# INVESTIGATION ON THE EFFECT OF *HIBISCUS SABDARIFFA CALYX* EXTRACT ON SODIUM FLUORIDE INDUCED FLUOROSIS IN RATS

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## ABSTRACT

Fluorosis was induced by the oral administration of Sodium Fluoride (10mg/kg) for 30 days. On 30th day the Flurosis was confirmed by studying the level of fluorine in serum and urine. Treatment was started from 30$^{th}$ day to 60$^{th}$ day by ingesting *Hibiscus Sabdariffa* calyx ethanolic extract 200 mg/kg and 400mg/kg. The treatment was continued for 30 days on the 60$^{th}$ day serum calcium, alkaline Phosphopate, Urine calcium, magnesium, Phosphorus, Fluorine, SGOT, SGPT of serum, WBC Count, hemoglobin and PCV of Plasma were estimated. It was found that serum calcium, WBC, Hemoglobin, PCV were decreased in positive control rat. Decrease levels inclined in treatment group dose dependently. The level of serum ALP, Urinary calcium, Phosphates, magnesium, Phosphorus, Fluorine, SGOT, SGPT were increased in positive control group and enhanced parameters were declined in treatment group dose dependently. On 60$^{th}$ day rats of all groups were sacrificed and histopathological parameters of kidney and liver were studied where we found the improvement in epithelium which was damaged by sodium fluoride. Less damage was seen in the treatment group. Histopathological studies of bone confirmed the increase of young cells in treatment group thus by assessing all the parameters we concluded that *Hibiscus Sabdariffa* calyx ethanolic extract can be used to treat fluorosis dose dependently.

**Key words:** Flurosis, *Hibiscus Sabdariffa*, Sodium Fluorides, Ethanolic extract.

## 1. INTRODUCTION:

Fluorosis is a disease caused by excessive ingestion of fluoride through water and or food. Fluorine is an element, a highly reactive and toxic gas, but it does not exist as such in nature. It is always combined with some other element or group of elements. When fluorine combines with a single element, generally a metal like potassium, calcium, aluminium, etc, it is a fluoride [1, 2].Flurosis continues to be an endemic problem. As per WHO standards permit only 1.5 mg/ml is considered as a safe limit of fluoride in drinking water for human consumption [3]. The problem of excessive fluoride in ground water in India was first reported in 1937 in the state of Andhra Pradesh. in India, approximately 62 million people including 6 million children suffer from Flurosis

because of consumption of water with high fluoride concentrations. Seventeen states in India have been identified as endemic for fluorosis [4]. Among the surveyed population 411,744 patients had disorders of calcium and bone metabolism. Of those 52 % had nutritional bone diseases, 43 % had endemic skeletal Flurosis and 5 % had metabolic bone diseases. Thus endemic skeletal fluorosis in India continues to be a national health problem and the drinking water remains the major source of intake of excess of fluoride. The most seriously affected states are Andhra Pradesh, Punjab, Haryana, Rajasthan, Gujarat, Uttar Pradesh, Bihar, Tamil Nadu, Kerala, Karnataka and Maharashtra. It is estimated that the fluoride skeletally affects about 100 million people, while furthermore not less than 200 million are at risk in India **[5].** *Hibiscus Sabdariffa* is commonly used as a culinary deliancy in this region. As it is rich calcium, phosphorus, iron, carotenoid.It is regarded as diuretic, cholerectic febrifugal and hypotensive, decreasing the viscosity of the blood, stimulating intestinal peristalsis, lowering blood pressure. Antispasmodic, anthelmintic, antibacterial, strengthening the immune system, prevents bladder infections and constipation [6]. *Hibiscus Sabdariffa Calyx* contains Calcium, Phosphorus, Iron, Carotene, Thiamine, Riboflavin, Niacin, Ascorbic acid, Protein, Fiber [7]. This is the reason for the selected the *Hibiscus Sabdariffa Calyx* in the treatment of Flurosis. *Hibiscus Sabdariffa Calyx* is commonly used as a culinary delicacy in this region. It is rich in calcium (12.63mg), phosphorous (273.2mg), Iron (8.9mg), carotene (0.029mg), vit C (6.7mg). As diet rich in calcium could displace fluorine from the body, we have investigated the role of aqueous and alcoholic extract of *Hibiscus Sabdariffa calyx* combating the effect of NaF.



