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RESEARCH ARTICLE

BIOLOGICAL EFFECTS OF TOPICAL APPLICATION OF MORINGA OLEIFERA EXTRACT VERSUS FLUORIDE ON UREMIC PATIENTS EXTRACTED TEETH.

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Abstract

Chronic renal failure can elicit a wide spectrum of oral manifestations in the hard and soft tissues. Moringa oleifera leaves have been reported to be a rich source of β-carotene, protein, vitamin C, calcium and potassium. Many studies have demonstrated the efficacy of casein phosphopeptide amorphous calcium phosphate with fluoride paste in promoting remineralization of enamel and dentin in vitro. This study was designed to determine the effect of renal insufficiency on patients' teeth (enamel and dentin) and study biological effects of topical application of moringa extract versus fluoride on extracted teeth. Fifty sound posterior teeth were used, divided into 3 groups. Each group was subdivided into 2 subgroups; A (control) and B (uremic). Group I (control group).Group II (fluoride group). Group III (Moringa oleifera group).All the specimens were scanned by using scanning electron microscope and elemental analysis of enamel and dentin surfaces was tested using energy dispersive analytical x-ray. Enamel of uremic teeth showed irregular enamel surface with deep depressions and pitting. Also, dentinal tubules showed different shaped outlines and diameter. Increase in calcium and phosphorus levels was statistically significant with Moringa as same as CPP-ACPF groups. **Conclusions:** CRF leads to alteration in the structure of enamel and dentin of permanent teeth with significant increase in mineral content (Ca& P) of permanent enamel and dentin when compared with healthy controls. Moringa has a protective effect on enamel and dentin remineralization similar/or better than fluoridated pastes.

Introduction:-

Chronic renal failure (CRF): is defined as a slow progressive loss of kidney function over the span of years, resulting in permanent kidney failure. **(Foley RN, Collins AJ, 2007).** Renal failure accompanied by noticeable symptoms is termed uremia **(Dr Per Grinsted, 2005)**. Classical signs of uremia are: progressive weakness and easy fatigue, loss of appetite due to nausea and vomiting, muscle atrophy, tremors, abnormal mental function, frequent shallow respiration and metabolic acidosis. **(Burtis, Ashwood and Bruns, 2012)**

A common oral symptom of CRF is the sensation of a dry mouth, odorous (uremic) breath and sensations of metallic tastes in the mouth (uremic fetor). **(La Rosa-García et al., 2006)**. Severe erosion on lingual surface of teeth due to frequent regurgitation and vomiting induced by uremia, medication and nausea from dialysis, a diminished prevalence of caries has been observed and is due to the protective effect of urea, which inhibits bacterial growth and neutralizes bacterial plaque acids **(Klassen, Krasko, 2002).** Enamel hypoplasia which can affect both primary and permanent dentition **(Davidovich et al., 2005)**.

Demineralization and remineralization have a crucial impact on the hardness and strength of tooth enamel. Demineralization occurs at a low pH when oral environment is undersaturated with mineral ions, relative to a tooth's mineral content **(Margeas, 2006).**

New technologies exist that create remineralization through various means of providing calcium and phosphate into saliva, changing the balance of the ratio toward remineralization. Recaldent (Casein phosphopeptide and amorphous calcium phosphate CCP-ACP): is a milk-derived peptide that is bound to amorphous calcium phosphate that binds to the tooth structure and is released during acidic challenges **(Reynolds, 1997).** With fluoride treatment, a noncavitated lesion can be remineralized with fluorapatite and have greater resistance to subsequent demineralization than hydroxyapatite **(Tenuta, 2009).**
Moringa oleifera commonly known as (Miracle Tree) or (Mother Best Friend) is most widely distributed species of moringaceae family, having an impressive range of medicinal uses with high nutritional value throughout the world **(Farooq et al., 2012).** The leaves and stems of Moringa olifera are known to have a large amount of their calcium bound in calcium oxalate crystals more vitamin than carrot, more calcium than milk more iron than spinach more vitamin c than orange and more potassium than in Banana **(Con Mark Olson, 2001).**

