 20). As a result, numerous traditional plant-based medicines were examined in experimental animal models for their potential to prevent liver damage and act as antioxidants.[15]. They identified Hepatoprotective phytoconstituents include phenyl compounds, coumarins, essential oils, steroids, alkaloids, monoterpenoids, diterpenoids, and triterpenoids. Natural substances derived from plants have long been employed as hepatoprotective agents. For example, the most studied substance with strong anti-hepatotoxic properties is silymarin, which comes from the seeds of Silybum marianum (L.) Gaertner (Asteraceae). Silymarin is created by combining the isomeric flavolignans silybin, silydianin, and silychristen. The impact of these pure compounds on the liver must be examined using both in vitro and in vivo models.[16]

2.3 COMPARISON OF HERBAL DRUG

The preventive effects of Phyllanthus niruri and curcumin against fluoride-induced liver damage in albino rats were compared. The evaluation concentrated on how well these substances might block fluoride-induced changes in liver histology and blood levels of important hepatic enzymes, such as alkaline phosphatase (ALP), alanine aminotransferase (GPT), and aspartate aminotransferase (GOT). When either Phyllanthus niruri or curcumin extracts were administered before or after fluoride exposure, the liver damage usually caused by fluoride was considerably lessened, as seen in both tissue structure and enzyme levels. But Phyllanthus niruri showed a stronger hepatoprotective effect when compared to the other.Fluoride treatment showed little effect on liver architecture and serum enzyme levels in rats that were pre-treated with Phyllanthus niruri for 28 days. On the other hand, fluoride still resulted in significant increases in serum enzyme activity—roughly 32% for GPT, 16% for GOT, and 26% for ALP—even with a comparable pre-treatment time utilizing curcumin.

Furthermore, Phyllanthus niruri post-treatment promoted a quicker regeneration of liver tissue than [17].

III. COMMONLY STUDIED HERBS

3.1 CURCUMIN

Turmeric's main phenolic ingredient, curcumin, has anti-disease properties. The rhizome of the Curcuma species frequently contains curcumin, which has long been utilized in herbal therapy. Curcumin has been shown in numerous studies to have protective properties against toxic substances in a variety of organs, including the liver.[18]. possesses strong pharmacological and biological qualities, including antitumor, anticancer, antioxidant, anti-inflammatory, antifungal, antibacterial, and anti-ischemic effects.

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Curcumin's antioxidant qualities The molecular mechanism of its hepatoprotective effect is explained by its inhibitory activity against nuclear factor (NF)-κB, which regulates numerous proinflammatory and profibrotic cytokines. According to research data, curcumin has a tremendous medicinal potential for treating liver illnesses. This is a thorough examination of the hepatoprotective qualities and possible mechanisms of action of curcumin.[19]

3.3 Pharmacology of curcumin

Turmeric's major component, curcumin, has been used medicinally for thousands of years. It was utilized as a traditional medicine for burns, bites, eye infections, wounds, and skin conditions (Gupta et al., 2013). Some such as , have medicinal for treating some of the most common illnesses in the world. Diabetes, heart problems, and certain malignancies are some of these conditions.[20]

3.4 Curcumin as hepatoprotective agent

Turmeric's yellow pigment, curcumin, has anti-inflammatory, hepatoprotective, anticarcinogenic, and antioxidant qualities (Bao et al., 2010). Curcumin's antioxidant qualities are primarily responsible for its beneficial benefits on the liver (Farombi et al., 2008). Consuming curcumin causes the liver's structural changes and serum protein levels to increase, total bilirubin to fall, and the activities of ALT, AST, ALP, LDH, and GGT to decrease.[20]A. It was discovered that curcumin reduced the amounts of free fatty acids, cholesterol, phospholipids, and serum aspartate transaminase and alkaline phosphatase activity. Tacrine is well-known for its hepatotoxicity and T-cell-destructive properties. Curcumin proved to be over ten times more efficient than the standard treatment, ascorbic acid, in a study using cultures of human hepatocytes that had been damaged by tacrine.[21]