## 2. MATERIALS AND METHODS:

**2.1. COLLECTION AND AUTHENTICATION OF PLANT MATERIAL:** The calyx of *Hibiscus Sabdariffa* was collected locally and authenticated by the botanist Prof. Laxman Reddy, N.G College, in Nalgonda, A.P, India.

**2.2 DRUGS AND CHEMICALS:** Calcium Kit (Transasia Bio-medicals Ltd, Mumbai), Phosphorus Kit (Transasia Bio-medicals Ltd, Mumbai), Alkaline Phosphatase (Transasia Bio-medicals Ltd, Mumbai), Creatinine Kit (Transasia Bio-medicals Ltd, Mumbai), Hydroxyproline Standard, Magnesium,Fluoride,Paradimethyl Aminobenzaldehyde, Hydrogen Peroxide, Sodium Hydroxide, Copper Sulphate, Sodium fluoride. All chemicals used are of analytical grade.

**2.3 PREPARATION OF PLANT EXTRACTION:** *Hibiscus Sabdariffa* calyx were collected and dried in a shade. 40 g of shade dried calyx of *Hibiscus Sabdariffa* was refluxed with 65% ethanol at $60^{o}$ C and the extract was concentrated under vacuum to produce a viscous residue. The percentage yield of *Hibiscus Sabdariffa* ethanol extract was found to be 14 % w/w. The ethanolic extract of *Hibiscus Sabdariffa* was dissolved in 1% acacia for the administration of extracts to the rats by oral route by oral feeding needles.

**2.4 EXPERIMENTAL ANIMALS:**

Thirty Male wistar rats of 3 months old weighing approximately 200 g were procured from National Institute of Nutrition, Hyderabad, India. The rats were housed in a room which provided alternating 12 h periods of light and dark with the room temperature of 23 ± 1 °C. All animals were allowed free access to distilled water and fed on a commercial diet. The experimental protocol has been approved by the institutional animal ethical committee and care of animals ((NCOP/IAEC/Approved 21/4/2010, date:10.04.2010) has been done as per the guideline laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

**2.5 ACUTE TOXICITY STUDY:** The safety dose of *Hibiscus Sabdariffa* has already been reported and it is found to be above 5000 mg/kg b.w [8]**.** Ethanolic Extract of *Hibiscus Sabdariffa* calyx dosage was fixed as 200 mg/kg and 400 mg/kg.

**2.6 INDUCTION OF FLUOROSIS WITH SODIUM FLUORIDE:** Fluorosis was induced by the oral administration of sodium fluoride (10 mg/kg b.w.) for 30 days. Induction was confirmed by the estimation of fluorine in serum on $30^{th}$ day after collecting the blood by retro orbital puncture and centrifuge at 4000 rpm for 15 minutes [9].

**2.7 EXPERIMENTAL DESIGN:**

Thirty animals were divided randomly into 5groups.

**Group I**-- Treated with Saline (negative control).

**Group II**-- These sodium fluoride induced rats used to induce of fluorosis (Positive control).

**Group III**-- These are sodium fluoride induced rats and treated with ethanolic extract of ***Hibiscus Sabdariffa*** calyx 200mg/kg b. w. dose.

**Group IV**-- These were sodium fluoride induced rats and treated with ethanolic extract of ***Hibiscus Sabdariffa*** calyx 400 mg/kg b.w. dose.

**2.8 EXPERIMENTAL SCHEDULE, COLLECTION OF BLOOD AND ESTIMATION OF PARAMETERS:** The animals were anesthetized with ketamine on day 30th day. Blood was withdrawn from the retro orbital plexus. Serum was separated after centrifuge at 4000 rpm for 10 minutes. Serum Calcium (OCPC Method) [10, 11, 12]. Alkaline Phosphatase (PNPP KINETIC Method) [13], Serum SGOT, Serum SGPT, White blood cells, PCV, Hemoglobin. These blood parameters were estimated by estimated by using kits.

