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# Effect of fluoride-induced toxicity on the rat nephrotoxicity and a therapeutic effect of Ocimum sanctum (Tulsi) leaf extract

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## ABSTRACT

Fluoride naturally occurs in soil, plants, animals, and water. A minimum amount of Fluoride is beneficial for the body, but an excessive amount can damage many organs, including the kidneys, liver, and brain. Chronic exposure to high levels of Fluoride causes oxidative stress, cellular damage, and histopathological damage. Experimental Animal: Take the Adult albino rats (150gm to 200gm) kept them in clean cage with suitable temperature and environment with proper food diet Most studies focused only in fluoride toxicity few research explore the protective and antioxidant effect of Ocimum Sanctum (Tulsi) leaf extract on Fluoride toxicity induce in rat model This show the biochemical, histopathological, body weight changes in animal model. To evaluate the protective and therapeutic potential of Tulsi (Ocimum Sanctum) leaf extract against the Toxicity of Fluoride in albino rats by analyzing Biochemical, Phytochemical, and histopathological parameters To study and analyze the change in the Hematology (blood) parameter, biochemical enzymes, and antioxidant enzymes on inducing Fluoride toxicity, and then study the protective and detoxifying effect of Tulsi leaf extract against toxicity on the param Toxicity of fluoride reduces the level of urea, creatinine, and liver enzymes, indicating severe oxidative and cellular damage, and also damages the kidney structure, function, and many tissues. This shows a chronic effect on the kidney as well as enzymes. Study concludes that Tulsi leaf extract effectively alleviates Fluoride toxicity by enhancing defense and protective integrity, so it is considered a natural therapeutic agent.

***Keywords:*** *Fluoride, Multi-organ toxicity, hepatotoxicity, splenic toxicity, glycemic index, histopathology, Kidney damage.*

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## 1. INTRODUCTION

Fluorine is the lightest member of the halogen group and is one of the most reactive of all chemical elements. It is not, therefore, found as fluorine in the environment. It is the most electronegative of all the elements.[1] Fluoride occurs naturally in soil, water, plants, and animals in trace quantities. In groundwater, natural fluoride concentrations range from trace quantities to over 25 mg/L.[2] A large amount of fluorine can be found in rocks of volcanic origin. It enters the environment through volcanic eruptions, rock dissolution, and numerous human activities (coal burning, ore processing, production and use of fertilizers, and industrial plants).[3] Fluorine in the environment is therefore found as fluorides, which together represent about 0.06–0.09 per cent of the earth's crust. The average crustal abundance is 300 mg $kg^{-1}$.[4] Fluoride is one of the very few chemicals that has been shown to cause significant effects in people through drinking water. Fluoride has beneficial effects on teeth at low concentrations in drinking water, but excessive exposure to fluoride in drinking water, or in combination with exposure to fluoride from other sources, can give rise to a number of adverse

effects.[5] Underground water sources are more likely to have higher levels of fluoride, whereas the concentration in seawater averages 1.3 ppm. Fresh water supplies generally contain between 0.01-0.3 ppm, while the ocean contains between 1.2 and 1.5 ppm. According to the World Health Organization (1984), the permissible limit of fluoride in drinking water is 1.5 ppm. Over 50% of the groundwater sources in India have been contaminated by fluoride.[6]

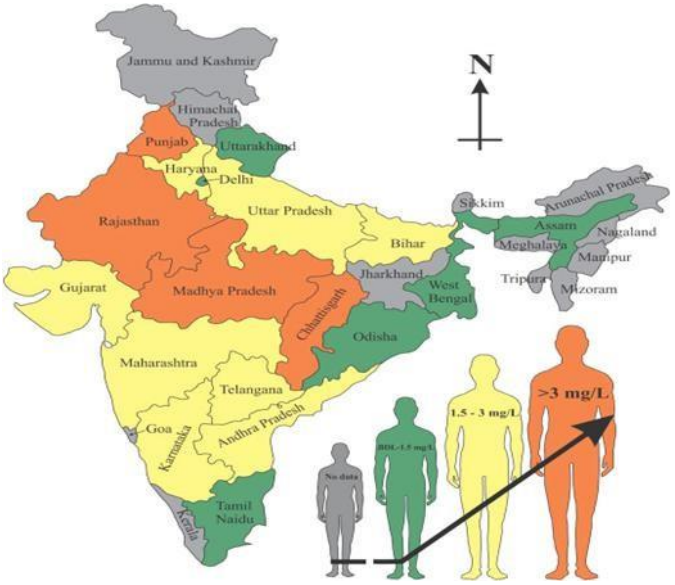



**Fig1. Concentration of Fluoride in Groundwater**

**Effect of fluoride:** Chronic F- toxicity is classified as dental, skeletal, and non-skeletal fluorosis on the basis of tissues affected. The earliest sign of chronic F- toxicity is expressed as dental fluorosis (Krishnamachari 1986). Fluoride in drinking water has a profound effect on teeth and bones (Razbe et al. 2013). In the areas with naturally high F- levels in water, the prevalence of dental fluorosis was significantly higher than in areas with lower levels (Gamarra et al. 2024). Long-term F- exposure results in skeletal fluorosis, forming bone deformities, calcification of ligaments, resulting in restricted movements (Vieira et al. 2005). The communities with high F- exposure, particularly those dependent on groundwater with naturally high F- concentrations, showed an increased risk of skeletal fluorosis (Shaji et al. 2024). The chronic F- exposure reduced the IQ in children, especially in areas with high levels of F- in drinking water (Saxena et al. 2012). The F exposure might also be associated with thyroid problems, including hypothyroidism, risk of kidney and liver damage, especially in areas with high levels of F in the water. [7]

**Fluoride in India**: Apart from India, other developed and developing nations have come under the threat of fluorosis. These are Argentina, USA, Morocco, Algeria, Libya, Egypt, Jordan, Syria, Turkey, Iraq, Iran, Pakistan, Kenya, Tanzania, South Africa, China, Australia, New Zealand, Japan, and Thailand. Nearly 12 million of the 85 million tons of fluoride Deposits on the earth's crust are found in India. It is not surprising; therefore, the fluorosis is endemic in 17 states of India (UNICEF 1999).In India, over 25 million people from 15 states, namely Rajasthan, Gujarat, Tamil Nadu, Maharashtra, Madhya Pradesh, Punjab, Haryana, Andhra Pradesh, Bihar, Delhi, Jammu and Kashmir, and Kerala, are suffering from fluoride. [8]