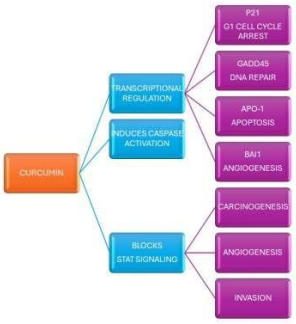

FIG 1: CURCUMIN MODE OF ACTION

IV. PHYLLANTUS NIRURI

For more than 2000 years, P. niruri has been well-known in herbal medicine systems like Indonesian Jamu, Traditional Chinese Medicine, and Indian Ayurveda.[22] Classification of plants:
Kingdom: Plantae
Division: Magnoliophyte
Class: Magnoliopsida
Family: Euphorbiaceae
Order: Euphorbiales
Genus: Phyllanthus
Species: Niruri

Bhumyaamalaki (Phyllanthus niruri Schum. & Thonn., Euphorbiaceae), which is found in many tropical and subtropical nations, including India, is primarily used in Indian Ayurvedic medicine to treat issues with the stomach, genitourinary system, liver, kidney, and spleen. In Ayurveda, Phyllanthus niruri is referred to by the Sanskrit terms Bhoomyaamalakee, Taamalakee, and Bhoodhatree. It was considered to possess the qualities of Guna, Veerya, Vipaaka, and Rasa. Shwaasahara (antispasmodic, antidyspneic), Kaphapittahara (relieving the Kapha Pitta Dosha), Pipaasaaghna (relieving Polydipsia), Raktapittahara (relieving hemorrhage disease), Kaamalaahara (relieving jaundice), Kushthaghna (indicated in leprosy), Daahaghna (refrigerant, relieving burning sensation), Kshatakshayaghna (indic in trauma), and Mootrarogahara (relieving urinary disorders).[23] Niruri's high concentration of flavonoids, tannins, lignans, and terpenes—all of which have antioxidative qualities—may be responsible for its antioxidant hepatoprotective action. The hexane

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extract of P. niruri contains lignans such phyllanthin and hypophyllanthin, which shielded rat hepatocytes against carbon tetrachloride and galactosamine-induced hepatotoxicity, according to one of the first in-vitro studies on the plant's antioxidative hepatoprotective effects.[24].

V. SILYMARIN

Silybum marianum is the scientific name for milk thistle, also referred to as St. Mary's thistle. It is native to the Mediterranean region and belongs to the Asteraceae family. Its oval leaves can reach a maximum length of 30 cm.in length, prickly branches, and a milky sap. The flowers have a diameter of up to 8 cm and are vivid pink. In its wild state, milk thistle can be found in the Middle East, northern Africa, and southern Europe. The plant is grown in South American nations like Argentina, Venezuela, and Ecuador, as well as in Hungary and China. Milk thistle is eaten as an additional food in Mexico.[25]

5.1 HEPATOPROTECTANT EFFECTS OF SILYMARIN

In mammals, the liver is an essential organ that carries out numerous physiological tasks. Animals' nutritional status is influenced by their liver's function and processing state in addition to what they ingest. Unfortunately, because of the ongoing use of chemicals (antibiotics), it is very difficult to recognize the early signs of liver imbalances and long-term illnesses (Handa, 1991). According to Trease and Evans (1983), the liver contains a complicated chemistry and is essential to physiology. Toxins that are consumed and absorbed from the stomach first go into the liver, where they can build up and cause illness. Liver damage, which can range from acute hepatitis to hepatocellular carcinoma, can be caused by immune response, inflammation, necrosis, fibrosis, apoptosis, altered gene expression, and regeneration. A. Although its precise mode of action is yet unknown, silymarin has been demonstrated to have immunomodulatory, antioxidant, antiproliferative, antifibrotic, and antiviral properties to treat such concerns. With a short half-life and rapid conjugation in the liver, silymarin is primarily excreted in bile. To lessen hepatic inflammation in vivo, it should be administered orally in high or frequent dosages. Italian researchers discovered that silymarin (silybin) given at a dose of 240 mg/day significantly decreased total bilirubin and blood liver enzymes ALT, AST, GGT, and ALP, which are indicators of liver stress and damage, in patients with chronic active hepatitis. These days, Amanita phalloides fungal poisoning is treated with silymarin. Silymarin is essential for hepatoprotection through processes such as blocking phalloidin inhibition, the α amanitinerohepatic cycle, α-amanitin binding to hepatocyte membranes, and preventing α-amanitin membrane transport.[26]