Materials and methods:-

Fifty sound posterior teeth were used in this study. The teeth were collected from healthy individuals (n=25), uremic patients (n=25), each tooth in group II and III were longitudinally sectioned into two halves perpendicular to the long axis of the root. Two specimens were prepared from each tooth. The specimens were divided into 3 groups. Each group was subdivided into 2 subgroups; subgroup A: healthy, and subgroup B: uremic. **Group I (control group):** The teeth were etched (n=10) each subgroup consists of 5 specimens**. Group II (fluoride group):** The specimens were etched then exposed to CPP-ACPF to the enamel and dentin surfaces and left for 5 minutes, then delicately removed with cotton tips and immersed in artificial saliva; (n=40) each subgroup consists of 20 specimens. **Group III (Moringa oleifera group):** Specimens were etched then exposed to Moringa oleifera solution and left for 5 minutes, then delicately removed with cotton tips and immersed in artificial saliva; (n=40) each subgroup consists of 20 specimens. The specimens of group II and III were subjected to the above mentioned steps twice daily for one week. The specimens were thoroughly rinsed with distilled water and dried with air spray for 30 seconds. Samples were coated with a thin layer of metal such as gold and prepared for SEM/ EDAX:

The treated enamel and dentin surfaces were scanned using SEM and images (photomicrographs) of areas of interest were captured. Elemental analysis of enamel and dentin surfaces of each specimen was tested.

Results: - SEM

Enamel; **Group I (control group):** Subgroup IA (healthy patients' specimens): Enamel showed enamel with type I etching pattern representing fish scales appearance. Enamel showed normal prisms and interprismatic substance with regular orientation showing parallel rows of protruded enamel prisms with interrod spaces, there are signs of rod resorbtion with variations in interrod space (Fig. 1A). Subgroup I B (uremic patients' specimens): The integrity of enamel prisms was severely affected and greater prism core dissolution compared with that in the interprismatic areas giving the enamel a honeycomb pattern (Fig. 1B)

Group II (fluoride group): Subgroup II A (healthy patients' specimens): eroded enamel specimens treated with CPP-ACPF paste showed filling of the interprismatic spaces with obvious formation of interprismatic substance seen as adherent granules or globules. These correspond to the redeposited minerals subsequent to mobilization of calcium and phosphate from the CCP-ACP (Fig. 1C). Subgroup II B (uremic patients' specimens): showed the enamel rods are attached to each other with gradual decrease in surface porosities with obvious formation of interprismatic substance (Fig. 1D).

Group III (Moringa group): Subgroup III A (healthy patients' specimens): showed increase mineral deposits in porous defects and smoothening of enamel surface (Fig. 1E). Subgroup III B (uremic patients' specimens): showed less advanced demineralization pattern compared to etching group with filling of the interprismatic spaces with obvious formation of interprismatic substance Fig. 1F).

Dentin; Group I (control group): Subgroup IA (healthy patients' specimens): showed normal dentinal surfaces appeared clear with no smear layer. Dentinal tubules showed uniform circular outlines (Fig 2A). Subgroup I B (uremic patients' specimens): Dentinal tubules showed different shaped outlines and diameter with variations in their diameter. Few lateral branching was found (Fig. 2B).

Group II (fluoride group): Subgroup II A (healthy patients' specimens): showing the diameter of dentinal tubules was decreased suggesting a remineralization effect of the CPP-ACPF paste (Fig. 2C). Subgroup II B (uremic patients' specimens): showed some of dentinal tubules were completely blocked showing nearly smooth surface (Fig. 2D).

Group III (Moringa group): Subgroup II A (healthy patients' specimens): showed occlusion of dentinal tubules due to formation of different sized mineral particles. Some of dentinal tubules were completely blocked (Fig. 2E). Subgroup III B (uremic patients' specimens): showed occlusion of dentinal tubules due to formation of different sized mineral particles. Most of dentinal tubules were completely blocked (Fig .2F).