**In Gujarat:** Gujarat is one of the most severely affected states in the country, with a high prevalence of fluorosis, where approximately 18 out of a total of 24 districts are prone to the condition due to high fluoride content in the drinking water. In Gujarat, various districts, namely Patna, Mehsana, Ahmedabad, Amreli, Baroda, and Banaskantha, are affected by high fluoride concentrations. Mehsana, Patna, and Banaskantha districts, located in North Gujarat and near to each other, are considered to be the most affected districts apart from Amreli, Ahmedabad, Sabarkantha, and Baroda. The study by the Gujarat Water Supply and Sewerage Board (GWSSB) indicates that 4,341 villages suffer from high fluoride content in water, 2,571 from salinity, and 1,336 from nitrate. [9] The Gujarat Ecology Commission's draft Action Environmental Program, prepared last year, said that in 1991, , the figure reached 2,836. Now, the GWSSB survey says the number of such villages has nearly doubled to 4,341 in 2010.[10]

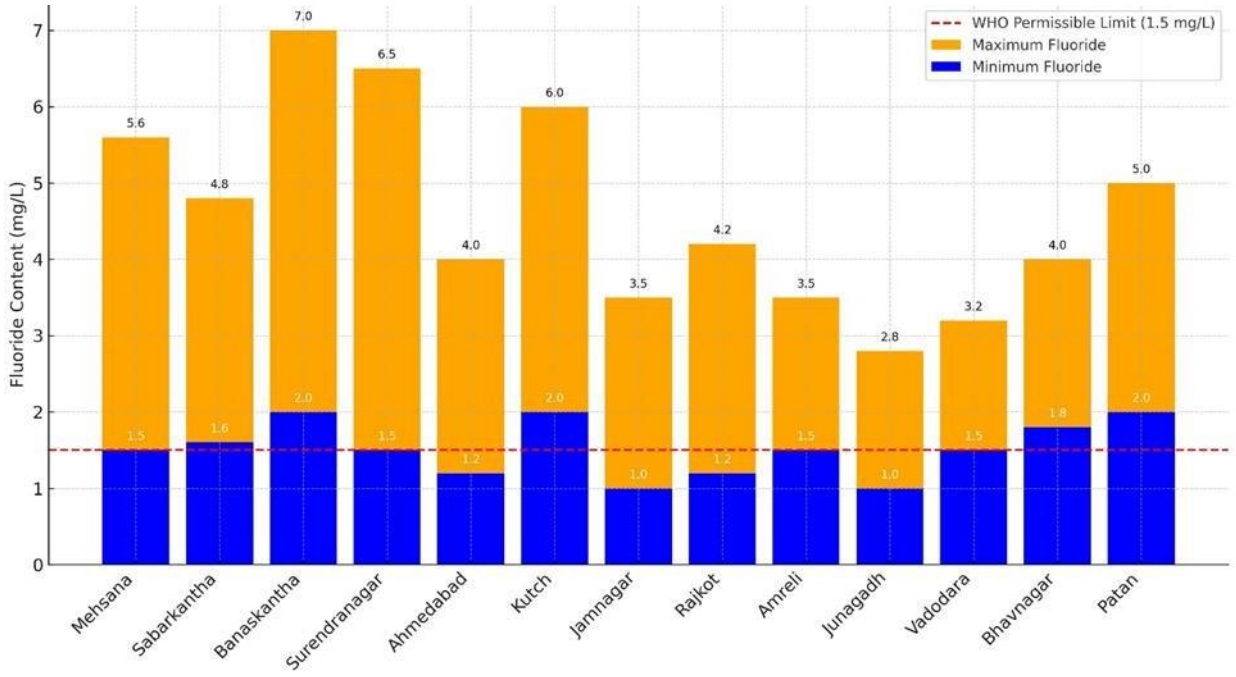



**Fig2. Fluoride concentration of water in Gujarat**

**In Uttar Pradesh,** in the Ganga alluvial plain of Uttar Pradesh (UP), fluoride content has been reported by various researchers and the State and Central Governments in the districts of Varanasi, Unnao, Kanpur, Agra, and Mathura. Fluoride contamination in many parts of UP (Unnao, 2 mg/l; Debraspur, 2.1 mg/l; Janghai, 3.2 mg/l; Kulpahar, 3 mg/l; Babera, 3.3 mg/l; Karchhana, 2.8 mg/l; Jhansi, 2.8 mg/l, and Etah, 3 mg/l) has been reported mainly in the Quarternary–Upper Tertiary deposits33. [11]

**In Rajasthan,** Rajasthan has 16,560 villages, which is more than 50%. Around 10% of fluoride-affected habitation in the world is in Rajasthan only. All 33 districts are partially or fully affected by the fluoride contaminant. Jalore, Jaipur, Ajmer, Nagaur, Pali, Jodhpur, and Sirohi districts are the worst affected by fluoride with an average concentration of 2mg/l High fluoride concentration can be seen on both sides of the Aravalli range in Tonk-Alwar-Bhilwara region in the east of Rajasthan, whereas, Nagor-Jodhpur-Pali-Jalor- Jalor belt in the west of the range also shows very high concentration of fluoride that renders water unusable for human drinking. The areas around Jhalwar-Kota-BaranBundi- Chittaurgarh region, and that around Churu, Bikaner, Barmer, and Jaisalmer have shown low concentration of fluoride in Rajasthan compared to other parts of the state in groundwater Value of fluoride has been observed at Bharatpur district as 8.70 mg/l. [12]

**Table 1: Occurrence of excess Fluoride in Rajasthan**

| Sr. No | The village has an excess of Fluoride | District of Rajasthan (Fluoride>1.5mg/L) |
|---|---|---|
| **1.** | **Upto 10%** | Shri Ganganagar, Bundi, Kota, Chittorgarh |
| **2.** | **10 – 20%** | Bikaner, Jhunjhunu, Udaipur, Dungarpur |
| **3.** | **20 – 40%** | Churu, Sikar, Karoli, Dausa, Alwar, Jaipur, Bharatpur, Swaimadhopur, Dholpur, Banswara, Serohi, Badmer, Jodhpur, Pali, Ajmer |
| **4.** | **>40 %** | Jaisalmar, Nagaur, Jalore, Bhilwara, Tonk |