5.2 Effect of silymarin on liver function

Rats given sodium fluoride showed significant cellular, molecular, and biochemical alterations, including fibrosis, liver damage, impaired mitochondrial function, inflammation, and DNA degradation. Serum GGT, ALT, ALP, bilirubin, and albumin levels were tested to gauge liver function. These metrics were used as indicators of the degree of silymarin's hepatoprotective effects. Serum ALT, ALP, and GGT activity as well as bilirubin concentrations significantly increased in response to sodium fluoride as compared to the control groups.[27]

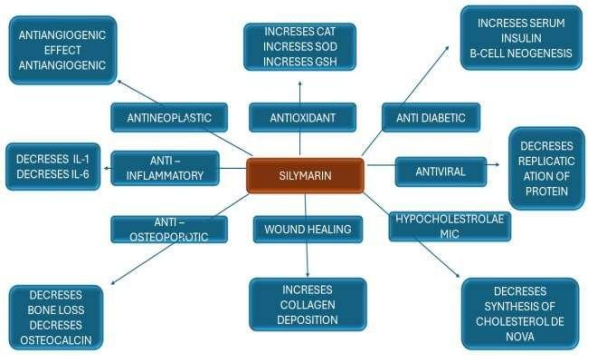

FIG 2: SILYMARIN ACTIVITY

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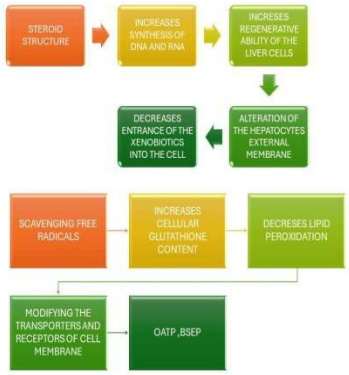

FIG 3: MODE OF ACTION OF SILYMARIN

VI. MATERIAL AND METHOD

Hematological, biochemical, lipid peroxidation, and histopathological characteristics were examined. Chemicals conducted the investigation over a 28-day period.

Chemical use:
Naf (sodium fluoride), Silymarin, Herbal drug: Phyllanthus niruri, curcumin

6.1 Biological kits
SGOT, SGPT, ALT

6.2 ANTIOXIDANT MARKERS
SOD, CAT, GSH

6.3 Experimental Animals
The experimental protocol was authorized by the Institutional Animal Ethical Committee (IAEC). The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, states that the experimental approach conforms with national regulations. Thirty Wistar strain rats from the reputable Laboratory Animal Breeding Center were employed in this study. In this investigation, rats weighing between 150 and 200 grams were employed.

6.4 Housing
According to CPCSEA regulations, all of the animals were housed in standard management circumstances. Twelve hours of light and twelve hours of darkness were given to them. Each animal was kept in 47 x 34 x 18 cm polypropylene cages with rice husk used as bedding. Each cage could hold no more than six animals.

6.5 Feeding
The normal pellet diet used to feed the animals was purchased from Swastik Feeds Ltd. in Maharashtra. 22.02% crude protein, 4.25% crude oil, 3.02% crude fiber, 7.5% ash, and 1.38% sand silica make up the pelleted diet. Throughout the experiment, they were provided with unlimited food and clean drinking water

TABLE 1: Design of experiments

Sr.no	Groups	Treatment
1	GROUP 1: CONTROL	NORMAL SALINE (10ml/kg) Routeorally
2	GROUP 2: NEGATIVE CONTROL	SODIUM FLUORIDE (20 mg/kg.b. wt) Route-orally
3	GROUP 3: POSTIVE CONTROL	SILYMARIN (100mg/kg) + NAF Route-orally
4	GROUP 4: TEST A	COMBINATION OF (A+B) CURCUMIN AND PHYLLANTUS NIRURI -A (200mg) +NAF Routeorally
5	GROUP 5: TEST B	COMBINATION OF (A+B) CURMCUMIN AND PHYLLANTUS NIRURI - B (400mg) +NAF Route orally

6.6 Collection of Material for Haematological and Biochemical Parameters
After the 28th day of the trial, the rats from each experimental group were appropriately sedated. The rats were weighed and given a 12-hour fast before being sacrificed. For hematological estimation, blood was drawn using the cardic puncture procedure and placed in glass vials containing 1% ethylenediamine tetra acetic acid (EDTA).

6.7 Biochemical parameters study
Using standard kits, the separated serum was used to estimate a number of biochemical markers, including total bilirubin, alkaline phosphate (ALP), aspartate

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aminotransferase (AST/SGOT), and alanine aminotransferase (ALT/SGPT).