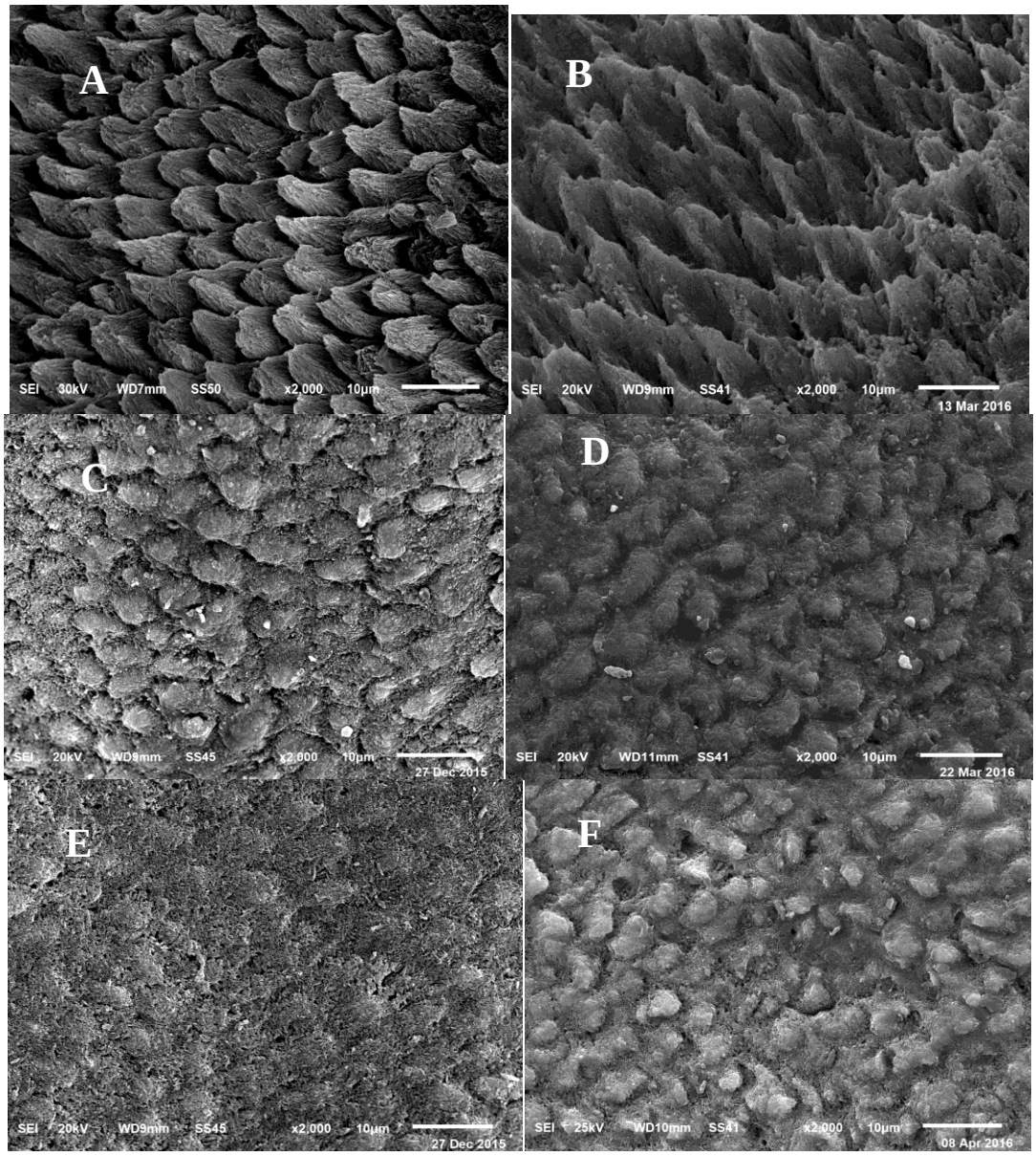

Fig.1:- SEM photomicrograph (x2000) **A (control group)**: subgroup 1A (healthy patients' specimens) showing enamel with type I etching pattern with eroded rod sheath giving fish scales appearance. B: subgroup 1B(uremic patients' specimens) showing enamel with type II etching pattern and and greater prism core dissolution compared with that in the interprismatic areas giving the enamel a honeycomb pattern. C (fluoride group): subgroup II A (healthy patients' specimens) showing insufficient enamel surface remineralization as adherent granules or globules after the treatment by CPP-ACPF. D: subgroup II B (uremic patients' specimens) showing enamel surface decrease in enamel surface roughnes with obvious formation interprismatic substance after the treatment with CPP-ACPF.. E (moringa group): subgroup III A (healthy patients' specimens) showing increase mineral deposits in porous enamel surface after the treatment with moringa oleifera. **F**: subgroup III B (uremic patients' specimens) showing appearance of mineralized deposits that were scattered along the porous enamel surface after the treatment with moringa oleifera

