Effect of *Moringa oleifera* leaves on hematological profile of fluorosis affected rats

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Abstract:
Fluorosis is a metabolic disease that is endemic in nearly 25 countries with India being one of the most affected. It primarily affects the bone and the teeth. *Moringa oleifera* (MO) leaves are known to reduce the effect of fluorosis on various tissues. Therefore, it is of interest to document the effect of *Moringa oleifera* leaves on the hematological profile of fluorosis affected rats. Twenty four Sprague Dawley rats were housed two per cage in a room with 12 hours light and 12 hours dark cycle. The rats were allowed to adjust to the laboratory environment for about one to two weeks before the beginning of the study. This study reveals that MO leaves is effective in reducing the plasma fluoride content. It also helps in improving the Hb % and RBC count in fluorosis affected rats. Data shows that *Moringa olifera* leaves powder is effective in reducing the plasma fluoride content. It also helps in improving the Hemoglobin percentage & Red Blood Cell count in fluorosis affected rats.

Keywords: Fluoride, anemia, intoxicification, premature erythrocytes death, drinking water, *Moringa Olifera*
Background:
Fluoride is known to act as a double edged sword and is the 13th abundant highly reactive, electronegative halogen with an atomic number 9 in the earth’s crust [1-2]. It helps to prevent the formation of dental caries at ingestion in low concentration (<1 ppm) but ingestion in high concentration leads to dental and skeletal lesions, commonly called as fluorosis [3]. There are 25 countries in Asia and Africa which are affected with fluorosis and India is the front manner with a highest prevalence. Present Indian statistics depicts that around 25 million people were affected by fluorosis and 66 million in future including children of age 14 years are at risk of developing fluorosis [4]. Studies show that 39% of population in Karnataka exhibited skeletal fluorosis [4]. Kolar located in the eastern most Karnataka has been reported to have 26,000 people suffering from dental and skeletal fluorosis [4]. Most common causes for fluorosis are through internal route and the main fluoride content is the drinking water. Other sources include exposure to fluoride rich effluents, dust and smoke from aluminum smelters plants, copper, glass, iron, super phosphate fertilizers plants and brick kilns areas [5-6]. The control of fluoride drinking-water is critical in preventing fluorosis. Removal of excessive fluoride from drinking-water is difficult and expensive. Fluorosis also leads to muscle fatigue, muscle weakness, hypothyroidism, anemia, oxidative stress that promotes atherosclerosis & myocardial cell damage, lung parenchymal inflammation, decreased GFR and diabetes mellitus [2]. Anemia has several complications in children and adults especially in pregnant women. During fluorosis anemia is caused due to reduced erythropoietin activity [2]. Some studies showed that fluoride toxicity may increase phagocytic activity of macrophages to engulf more RBC in spleen which contributes to anemia and increased white blood cells causing hematological alterations in rabbits [7]. A study reported that fluoride intoxication may lead to anemia by early hemolysis [8]. Most of these manifestations are, no doubt, nonspecific, but their occurrence in subjects living in fluorosis-endemic areas should alert suspicion. These early warning signs have been extremely helpful in early detection of large numbers of cases in rural areas; prompt intervention programmes (i.e. providing safe drinking water) in these cases have provided considerable relief within a short span of time [9]. Several inorganic and organic treatment methods such as reverse osmosis, nano filtration, electrodialysis, donan dialysis, ultra filtration, ion exchange and adsorption were tried to reduce fluorosis [10-11]. Some studies found that beneficial effect of aqueous extract of Moringa seeds & dried leaf powder to minimise fluoride toxicity in rabbits and calves [12]. Moringa oleifera (MO) leaves belongs to Moringaceae family commonly known as “The Miracle Tree,” “Horseradish-tree,” or “Ben oil tree” [13]. It is a multipurpose crop, widely cultivated in Africa and Southern Asia and has medicinal and nutritional properties [14, 15]. Leaves of the MO tree are noted for high crude protein, energy and appreciable levels of carotene, ascorbic acid, iron, methionine and cysteine with negligible amounts of tannins [16]. Earlier studies have found MO is nontoxic and recommended for therapeutic use in developing countries [17]. Therefore, it is of interest to document the effect of MO leaves on haematological profile of fluorosis affected rats.

Materials & Methods:
Design of study: Prospective case control study

Number of animals:
Twenty four male Sprague Dawley (SD) rats are included for the study and were categorized into four groups (Group I, Group II, Group III & Group IV) of six animals each which was approved by institutional animal ethics committee (IAEC/PHARMA/SDUMC/2017-18/10a). Group I (n = 6): Control animals had free access to RO water for a period of 30 days (reference range of fluoride is 0.3-0.5mg/litre) [18]. Group II (n=6): Sodium fluoride was administered ad libitum in the drinking water at a concentration of 50mg/kg body weight for a period of 30 days [19,22]. Group III (n = 6): This group animals received 50mg/kg of body weight of fluoride in drinking water, supplemented with 200mg/kg of MO orally by mixing with water through an oral gavage bent needle for a period of 30 days [21]. Group IV (n = 6): Animals with continuous access of food and RO water supplemented with MO by mixing with water through a oral gavage bent needle as a vehicle for a period of 30 days.

Study Location:
This study was done in the department of physiology, Sri Devaraj Urs Medical College. SD rat’s 10-12 weeks old, weighing 180-220 gms, were housed two per cage in a room with 12 hour’s light & 12 hours dark cycle. The rats were allowed to adjust to the laboratory environment for around one to two weeks before the start of the study. A standard animal feed and drinking water were provided ‘ad-libitum’.