Urine was collected in metabolic cages and estimation of calcium (OCPC Method), phosphorus (MOLYBDATE U.V Method), and creatinine (Modified Jaffe's Kinetic Method) was done using erba kits. Urine hydroxyproline is estimated by colorimetric method Modified Neuman and Logan Method). The levels of serum and urine chemistries were measured by standard colorimetric methods (using a semiautomatic analyzer (Erba Chem-5 Plus V2, Transasia Bio-Medicals Ltd, Mumbai). **Parameters:** Urine fluoride, Urine Calcium (OCPC Method) [10]. Urine Magnesium [14, 15, 16], Urine Phosphorus (MOLYBDATE U.V Method). [24] Urine Creatinine (MODIFIED JAFFE'S KINETIC Method) Urine Hydroxy proline (MODIFIED NEUMAN AND LOGAN Method) All these urine parameters were estimated by estimated by using kits.

**2.9 HISTOPATHOLOGICAL EXAMINATION:** Animals were sacrificed on 60th day. Liver and kidney were fixed in 10 % v/v formalin solution, dehydrated in graded ethanol cleaved in xylene and embedded in paraffin blocks 5 micron-thick sections obtained were stained with hematoxylin and eosin dye and examined for pathological changes **[20]**.

**2.10 STATISTICAL ANALYSIS:**

Data are expressed as mean ± SEM. Analysis of data was done by One-Way ANOVA followed by Dunnett comparison test using Graph Pad InStat version 3.10 for Windows 2009 (Graph Pad Software). The statistical significance was set as 0.05 level ($P<0.05$).

# 3 RESULTS:

## 3.1 Effect of *Hibiscus Sabdariffa* calyx ethanolic extract on urine calcium in sodium fluoride induced rats:

| S.NO | GROUP | TREATMENT | DOSE | CALCIUM (mg/dl) |
|---|---|---|---|---|
| 1 | GROUP I<br>-Ve control | saline | ___ | 12±0.022 |
| 2 | GROUP II<br>+Ve control | Sodium fluoride | 10 mg/kg | 5±0.012 |
| 3 | GROUP III | NaF + *Hibiscus Sabdariffa* | 200 mg/kg | 9±0.013* |
| 4 | GROUP IV | NaF + *Hibiscus Sabdariffa* | 400 mg/kg | 11±0.016* |

Values are mean ± SEM, n=6, NaF: Sodium fluoride, * p<0.05 compared with the positive control group.

## 3.2 Effect of *Hibiscus Sabdariffa* calyx ethanolic extract on urine magnesium in sodium fluoride induced rats:

| S.NO | GROUP | TREATMENT | DOSE | MAGNESIUM (mg/dl) |
|---|---|---|---|---|
| 1 | GROUP I<br>(negative control) | saline | ___ | 3.8±0.022 |
| 2 | GROUP II<br>(positive control) | Sodium fluoride | 10 mg/kg | 3.2±0.009 |
| 3 | GROUP III | NaF + *Hibiscus Sabdariffa* | 200 mg/kg | 3.6±0.015 |
| 4 | GROUP IV | NaF + *Hibiscus Sabdariffa* | 400 mg/kg | 3.9±0.023* |

Values are mean ± SEM, n=6, NaF: Sodium fluoride, * p<0.05 compared with the positive control group.

### 3.3 Effect of *Hibiscus Sabdariffa* calyx ethanolic extract on urine PVC in sodium fluoride induced rats:

| S.NO | GROUP | TREATMENT | DOSE | PACKED CELL VOLUME |
|---|---|---|---|---|
| 1 | GROUP I<br>-Ve control | saline | ___ | 42±0.010 |
| 2 | GROUP II<br>+Ve control | Sodium fluoride | 10 mg/kg | 38±0.012 |
| 3 | GROUP III | NaF + *Hibiscus Sabdariffa* | 200mg/kg | 39±0.012 |
| 4 | GROUP IV | NaF + *Hibiscus Sabdariffa* | 400mg/kg | 40±0.011* |

Values are mean ± SEM, n=6, NaF: Sodium fluoride, * $p<0.05$ compared with the positive control group.

### 3.4 Effect of *Hibiscus Sabdariffa* calyx ethanolic extract on Hemoglobin in sodium fluoride induced rats:

| S.NO | GROUP | TREATMENT | DOSE | HEMOGLOBIN |
|---|---|---|---|---|
| 1 | GROUP I<br>-Ve control | saline | ___ | 14.2±0.001 |
| 2 | GROUP II<br>+Ve control | Sodium fluoride | 10 mg/kg | 10.2±0.005 |
| 3 | GROUP III | NaF + *Hibiscus Sabdariffa* | 200mg/kg | 11.8±0.003 |
| 4 | GROUP IV | NaF + *Hibiscus Sabdariffa* | 400mg/kg | 13.6±0.001* |

Values are mean ± SEM, n=6, NaF: Sodium fluoride, * $p<0.05$ compared with the positive control group.