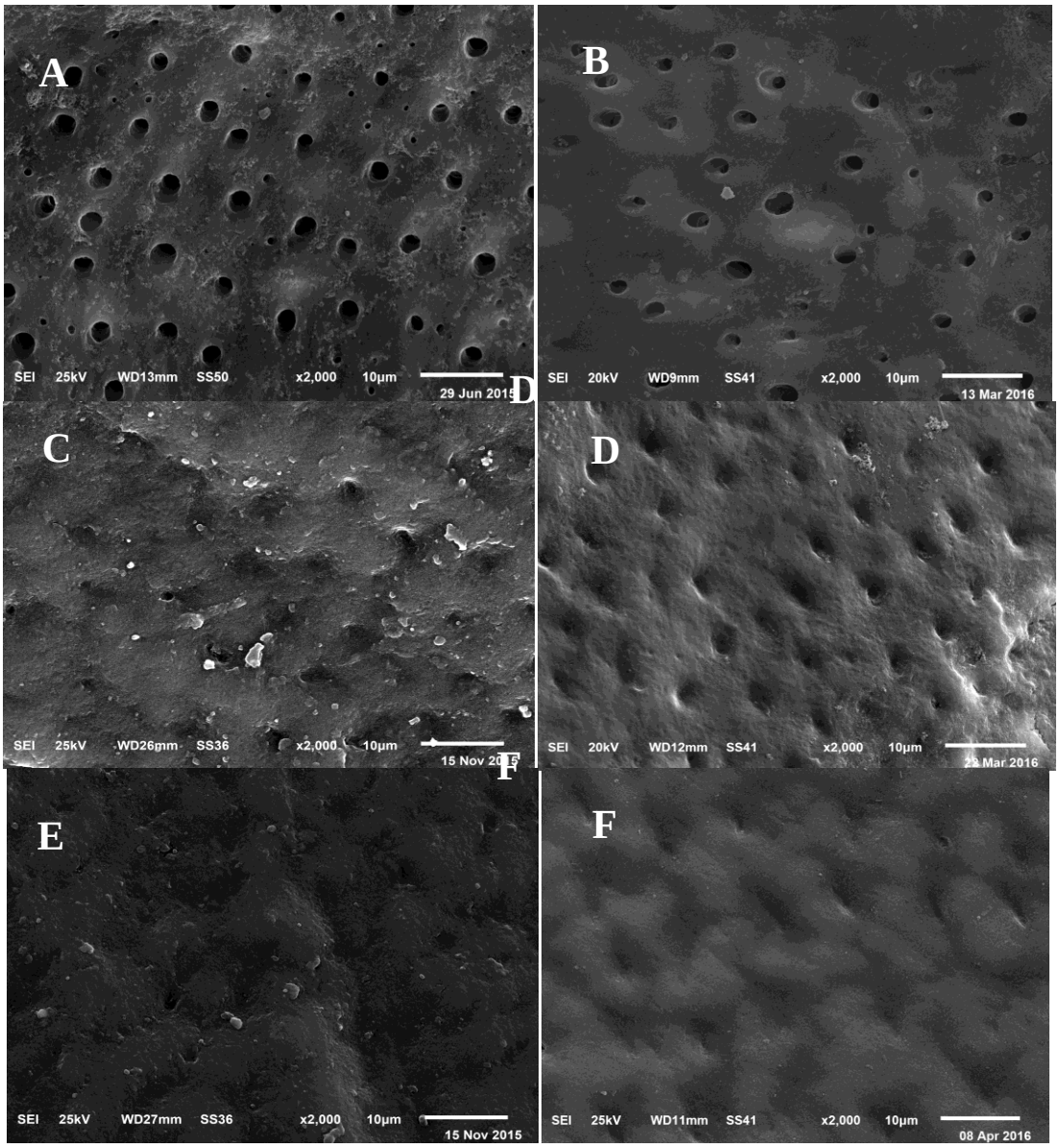

Fig.2:- SEM photomicrograph (x2000) **A (control group)**: subgroup 1A showing dentin etching of subgroup 1A (healthy patients' specimens) showing normal appearance of dentinal tubules. **B:** dentin etching of subgroup 1B (uremic patients' specimens) showing variations in size & shape of dentinal tubules. **C (fluoride group):** dentin remineralization with CPP-ACPF of subgroup IIA (healthy patients' specimens) showing blockage of most opened dentinal tubules**. D:** dentin remineralization with CPP-ACPF of subgroup IIB (uremic patients' specimens) showing occlusion of dentinal tubules. **E (Moringa group):** subgroup IIIA (healthy patients' specimens) showing occlusion of dentinal tubules after treatment with Moringa oleifera. **F:** dentin remineralization with Moringa of subgroup IIIB (uremic patients' specimens) showing blockage of most of dentinal tubules.

Discussion:-

Renal failure may lead to changes regarding oral health. Chronic renal failure has well documented effects on oral tissues such as calcifications leading to obliteration of pulp chambers and canals, enamel hypoplasia, and production of poorly-formed enamel as a result of ameloblast disruption. This may put patients at a slightly greater risk of developing dental caries **(Davidovich et al., 2005)**

Other studies showed the levels of dental caries were dramatically less than that seen in healthy groups. Reduced caries prevalence has been reported in ESRD patients. This is attributed to the protective effect of metabolism of

urea in saliva, which inhibits bacterial growth and neutralizes bacterial plaque acids **(Martins, Siqueira, Guimaraes 2008)**.