**Table 2: Major states of India with excess of fluoride concentration.**

| Sr.No | States | High Fluoride concentration cities and districts | Approx. Range (mg/L) |
|---|---|---|---|
| **1.** | **Rajasthan** | Nagaur, Jaipur, Dungarpur, Jodhpur, Ajmer | 2.0 - 10.0 |

| | | | |
|---|---|---|---|
| **2.** | **Gujarat** | Mehsana, Banaskantha, Rajkot, Ahmedabad, Surendranagar | 1.5 - 9.0 |
| **3.** | **Tamil Nadu** | Dharmapuri, Krishnagiri, Salem, Namakkal, Vellore | 1.0 -6.0 |
| **4.** | **Maharashtra** | Yavatmal, Chandrapur, Nanded, Beed, Jalgaon | 0.8 - 5.0 |
| **5.** | **Madhya Pradesh** | Dindori, Mandla, Betul, Seoni, Chhindwara, | 1.0 - 6.0 |
| **6.** | **Punjab** | Mansa, Bathinda, Muktsar, Sangrur, Faridkot | 0.8 – 4.5 |
| **7.** | **Haryana** | Rewari, Mahendragarh, Bhiwani, Hisar, Palwal | 1.0- 6.0 |
| **8.** | **Andhra Pradesh** | Nalgonda, Anantapur, Prakasam, Guntur, Kadapa | 1.2 – 8.0 |
| **9.** | **Bihar** | Nawada, Gaya, Rohtas, Aurangabad, Nalanda | 0.8 – 3.0 |
| **10.** | **Delhi (NCT)** | Najafgarh, Dwarka, South-West Delhi, Palam, Rohini | 0.8 – 3.0 |
| **11.** | **Jammu & Kashmir** | Udhampur, Jammu, Kathua, Rajouri, Samba | 0.5 – 2.0 |
| **12.** | **Kerala** | Palakkad, Idukki, Wayanad, Thrissur, Malappuram | 0.3 – 1.5 |

**Plant: (Tulsi)**- ***Tulsi Botanical /Scientific name*** : Ocimum sanctum L. (also known as Ocimum tenuiflorum L.)Tulsi is an aromatic shrub in the basil family Lamiaceae (tribe ocimeae) that is thought to have originated in north central India and now grows native throughout the eastern world tropics. Within Ayurveda, Tulsi is known as "The Incomparable One," "Mother Medicine of Nature" and "The Queen of Herbs," and is revered as an "elixir of life" that is without equal for both its medicinal and spiritual properties. This emerging science on Tulsi, which reinforces ancient Ayurvedic wisdom, suggests that Tulsi is a tonic for the body, mind and spirit that offers solutions to many modern-day health problems.[13]

***Tulsi is considered both a medicinal plant and an herb plant :***Herb: Because it is a small, aromatic plant with soft stems, commonly used in daily life — teas, cooking, and herbal remedies. Medicinal Plant: Because it contains bioactive compounds (like eugenol, ursolic acid, rosmarinic acid, and flavonoids) that have therapeutic properties.


**Fig3: Tulsi Plant**

Tulsi is recommended as a treatment for a range of conditions, including anxiety, cough, asthma, diarrhea, fever, dysentery, arthritis, eye diseases, otalgia, indigestion, hiccups, vomiting, gastric, cardiac, and genitourinary disorders, back pain, skin

diseases, ringworm, insect, snake, and scorpion bites, and malaria.[14] These studies reveal that Tulsi has a unique combination of actions that include: Antimicrobial (including antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, anthelmintic), mosquito repellent, anti-diarrheal, anti-oxidant, anti-cataract, anti-inflammatory, chemopreventive, radio protective, hepato-protective, neuro-protective, cardio-protective, anti-diabetic, anti-hypercholesterolemia, anti-hypertensive, anti-carcinogenic. [15]

**Table 3: Some important types of Tulsi (*Ocimum Sanctum*)**

| Sr. No | Types of Tulsi Plant | Image |
|---|---|---|
| 1. | **Rama Tulsi (Green Tulsi)**<br>Rama Tulsi has green leaves, purple flowers, and a clove-like scent and flavor. It is used for daily balance and to promote calm. |  |
| 2. | **Krishna/Shyama Tulsi (Purple Tulsi)**<br>This Tulsi has dark green to purple leaves and a spicy, peppery taste. It is used for mental focus, stress resilience, and as an antioxidant. |  |
| 3. | **Vana Tulsi (Wild Tulsi)**<br>Wild Tulsi plant with large green leaves and a spicy, anise-like fragrance and flavor. It is used to improve vitality, mood, and endurance. |  |
| 4. | **Amrita/Kapoor Tulsi (Camphor Tulsi)**<br>This is an annual herb with a floral, light, camphor-like aroma and taste. It is used for respiratory support and immune defense. |  |

## 2. MATERIAL AND METHOD:

**Chemicals-** Mayer reagent, Wagner's reagent, Lead ethanoate, Alkaline reagent, Ferric chloride, Molisch's reagent, Alkaline reagent, Barford's reagent, Iodine solution, Ninhydrin solution, sodium hydroxide, all chemicals were used to find out the presence of phytochemical constituents, which were obtained from the research lab of Nims University. Jaipur Rajasthan.

**Plant Material-** Fresh Leaves of the selected medicinal herb Ocimum sanctum (Tulsi) were harvested from the herbal garden of the Nims University. Jaipur, Rajasthan. After 4-5 days for obtaining aqueous extract, the properly dried leaves were then ground into a fine powder by using the grinding machine than the powder material of Tulsi leaves was weighed properly. The fine powder of Tulsi leaves was stored in a clean and tightly closed container for extraction.

**Preparation of aqueous extract of Ocimum sanctum (leaves)-** The extract of leaves were obtained in sufficient quantity by using distilled water. In this process firstly 20 g powdered leaves of Ocimum sanctum were placed in 200 ml of beaker and 100 ml of distilled was poured into beaker after addition of water kept for overnight at the room temperature approximately 22 hours for thorough mixing and also complete elucidation of active materials to dissolve in the respective solvent then, extract was filtered by using muslin cloth followed by Whatman no 1 filter paper then the green color filtrate was obtained, after done this process filtrate was dried. Finally, the residues were collected and used for the experiment.