VI. COMPARATIVE OUTCOME

A. Biochemical Markers (ALT, AST, ALP, Bilirubin)

Treatment	Outcome
Silymarin-	Most notable decrease in ALT, AST, and ALP; returns most closely to normal levels
Curcumin-	Strong reduction; slightly less efficient than silmarin
Phyllanthus niruri-	Moderate decline; levels increased but did not return to normal

B. Oxidative Stress Markers (MDA, SOD, CAT, GSH)

Marker	Fluoride Effect	Silymarin	Curcumin	P. niruri
MDA	↑↑ (high)	↓↓↓ (strong decrease)	↓↓	↓
SOD/CAT/GSH	↓↓	↑↑↑	↑↑	↑

C. Histopathology (Liver Tissue Restoration)

Treatment	Histological Outcome
Silymarin	Hepatic architecture is almost normal, with no necrosis or vacuolation.
Curcumin	Mild alterations; hepatocytes largely preserved
Phyllanthus niruri	Moderate improvement; some necrosis is still present

1. Silymarin (Standard Drug)

- Nearly total hepatoprotection,
- Best recovery in enzyme levels, antioxidant status, and tissue shape.
- Most successful in reversing fluoride toxicity[29].

2. Curcumin (Herbal Extract)

- Strong hepatoprotection, comparable to silymarin at larger doses.
- Outstanding anti-inflammatory and antioxidant properties.
- A significant restoration of liver structure and function[30].

3. Phyllanthus niruri (Herbal Extract)

- It is best used as an adjunct or supporting herb.
- It has a moderate protective impact and improves liver enzymes and oxidative indicators, but not to normal levels[31].

VII. LIMITATIONS IN EXISTING STUDIES

1. Lack of Standardized Fluoride Dosing Protocols

- Direct comparison is challenging because studies differ greatly in fluoride dose (5–100 mg/kg), exposure method, and duration[32].
- A lot of animal models don't accurately reflect the long-term, low-level exposure that humans experience[33].

2. Variability in Herbal Extract Preparation

- Curcumin and Phyllanthus niruri are two examples of herbal extracts that differ in terms of purity and active chemical content, as well as the extraction process (aqueous, ethanol, or hydroalcoholic)[34].
- Pharmacological efficacy is uneven between investigations due to formulation (raw vs.
- standardized extract)[35].

3. Limited Use of Standardized Positive Controls

- Despite the widespread usage of silymarin, certain studies lack a consistent reference medication, making it more difficult to quantify relative efficacy objectively [36].

4. Small Sample Sizes

- Six to eight rats are used in each group in many animal experiments, which may reduce statistical power and generalizability[37]

5. Short Duration of Treatment
- The majority of studies last 14–28 days, which might not accurately reflect chronic fluoride toxicity or long-term hepatoprotective effects[38].

6. Focus on Biochemical Markers Only
- ALT, AST, ALP, and oxidative stress indicators are highlighted in many publications, but further mechanistic research, including gene expression profiling, is lacking[39].
- Assays for mitochondria Mapping of inflammatory pathways (NF-κB, Nrf2, etc.)[40]

7. Limited Histopathological Quantification
- A lot of research use histology photos, but they don't have quantitative grading systems, which makes tissue evaluation less objective[41].

8. Poor Bioavailability of Curcumin Often Ignored
- Because of its quick metabolism and poor absorption, curcumin's effects could be overestimated or underestimated until improved formulations (such piperine or nano-curcumin) are utilized[42].

9. Few Direct Comparative Studies
- Comparative results are challenging because there are few studies thatexamine silymarin, curcumin, and Phyllanthus niruri at the same time[43].

10. Lack of Human Cliical Data
- The majority of studies originate from rodent models; translation to human populations is still unclear, particularly with regard to long-term safety, metabolism, and dosage[44].

11. No Standardized Biomarkers for Herbal Efficacy
- The biological activity of herbal substances varies, but no research has established conventional biomarkers to assess hepatoprotection in fluoride poisoning[45].

12. Potential Interaction Between Extracts Not Studied
- There is little research on the combined or synergistic use of herbal compounds (such as curcumin + P. niruri), which could have therapeutic advantages[46].