In this study, numerous differences were found between uremic and healthy patients specimens in control group. SEM analysis of healthy control enamel after etching with 37% phosphoric acid gel showed normal prisms and interprismatic substance. While enamel of uremic patients' specimens showed decrease in dissolution after preparation for etching that have a tendency for close packing with shallow porosities in between, which indicates a resistance to demineralization.

These results were in agreement with **Kho et al., (1999)** who explained that uremia makes enamel more resistant to demineralization and agrees with the findings of a previous study that reported that the pH of the saliva of uremic patients receiving hemodialysis was alkaline because of the high concentration of ammonia as a result of ureal hydrolysis. However these results were in contrast with **Al Nowaiser et al. (2005) and Davidovich et al. (2003)** who reported that enamel of uremic patients is hypoplastic, and has been attributed to the production of poorly-formed enamel as a result of ameloblast disruption. This may put patients at a slightly greater risk of developing dental caries than normal patients.

In the present study for dentin of uremic patients' teeth specimens, SEM analysis showed variation in circular outline of dentinal tubules. Few lateral branching was found in between the dentinal tubules, this means that dentin is denser in uremic patients' teeth than healthy one. These results come in agreement with previous studies, which showed that characteristic changes were detected in dentin of erupted teeth in patients with CRF. **Van Meerbeek et al (1994)** suggested that demineralization is more difficult in both the peritubular and intertubular regions of sclerotic dentin.