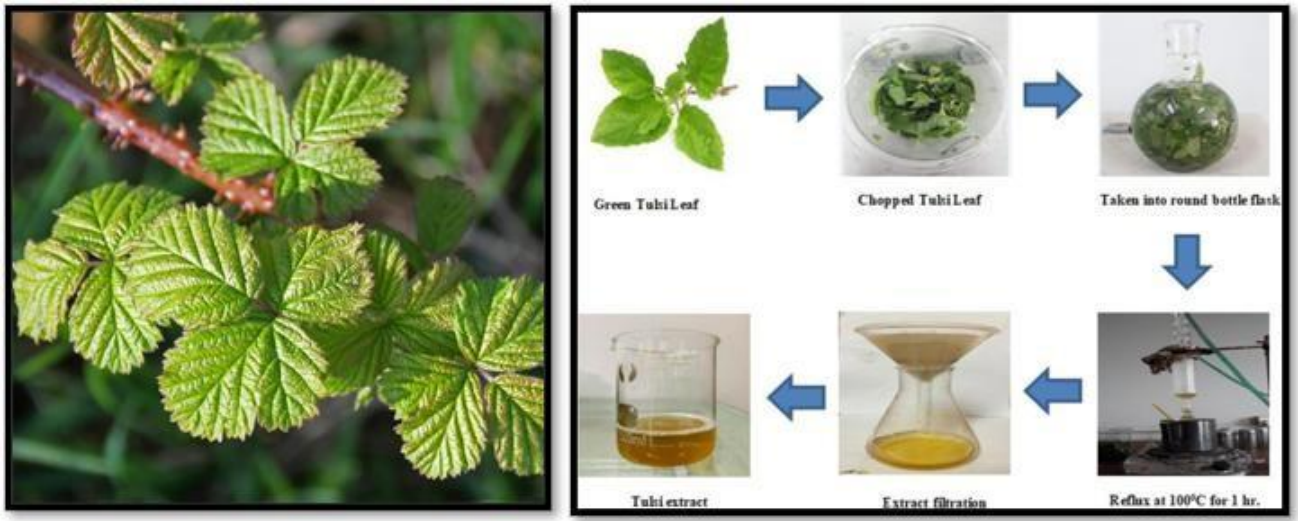



**Fig4. Aqueous extract of Ocimum Sanctum (*Tulsi Leaf*)**

**Phytochemical Test:** Phytochemical analysis of this medicinal herb can identify the nature of compounds present in the extract of Ocimum sanctum. It is also for identifying the bioactive compound and their effect. They are commonly helpful as a model for the synthesis of new medicines.

**Phytochemical analysis of aqueous extract of Ocimum Sanctum:**

The aqueous extract of Ocimum sanctum was subjected to phytochemical analysis to determine the presence and absence of phytochemical constituents. Flavonoid synthesis in plants is induced by light color spectrums at both high and low energy radiations. Low energy radiations are accepted by phytochrome, while high energy radiations are accepted by carotenoids, flavins, cryptochromes in addition to phytochromes. The phytochemical tests employed for alkaloids, flavonoids, glycosides, proteins, fixed oil, carbohydrate and tannins, Cardiac glycosides, saponins and flavonoids, and terpenoids.

**Test for alkaloids:** A. Mayer test- 5 mg extract of Ocimum Sanctum (Tulsi) was transferred in the test tube, and then 1% hydrochloric acid HCl) the obtained solution was gently heated. Red color indicates the presence of alkaloids because Potassium mercuric iodide is present in Mayer's reagent. B Wagner's test- In this test, 5 mg extract of Ocimum sanctum was taken in a test tube than 0.5 of Wagner reagent was added to the solution, shaken well. Appearance of reddish brown color shows that the alkaloids are present. Reddish brown color because iodine forms a complex is insoluble and has the color brown redish. Dragendorff test-5 mg extract of Ocimum sanctum Tulsi was taken in a tube. And then one drop of Dragendorff reagent was added in the test tube. orange-red color, showing the presence of alkaloids. Dragendroff reagent was prepared using Bismuth nitrate, Nitric acid, iodine and water because of these chemicals it gives orange red color in the presence of alkaloids.

**Test for flavonoids:** À. Shinoda test: Firstly 5mg extract was added in the test tube then small amount of magnesium was mixed in this solution, also added the few drops of concentrated Hydrochloric acid. It should be indicate the pink color with the flavonoids. Colors varying from orange to red indicated flavones, red to crimson indicated flavonoids, crimson to magenta indicated flavonones. Catechins when treated with vanillin solution in hydrochloric acid give red pink color. Lead

ethanoate test for flavonoids- placed 5 mg of aqueous extract of Tulsi in test tube then 1ml of lead ethanoate solution was added. It gives a buff colored solution if the alkaloids are present.

B. Sodium hydroxide test for flavonoids- 5 mg extract was taken in 1 ml of the 10% solution of sodium hydroxide was For the appearance of a yellow color solution after addition of 1ml of dilute Hydrochloric acid, in the presence of alkaloids the color should be changed from yellow to colorless after addition of 2 ml of dilute hydrochloric acid.

Ç. Alkaline reagent test for flavonoids- 5 mg extract of Ocimum sanctum was placed in the test tube mixed then 2 2ml of 2% solution of Sodium hydroxide was poured in it, if the formation of yellow which turned into colorless after addition of few drops of diluted acetic. It means that alkaloids are present in the holy Basil

D. Ferric chloride test- Ferric chloride test was performed for checking the presence of flavonoids in the aqueous extract of Ocimum sanctum. Firstly 5 mg extract was mixed with 1ml of distilled water than 0.5ml of dilute ammonia solution was added into it. After addition of dilute ammonia few drops of concentrated Sulfuric acid was mixed later. Formation of yellowish with flavonoids. **Test for glycoside:** À. Liebermann's test- Liebermann' test for the analysis of glycoside are present or not in aqueous extract of Ocimum sanctum in this test 5 mg extract of Ocimum sanctum was mixed properly with 2ml of chloroform and then 2ml of acetic acid were mixed in the. Solution than it was cooled in ice. After cooling 1 ml of concentrated Sulfuric acid was added. The color will be change from violet to green with the presence of alkaloids in the extract.

**Test for tannins :** À. Ferric chloride test- 5 mg aqueous extract of Ocimum sanctum was mixed with 0.5 ml of ferric chloride solution. Formation of blackish precipitate in the presence of tannin.

B. Gelatine test- Gelatine test was performed for checking the presence of tannin in the extract. In this test 5 mg extract was mixed with Gelatine and 1ml of water was added into the solution. White precipitate should be produced.

C. Lead acetate test - Lead acetate test was performed to estimate the presence of tannin in which 5 mg of test samples was taken in test tubes. Few drops of basic lead acetate was added in the sample solution, if a brown bulky precipitate will be found it means tannin are present in test sample.

**Test for saponins:** À. Foam test- This test was performed for the identification of saponin in the aqueous extract, in which 1ml extract was dissolved in 5ml of distilled water. After the addition of distilled water, it was shaken for proper mixing till foam was observed. A few foams were added with 2 drops of olive oil, and it was shaken vigorously. It should be produced emulsion with the saponins.

**Oil test:** À. Stain test- few quantity of aqueous extract was spread onto the filter paper formation of oil on the filter paper will indicate the presence of oil in the aqueous.

B. Saponification test- A Few drops of alcoholic potassium hydroxide and 0.5 ml of extract were taken into a test tube and mixed well. 1-4 drops of phenolphthalein were added to the mixed solution. It was heated in a water bath for 1 hour. Formation of partial neutralization of alkali, which indicates the presence of oils and fats.