### 3.5 Effect of *Hibiscus Sabdariffa* calyx ethanolic extract on urine SGOT in sodium fluoride induced rats:

| S.NO | GROUP | TREATMENT | DOSE | SGOT |
|---|---|---|---|---|
| 1 | GROUP I<br>-Ve control | saline | ___ | 98±0.005 |
| 2 | GROUP II<br>+Ve control | Sodium fluoride | 10 mg/kg | 145±0.015 |
| 3 | GROUP III | NaF + *Hibiscus Sabdariffa* | 200mg/kg | 121±0.009* |
| 4 | GROUP IV | NaF + *Hibiscus Sabdariffa* | 400mg/kg | 101±0.006* |

Values are mean ± SEM, n=6, NaF: Sodium fluoride, * $p<0.05$ compared with the positive control group.

### 3.6 Effect of *Hibiscus Sabdariffa* calyx ethanolic extract on urine SGPT in sodium fluoride induced rats:

| S.NO | GROUP | TREATMENT | DOSE | SGPT |
|---|---|---|---|---|
| 1 | GROUP I<br>-Ve control | saline | ___ | 32.89±0.004 |
| 2 | GROUP II<br>+Ve control | Sodium fluoride | 10 mg/kg | 78±0.013 |
| 3 | GROUP III | NaF + *Hibiscus Sabdariffa* | 200mg/kg | 54±0.009* |
| 4 | GROUP IV | NaF + *Hibiscus Sabdariffa* | 400mg/kg | 39±0.005* |

Values are mean ± SEM, n=6, NaF: Sodium fluoride, * $p<0.05$ compared with the positive control group.

## 4. DISCUSSION:

Fluorosis is a disease caused by excessive ingestion of fluoride through water and or food. Fluorine is an element, a highly reactive and toxic. High intakes of fluoride and include periosteal and endosteal reactions, coarse axial trabcculations and osteopenia in studying the level of fluorine in serum and urine. Treatment was started from 30$^{th}$ day to 60$^{th}$ day by ingesting *Hibiscus Sabdariffa calyx* ethanolic extract 200 mg/kg 400mg/kg. The treatment was continued for 30 days on the 60$^{th}$ day serum calcium, alkaline Phosphopate, Urine calcium, magnesium, Phosphorus, Fluorine, SGOT, SGPT of serum, WBC Count, Hemoglobin and PCV of Plasma were estimated. It was found that serum calcium, WBC, Hemoglobin, PCV were decreased in positive control rat. Decrease levels inclined in treatment group dose dependently. The level of serum ALP, Urinary calcium, Phosphotase, Urine calcium, magnesium Phosphorus, Fluorine, SGOT, SGPT were increased in positive control group and enhanced parameters were declined in treatment group dose dependently. Thus by assessing all the parameters we concluded that *Hibiscus Sabdariffa calyx* ethanolic extract can be used to treat fluorosis.the metaphyseal regions, sclerosis, and modeling abnormalities of the epiphyses, carpal and other bones of the hand, more particularly observed in growing children. In fluorosis the decreased mineral content was observed. Hibiscus *Sabdariffa* found as rich source of calcium, magnesium, phosphorus, ascorbic acid, tannins. Fluorosis was induced by the oral administration of Sodium Fluoride (10mg/kg) for 30 days. On 30th day the fluorosis was confirmed by



## 6. CONCLUSION:

Ethanolic extract of *calyx of Hibiscus Sabdariffa* found as rich source of calcium, magnesium, phosphorus, ascorbic acid, tannins. It shows a significant improvement in serum calcium and urine calcium, magnesium levels and there are decreased levels of phosphorus, hydroxyproline and creatine was observed with ethanolic extract of *calyx of Hibiscus Sabdariffa.* A decreased liver toxicity was observed with *Hibiscus Sabdariffa* by estimating ALP, SGOT, SGPT on sodium fluoride induced fluorosis rats. *Hibiscus Sabdariffa* is effective for the treatment of fluorosis and dose dependently confirming its use and minimizing fluoride induced toxicity.

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