Remineralization is defined as natural repair process for non-cavitated lesions. It relies on calcium and phosphate ions, assisted by fluoride, to rebuild a new surface on the existing crystal remnants in the subsurface **(Featherstone, 2008).** Many studies described CPP-ACP properties on enamel, but few studies have been performed about its effects in dentin. It was postulated the erosion-inhibiting capability of CPP-ACP is valid only if erosive demineralization occurs (**Borges et al. 2013).**

The SEM analysis of healthy enamel specimens' subgroup after application of CPP-ACPF paste showed decrease in surface porosities with appearance of mineralized deposits that were scattered along the porous defects. The same results were found in uremic enamel specimens' subgroup which showed gradual decrease in surface porosities with obvious formation of interprismatic substance.

These results were in agreement with **Badr& Ibrahim (2010)** who stated that the protective effect of CPP-ACP lies in the fact that it provides a recervoir of bioavailable Ca and P that maintain supersaturated levels, thus inhibits enamel demineralization. While this results disagree with **Beerens et al. (2010)** who observed that there was no clinical advantage for use of the CPP-ACPF paste supplementary to normal oral hygiene when used to treat white spot lesions and dental plaque after orthodontic treatment.

The comparisons between fluoride group and control group of uremic subgroup showed high significant decrease in surface roughness in the enamel surface. These observations indicate the protective effect of CPP-ACPF against enamel demineralization. And also indicate the protective effect of uremia on patients' teeth against demineralization.

These results disagree with results of **Fejerskov, Thylstrup and Larsen. (1977)** who stated that there are similarities between the appearance of fluorosed enamel and the hypoplastic enamel defects of uremia. In patients with renal insufficiency, the decreased ability to excrete F leads to increased retention of this anion. Indeed, the serum levels of F are elevated in children and adults with impaired renal function.

In the present study, the healthy patients' dentin specimens treated with CPP-ACP showed decrease in the diameter of dentinal tubules, some of dentinal tubules were completely blocked by mineral deposits with normal appearance of peritubular dentin. The same results were found in dentin specimens of uremic patients specimens which showed dentinal tubules have occluded due to formation of different sized mineral particles. These observations indicate the protective effect of CPP-ACPF against dentin demineralization.

These results were in agreement with the study of **Al Zraikat et al (2011)** who demonstrated that the release of CPP-ACP and fluoride from CPP-ACP containing glass ionomer cement was associated with enhanced protection of the adjacent dentin during acid challenge in vitro. Moreover, **Rahiotis& Vougiouklakis (2007)** stated that the presence of CPP-ACP agent on dentin caused decrease in demineralization and increase in remineralization of dentin as compared to other paste that was of CPP-ACP free.

Moringa leaves have been reported to be a rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants. **(Siddhuraju, Becker, 2003).**

The SEM analysis of healthy enamel specimens' subgroup after application of Moringa showed blockage of enamel prisms with appearance of mineralized deposits along the porous defects. Dentin specimens showed that the diameter of dentinal tubules has decreased.

The SEM analysis of uremic enamel specimens' subgroup after application of Moringa specimens showed protruded enamel prisms with head emerging and filling of the interprismatic spaces with obvious formation of interprismatic substance. Dentin specimens showed the diameter of dentinal tubules has decreased. These observations indicate the protective effect of Moringa against enamel and dentin demineralization.

The comparisons between Fluoride group and Moringa group showed no difference in surface roughness and calcium levels in enamel and dentin specimens. These results indicate that fluoride and Moringa have the same remineralizing potential on enamel and dentin.

This results agree with **Sanganna C. Burali et al (2010)** study which proved that treatment of experimental animal groups under calcium and estrogen deficient conditions with Ethanolic Extact of Moringa Oliefera could effectively restore the reduced calcium level, correct the high rate of bone turnover prevented ovariectomy induced bone loss in rats, thus indicates its potential in preventing osteoporosis in a natural way through herbal resources.

Conclusions:-

CRF leads to alteration in the structure of enamel and dentin of permanent teeth with significant increase in mineral content (Ca& P) of permanent enamel and dentin when compared with healthy controls.

Moringa oleifera has a protective effect on enamel and dentin remineralization that is similar to CPP-ACPF paste in preventing erosion of enamel and dentin by localizing Ca and P at the tooth surface.

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