**Test for carbohydrates:** A. Benedict's test- Benedict's reagent was used for the analysis of carbohydrates. the 5 mg extract was mixed with a few drops of Benedict's reagent, then allowed to boil. The reddish brown precipitate was found in the presence of the carbohydrates (absent).

B. Molisch's test- initially, 5 mg of extract was taken in a test Tube and then 1 ml of Molisch's reagent was added to it. The mixture was shaken properly. After that, 2ml of concentrated Sulfuric acid was poured carefully along the side of the test tube. The appearance of a violet ring at the interface indicated the presence of carbohydrate.

## 3. RESULT AND DISCUSSION

Phytochemical studies Qualitative phytochemical investigation discovered presence of alkaloids compounds [Appearance of red color]; fl avonoids and tannins [The pink color shows the presence of fl avonoids and blackish precipitate indicated the presence of tannins] and absence of flavonoids [Not observed pink coloration] in all mentioned extracts of plant. Salkowski's test- [formation of brownish red color] showed positive result for aqueous extract. It showed negative result in case of protein, saponin, oil, steroids. The presence of these phytochemical components may be responsible for the observed antibacterial activity of the plant leaf extract. Flavonoid has also been reported to have greater potential benefit to human Health. The medicinal plants Ocimum sanctum is being used traditionally for the treatment of inflammation, wound healing, toothache, antiseptics, carminative, cough, expectorant, stomatitis and some fungal infection. The antibacterial activity has been attributed to the presence of some active constituents in the extracts.

**Table 4: Qualitative analysis of phytochemicals in Ocimum Sanctum extract.**

| No. | Phytochemical Name | Test | Interfere |
|---|---|---|---|
| 1. | Alkaloids | Mayer's testWagner's test | } Present |

| | | | |
|---|---|---|---|
| 2. | Flavonoids | Sodium hydroxide testShinoda testAlkaline reagent test | } Present |
| 3. | Glycosides | Lieberman testSalkowski testKeller-Kiliani test | } Absent |
| 4. | Tannin | Ferric Chloride testGelatine test | } Present |
| 5. | Saponin | Foam test | } Present |
| 6. | Oil | Stain test | } Absent |
| 7. | Carbohydrates | Saponification testMolisch's testBenedict test | } Absent |
| 8. | Steroids | Chloroform testSalkowski test | } Absent |
| 9. | Test for protein | Biuret testMillions of testsNinhydrin test | } Absent |

**Table5. Results show the Organoleptic properties of Ocimum sanctum.**

| No. | Properties of *Ocimum sanctum* | Observation |
|---|---|---|
| 1. | Color | Green |
| 2. | Odour | Aromatic |
| 3. | Taste | Slightly Pungent |
| 4. | Texture | Smooth |

It showed aromatic- odour, taste – slightly pungent, the texture- smooth of Ocimum sanctum were found. The phytochemical screening of aqueous leaf extract of O. sanctum, revealed the presence and absence of alkaloids, fl avoids and tannin compounds.


**Fig5. Result of Phytochemical Test**

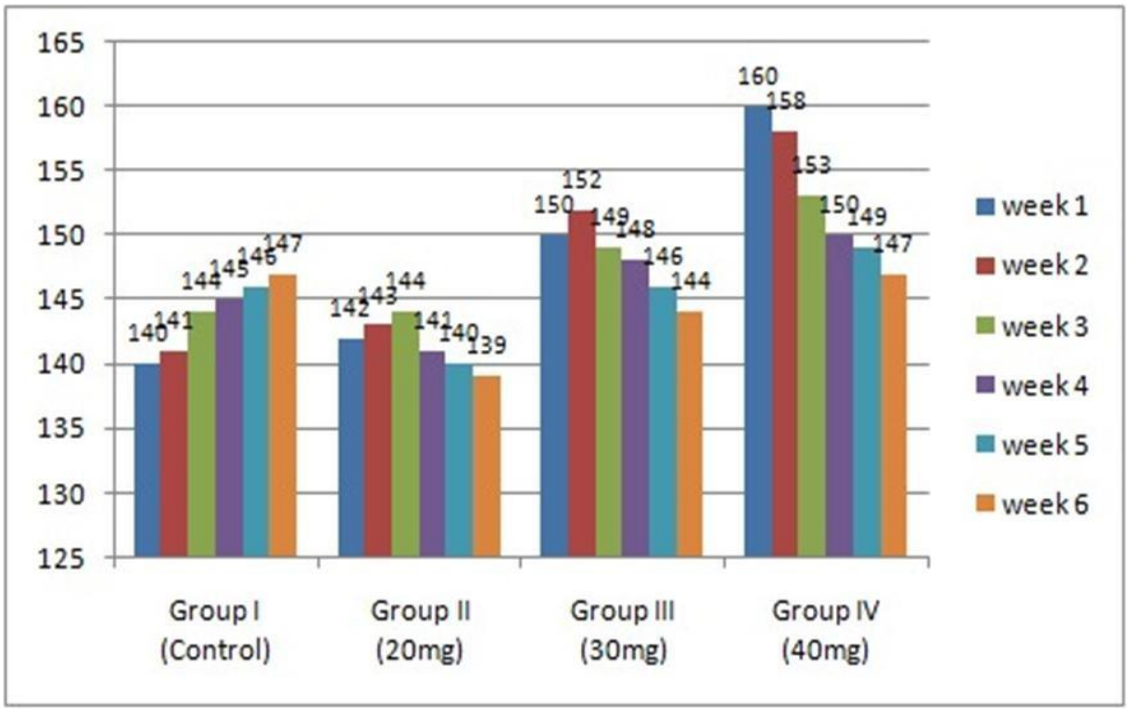
**Biochemical test:** Biochemical Testing Experience in the Lab, Biochemistry, NIMS University, Jaipur, Rajasthan.

**Bile Esculin Agar Test:** Positive result (turned black), indicating the organism's ability to hydrolyze esculin. **Citrate Test:** Positive result (turned blue), showing the organism's ability to utilize citrate as a carbon source. **Triple Sugar Iron (TSI) Test:** Result turned red, suggesting no fermentation of sugars or gas production. **Indole Test:** Negative result, indicating the organism does not produce indole from tryptophan.

**Antioxidant Test on Fluoride:** DPPH -2,2 Diphenyl-1-picrylhydrazyl Radical Scavenging Assay.ABTS-2,2-Azino-bis(ethylbenzothiazoline-6-sulfonic acid) Radical Cation Decolorization essay. FRAP - Ferric Reducing Antioxidant Power Assay. **Organ affected by florid and improved by giving Tulsi leaf extract.** Fluoride affects several organs in the body, but Tulsi (Ocimum sanctum) leaf extract has shown a remarkable ability to improve their function and reduce toxicity.