IX. MECHANISTIC INSIGHT

1. Silymarin – Mechanism of Action (Standard Drug)

One of the most potent natural hepatoprotectants is silymarin, a flavonolignan complex derived from Silybum marianum[47].
Key Mechanisms:

A. Antioxidant Defense Enhancement
- Silymarin, a flavonolignan complex obtained from Silybum marianum, is one of the most effective natural hepatoprotectants[48].

B. Membrane Stabilization
- Prevents the entry of toxins by binding to the membranes of hepatocyte cells[49].
- Prevents alterations in permeability brought on by fluoride[50].

C. Protein Synthesis Activation
- Promotes the production of ribosomal RNA by stimulating RNA polymerase I.
- Encourages hepatic regeneration[51]

D. Anti-inflammatory Action
- Blocks the NF-κB, TNF-α, IL-6, and COX-2 pathways[52].
- Lessens the infiltration of inflammation in the liver tissue[53].

E. Anti-fibrotic Effect
- Inhibits stellate cell activation and collagen deposition[54].

2. CURCUMIN

Curcumin is a polyphenolic compound known for potent antioxidant and anti-inflammatory activities[55].

A. Potent Antioxidant Activity

Directly scavenges ROS and reactive nitrogen species (RNS)[56].
- Enhances endogenous antioxidants: GSH, SOD, CAT [57].

- Blocks lipid peroxidation caused by fluoride[57].

B. Anti-inflammatory Action
The primary regulator of inflammation, NF-κB, is strongly inhibited. Pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, are downregulated.

- Inhibits the COX-2 and iNOS enzymes [58].

C. Mitochondrial Protection
Maintains the potential of the mitochondrial membrane.

- Inhibits caspase-3 to stop fluoride-induced apoptosis[59].

D. Detoxification Enhancement
- Activates Nrf2 to upregulate phase II detoxification enzymes.
- Encourages pathways for liver detoxification [60]

E. Metal/Toxin Chelation
- Curcumin can bind fluoride ions and reduce their reactivity [61].

3. PHYLLANTHUS NIRURI
Mechanism of Action (Traditional Herbal Hepatoprotectant)
Phyllanthus niruri contains phyllanthin, hypophyllanthin, ellagic acid, and flavonoids [62].

A. Antioxidant and Free Radical Scavenging
- Boosts GSH, SOD, and CAT, three antioxidant enzymes.
- Lowers levels of lipid peroxidation and MDA.
- Bioactive lignans counteract oxidative damage caused by fluoride [63].

B. Hepatocyte Membrane Protection
Lysosomal and plasma membranes are stabilized, and enzyme leakage (ALT, AST) is prevented [64].

C. Anti-inflammatory Activity
- Inhibits TNF-α and IL-6, two inflammatory mediators.
- Lessens the invasion of neutrophils and macrophages into hepatic tissue[65]

D. Improvement of Bile Flow and Detoxification
- Reduces the invasion of neutrophils and macrophages into hepatic tissue;
- Inhibits two inflammatory mediators, TNF-α and IL-6[66].

CONCLUSION

As demonstrated by higher blood liver enzymes, increased lipid peroxidation, depletion of antioxidant defenses, and severe histological abnormalities in hepatic tissue, the current study suggests that chronic fluoride exposure causes considerable hepatotoxicity in rats. These results verify that oxidative stress is a major factor in liver damage caused by fluoride. Hepatic damage caused by fluoride was significantly reduced after treatment with specific plant preparations. Antioxidant enzymes such superoxide dismutase, catalase, and reduced glutathione were restored, biochemical liver indicators were returned to normal, and lipid peroxidation levels significantly decreased in the herbal-treated groups. These findings were corroborated by histopathological analysis, which showed that treated mice had better hepatic architecture, less inflammatory infiltration, and hepatocyte regeneration. Differential hepatoprotective efficacy was noted across the assessed herbal extracts, with one extract exhibiting protective effects similar to those of the common medication silymarin. These herbal extracts have a hepatoprotective effect because they include bioactive phytochemicals such flavonoids, phenolics, and antioxidants that may improve liver detoxification processes, maintain cell membranes, and combat oxidative stress caused by fluoride.

The study concludes with strong experimental evidence that herbal extracts have a substantial protective potential against hepatotoxicity caused by fluoride. These results suggest that herbal formulations may be used as safe, affordable, and efficient therapeutic agents to treat fluoride toxicity. To confirm these results and investigate their translational usefulness, additional molecular and clinical research is advised.

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