Plant materials:
Semi ripen leaves of M O were washed with clean water to remove dirt and soil. The leaves which are having any outer observable lesions or decayed ones were discarded. The leaves were dried at 60°C upto a constant mass. These dried materials further processed into powder form by passing through grinder, it was kept in air tight sachets till further use [1]. Blood sample were collected by retro orbital puncture from all the groups for complete blood picture & fluoride levels estimation. Plasma was separated from the EDTA blood samples for the estimation of fluoride.

Complete blood picture & blood smear analysis:
After collecting the EDTA blood samples from all the groups, haemoglobin (Hb) (g/dL), Red blood cell count (RBC), Total leucocyte count (TLC), Packed cell volume (PCV), Differential leucocyte count (DLC) & Reticulocyte count were estimated by automated haematology system analyzer method; peripheral blood smear was studied to know the morphology of cells. Erythrocyte indices such as Mean corpuscular volume (MCV), Mean corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin concentration (MCHC) were calculated.

Estimation of fluoride:
The fluoride concentration of plasma samples were measured by Ion selective electrode method. This method was adopted by Cernik et al. with modifications of orien model [1].
Comparison of findings with Group II and Group IV:

There was no significant difference in other parameters such as MCV, compared with Group II. Similarly, Fluoride content was also not statistically significant.

Comparison of findings with Group I & Group IV:

Parameters such as MCH, MCHC, MCH, TLC, PLT & Rets % were decreased in Group II compared to Group I, but the decrease was not statistically significant.

Comparison of findings with Group I & Group III:

RBC count and Hb % were significantly decreased (p < 0.05) in Group II as compared with Group I. Fluoride content was increased in Group II compared with Group I and was statistically significant. Parameters such as MCV, MCHC, MCH, TLC, PLT & Rets % were decreased in Group II compared to Group I, but the decrease was not statistically significant.

Comparison of findings with Group I & Group IV:

There was no significant difference in parameters between Group I & Group III.

Comparison of findings with Group I & Group IV:

RBC count and Hb % were significantly increased in Group IV compared with Group I. Similarly, Fluoride content was significantly decreased in Group IV compared with Group II. There was no significant difference in other parameters such as MCV, MCHC, MCH, TLC, PLT & Rets %.

Comparison of findings with Group II and Group IV:

RBC count and Hb % were significantly increased in Group IV compared with Group II. Fluoride content was significantly decreased in Group IV when compared with Group II. There was no significant difference in other parameters such as MCV, MCHC, MCH, TLC, PLT & Rets %.

Analysis & Statistical Methods:

Data was coded and entered into Microsoft excel data sheet. Quantitative data was represented as mean, confidence interval and categorical data by percentages. Data was analyzed by using two-way analysis of variance (ANOVA) & post hoc analysis to compare between the groups. A p value of less than or equal to 0.05 is considered as statistically significant.

Results & Discussion:

The results were analyzed using the licensed version of SPSS statistics 20, Mean ± SD was calculated.
looses calcium content. The membrane which is lacking in calcium content is flexible and is thrown into folds. The shape of erythrocytes is changed. Such RBCs are called echinocytes, which will be found in circulation. The echinocytes are eliminated from circulation by means of phagocytosis. This would lead to low haemoglobin levels in fluoride toxicity [27]. Some studies suggest that dietary supplement of MO may have the potential of reversing anemia within a short period of administration, because it has been known to contain alkaloids, flavonoids, phytosterols and saponins which are identified to have hemapoietic property. Apart from these bioactive substances in the leaves of MO, it has also been said to be an outstanding source of vitamin A, B, C, minerals like iron as well as protein, which may all contribute to its observed effects on red blood cells [28].

Maryam et al. in their study showed that administration of fluoride orally to rabbits leads to reduction in RBC count, leukocytopenia, monocytosis, eosinopenia, neutrophilia and thrombocytosis and stated that fluoride toxicity may increase phagocytic activity of macrophages to engulf more RBC in spleen which contributes to anemia causing haematological alterations [7]. The present study findings are consistent with the data documented by Maryam et al. and Mandal et al. [1, 7]. We could observe that in fluoride supplemented group significantly reduced was observed only in Hb% and RBC count but not with other blood parameters. Studies conducted by Susheela et al. reported that fluoride intoxication leads to anemia by premature erythrocyte deaths [9]. Peripheral smear examination reveals normocytic normochromic anemia. Mandal et al. have showed that the calves reared in flourotic zone had decreased Hb, PCV, TLC and increased fluoride content supplementation of dried MO fruit powder to those calves resulted in significant reduction in fluoride levels and increase in Hb%, PCV, TLC. They showed that Supplementation of MO fruit powder was able to reduce the plasma fluoride level in affected calves. Interference with fluoride absorption from the gut might have allowed the calves to reduce the plasma fluoride level in affected calves. Therefore, the usage of local and easily available plants like MO in reducing fluoride levels & improved haematological effects due to fluorosis is reported.

Conclusion:
Data shows that MO leaves powder is effective in reducing the plasma fluoride content. It also helps in improving the Hb % & RBC count in fluorosis affected rats. Thus, the usage of local and easily available plant products like MO in reducing fluoride levels & improved haematological effects due to fluorosis is reported.

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