1. Exposure causes liver damage by increasing oxidative stress and altering enzyme levels. When Tulsi leaf extract is given, it boosts the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH). This helps restore liver enzyme balance and protects liver cells from degeneration.
2. In the kidneys, fluoride leads to tubular injury and reduces filtration ability. Tulsi Helps protect kidney tissue by reducing oxidative stress and promoting detoxification, allowing the kidneys to function more efficiently.
3. Fluoride also affects the brain, causing oxidative damage and memory problems. The antioxidants present in Tulsi, like eugenol and flavonoids, reduce the generation of free radicals in brain tissue, improve neuronal health, and help restore normal cognitive function.
4. In the bones, chronic fluoride exposure causes skeletal fluorosis, leading to stiffness and pain. Tulsi extract helps reduce fluoride accumulation and improves calcium and phosphorus metabolism, making bones stronger and more flexible.
5. For teeth, excessive fluoride results in dental fluorosis—discoloration and pitting of enamel. Tulsi's antioxidant and mineral-balancing properties help protect tooth enamel and prevent further fluoride damage.
6. The thyroid gland is also affected by fluoride because it blocks iodine uptake and disturbs hormone production. Tulsi helps by supporting thyroid function and reducing the inhibitory effects of fluoride on iodine absorption.
7. Finally, fluoride toxicity can damage the reproductive organs, leading to reduced sperm count, motility issues, and hormonal imbalances. Tulsi leaf extract protects reproductive tissues through its antioxidant and hormone-regulating properties, thus improving fertility and reproductive health.

**Body weight analysis:** On the basis of fluoride toxicity in the body weight is analyzed. During the duration of 6 weeks of experiment, in the control condition (only mineral water) no change in the body weight of rat. In the given (20mg) fluoride toxicity initial weeks experiment the weight gain then decreased in the last weeks. When the dose increases by 30mg) weight is increase in 1st week and decrease in the remaining weeks. High dose of toxicity (40mg) only decreased in the weight of the rats




**Graph 1: Body Weight Analysis**

During the period of experiment on fluoride on Albino rats for 6 weeks, we divided the rats into four groups with different toxicity levels of fluoride, like **Group I:** only gave mineral water to the rats with the help of a cannula. It's a control step. **Group II:** In this group (20mg), toxicity is induced in the rat. **Group III:** Toxicity level increased (30mg) in the rats. **Group IV:** The level of toxicity is high (40mg) in the rat.

**Hematology parameter:**

**Table 6: Hematology parameter in blood include ,WBC , RBC , Hb , PCV (Packed cell volume), MCV(Means cellular volume),MCH(Means cellular Hemoglobin), MCHC(Mean cellular Hemoglobin concentration), Platelets.**

| Sr. No | Parameter | WBC ($10^3$/µL) | RBC ($10^6$/µL) | Hb (g/dL) | PCV % | MCV (fL) | MCH (Pg) | MCHC (g/dL) | PLATELET ($10^5$/mm²) |
|---|---|---|---|---|---|---|---|---|---|
| 1. | Control | 8.5 ± 0.4 | 5.2 ± 0.3 | 14.0 ± 0.6 | 42 ± 2 | 85 ± 2 | 27 ± 1 | 33 ± 1 | 250 ± 15 |
| 2. | Fluoride toxicity (20mg ) | 7.2 ± 0.5 | 4.6 ± 0.4 | 12.5 ± 0.7 | 38 ± 2 | 78 ± 3 | 26 ± 1 | 31 ± 1 | 230 ± 18 |
| 3. | Increase toxicity (30mg) | 6.1 ± 0.6 | 4.0 ± 0.5 | 11.0 ± 0.8 | 34 ± 3 | 72 ± 4 | 25 ± 1 | 30 ± 1 | 210 ± 20 |
| 4. | High Fluoride toxicity (40mg) | 4.0 ± 5.0 | 3.3 ± 0.6 | 9.2 ± 0.9 | 29 ± 3 | 66 ± 5 | 23 ± 2 | 28 ± 2 | 190 ± 22 |

**Effect on Histopathology Organ :** Fluoride toxicity effect on various body part in Human as well as animals , plants and many more microorganisms or macroorganisms. When the Toxicity is induce in the (Albino) rats it effect many organs like Liver , Kidney, Teeth , Bone, Reproductive organ etc The major targeted organ are kidney and teeth that show high effect of toxicity in rats..

**Effect on the kidney:** In structural damage Fluoride fluoride-treated rats show degeneration of renal tubules, glomerular shrinkage, cytoplasmic vacuolation, and necrosis of kidney cells. This also causes impaired kidneys, reflected in the elevated or high levels of urea, uric acid, and creatinine in the blood. This also leads to a reduction the antioxidant enzymes like SOD, CAT, GPx, which lead to lipid peroxidation and oxidative damage of renal tissues. High exposure lead to inflammation and widespread fibrosis that indicates tissue injury and dysfunction.

**Effect on Teeth:** Fluoride also exerts harmful effects on the teeth of albino rats. Continuous exposure leads to dental fluorosis, characterized by chalky white or brownish discoloration, enamel hypomineralization, and surface pitting. Microscopic examination of the teeth shows irregular enamel and dentin formation, disorganization of odontoblasts, and degeneration of the dental pulp cells. These changes occur due to fluoride interfering with the process of mineralization and inducing oxidative stress in tooth-forming cells, leading to weaker and demineralized enamel. **Improvement after giving Tulsi extract:** Tulsi (Ocimum Sanctum) leaf extract, when induced in the fluoride-treated rat, shows a better improvement in the Kidney and teeth by reducing Toxicity as it has bioactive compounds like eugenol, ursolic acid, rosmarinic acid, and flavonoids, which possess powerful antioxidant, anti-inflammatory, and detoxifying properties.

**In the Kidney:** Tulsi leaf extract helps to neutralize free radicals, reduce lipid peroxidation, and restore the activities of antioxidant enzymes (SOD, CAT, GPx). This results in regeneration of renal tubules, normalization of glomerular structure, and a significant reduction in blood urea and creatinine levels, showing recovery of the Kidney of the Albino rat

**In Teeth :** Here also, Tulsi shows its antioxidant effect on fluoride by restoring enamel structure, reducing oxidative damage, and improving mineral composition.

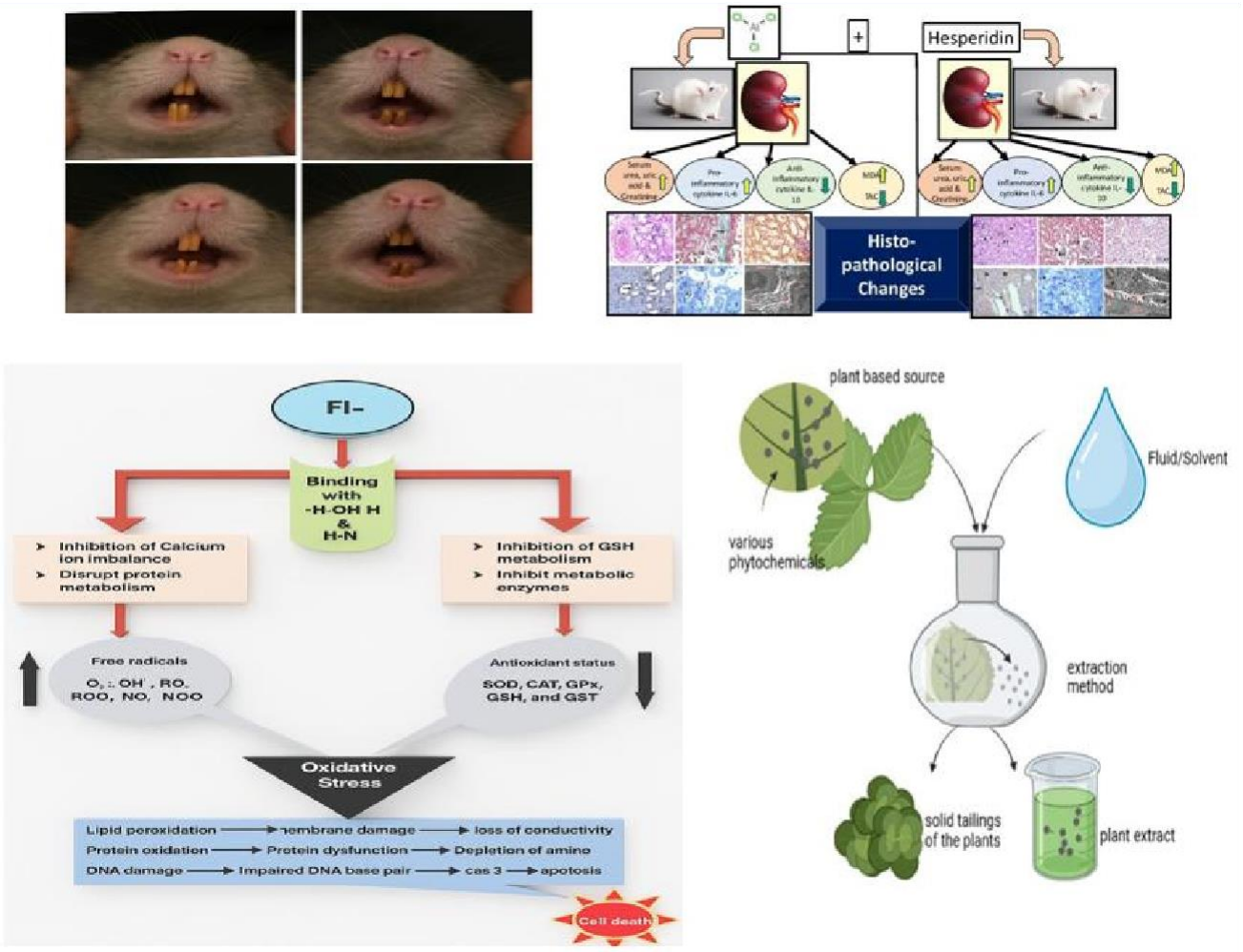



**Fig6. Effect of Fluoride on rat teeth and kidney and FI- Oxidative Stress**

## 4. RESULT &DISCUSSION

**Blood Collection:**

Fluoride and Tulsi (10 ppm/1 ml/ 40mg 60days/rat) dissolved in distilled water. Blood samples were collected after fasting on day 0 and again after the experiment using a heart puncture technique with Chloroform and IP. We collected blood samples from rats and divided them into three vials. The first vial was an EDTA vial, the second was a plain vial, and the third was a fluoride vial. The EDTA vial was used for whole blood testing, the plain vial was used for insulin testing, the fluoride vial was utilized, and for liver function tests {LFT} and renal function tests {RFT}. After centrifuging the blood samples for 15 minutes at 3000 rpm to separate the serum, the following metrics were evaluated: Glycaemic index, which includes insulin resistance, serum insulin levels, and glucose levels; ALT and AST activity were measured using ALT and AST; and direct, indirect, and total bilirubin levels were also evaluated. To assess weight gain, the animals' body weights were recorded both at the beginning of the study and later measured weekly until the study concluded.


**Fig7. Light Microscope, microtome, tissue, albino rat blood collection**

**Histopathology organ effects of fluoride toxicity. Kidney:** When fluoride toxicity is induced in the Albino rats for 6 weeks in different amounts, many organs, tissues are affected. More effects can be seen on the kidneys. The kidney is broken down and mixed in a Tissue homogenizer and make a smooth mixture called a Homogenate. This Homogenate is then observed under a microscope with resolution powers of 50X, 120X, and 150X. (Done in the laboratory of NIMS University) Results are given below

**A.** This shows the control or normal Glomeruli are intact, and well-organized tubules have a clear lumen and normal epithelial lining. **B.** Moderate amount of toxicity: Black arrow towards Glomeruli that appear shrunken and irregular in shape, tubular cells are swelling, loss of cytoplasmic clarity, and degeneration lumen of Renal tubules appear dilated, and cell boundaries are less distinct. **Ç.** In this picture, Tulsi leaf extract induces in the rat show protective and antioxidant effects against Fluoride toxicity, and shows the improvement in the structure of tissue. Compared to B, The Glomeruli and tubules are more defined, and cellular organization is partly restored.

**D.** High dose of toxicity: In this Glomeruli are severely damaged and distorted, with loss of tubular epithelium and necrosis.

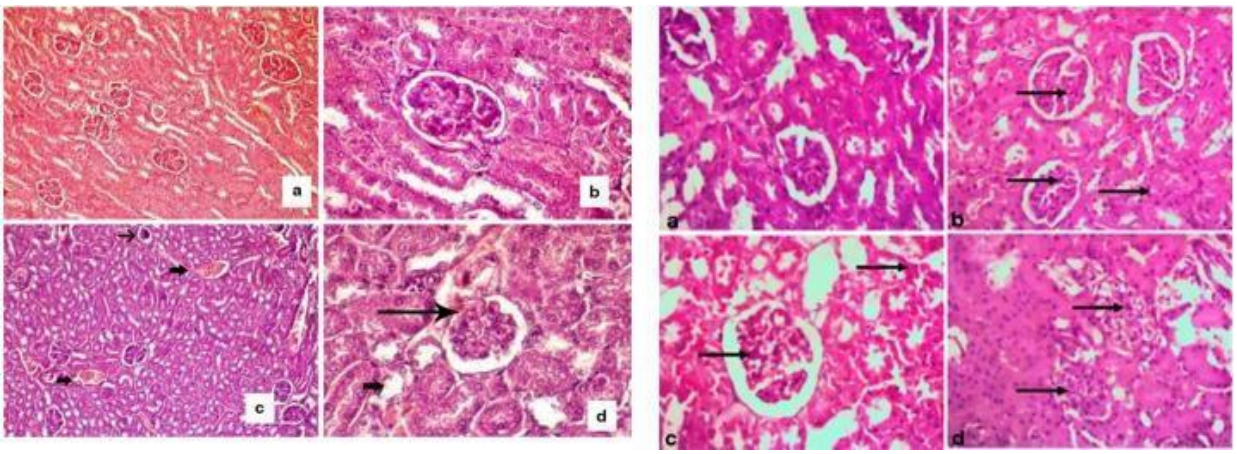



Image1. This demonstrates that well-organized tubules have a clear lumen and a normal epithelial lining, and that the control or normal Glomeruli are intact. Level of toxicity that is moderate: The lumen of renal tubules seems dilated, tubular cells are swollen, the cytoplasmic clarity is lost, and the cell borders are less clear. There is a black arrow pointing towards glomeruli that appear shrunken and irregular in shape.

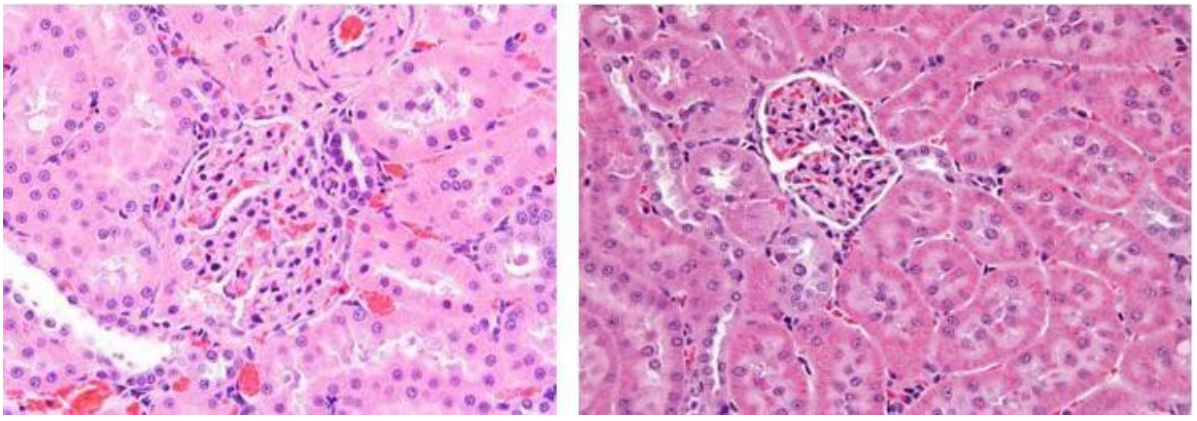


Image2. In this picture, Tulsi leaf extract induces in the rat show protective and antioxidant effects against Fluoride toxicity, and shows the improvement in the structure of tissue. Compared to B, The Glomeruli and tubules are more defined, and cellular organization is partly restored.

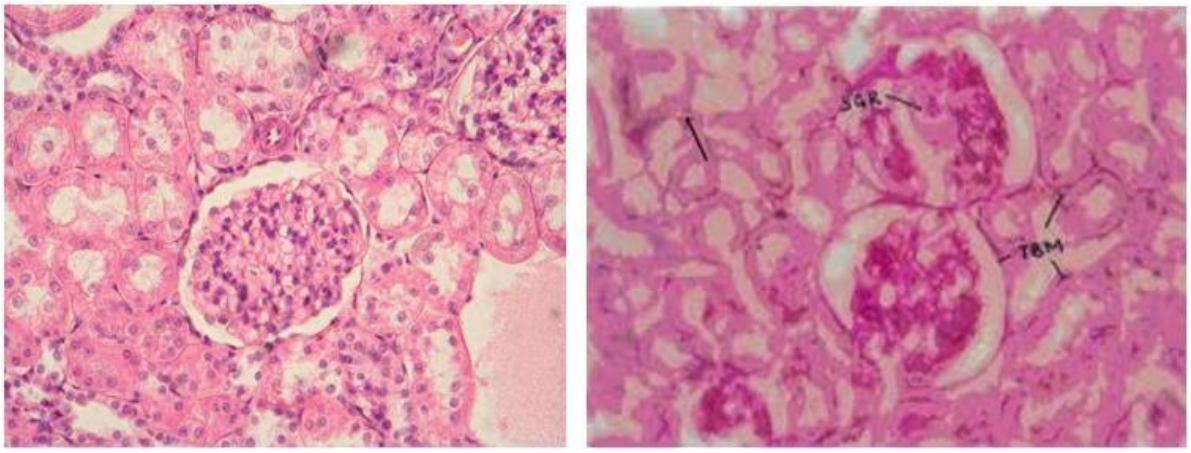



Image3. High dose of toxicity: In this Glomeruli are severely damaged and distorted, with loss of tubular epithelium and necrosis.

## 5. CONCLUSION

Fluoride is a naturally occurring ion. A minimum amount of Fluoride (0.7 – 1.5 mg) is important for the body for preventing tooth decay in children, Strengthens Tooth Enamel, Supports Bone Health, and acts as an Antibacterial Agent. High amounts (>1.5mg) of fluoride affect many vital organs of the body, like the kidneys, Liver, teeth, bones, etc., also causing fluorosis and oxidative stress in the kidneys and liver. During the experimental phase of six weeks, different groups are divided with different toxicity levels of fluoride, like control, 20mg, 30mg, and 40mg. This Toxicity is induced in the rats then observe the effect on body weight, highly targeted or affected organ. To reduce the effect of fluoride, an antioxidant (Ocimum Sanctum) Tulsi leaf extract is induced in the rat, showing better results by reducing the Toxicity level in the kidney and teeth and helps to regain their original shape and proper function of the tissue, and also prevents dental and skeletal fluorosis as well as kidney and liver stress.

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