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Reversal of Fluoride Induced Teratogenicity in Swiss albino mice using Quinoa and Aloe-vera- A comparative study

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ABSTRACT

This research paper investigates the use of Chenopodium quinoa Willd and Aloe-vera as an antidote for fluoride-induced teratogenicity in Swiss albino mice. The study used chemically standardised plant materials, with powdered seeds of Quinoa orally given to mice at a dose of 250 mg/kg body weight and gel of Aloe-vera leaf at a dose of 300 mg/kg body weight. Animals in the Sodium Fluoride (NaF)-treated groups were fed a therapeutic diet after standardization of administration and dose of aqueous extracts. Foetal viability status and weights were reported after caesarean section. Protective activity was assessed by estimating prenatal morphology and skeletal parameters of mice foetuses. Out of thirty-two female animals, sixteen were autopsied on 19th day of gestation and the rest were allowed to deliver on 21st day of gestation for postnatal observations. Dissection at 19th days showed significant differences in maternal reproductive performance. The study found a significant decrease in the number of live fetuses in the 300ppm NaF group, with a reduction in average fetal weight. However, in the Sodium fluoride + medicinal plant treated groups, there was a significant increase in all parameters compared to the single Sodium fluoride group.

1. Introduction

Fluoride (F) is widely used to sterile drinking water against bacterial infection as well as for normal cleaning of teeth. Fluorosis is caused by high fluoride consumption over an extended period of injured bone and soft tissues, even if fluoride intake in modest levels is necessary to prevent dental caries (Susheela, 1999; Gadallaah, 2016). Fluoridation of community water has become a common intervention since fluoride's use as a caries-preventive agent was discovered in the middle of the 20th century. It is sometimes touted as a cornerstone of contemporary public health (Grandjean, 2019). The high permeability of the blood-brain barrier and placenta to fluoride during pregnancy and lactation may be critical to morphological and neurological development. Long-term exposure to high concentrations of fluoride can harm mineralized and soft tissues such as bones, liver, kidney, intestine, and nervous system in adult rats. (Ferreira et al., 2021). nearly all of the body's vital systems and organs, including the thyroid, kidney, cardiovascular, gastrointestinal, endocrine, neuron-reproductive, developmental, molecular level, and immunity. (Choudhary and Mathur, 2020).

The medicinal benefits of many herbs used in both conventional and modern medicine are largely attributed to saponins. Numerous saponins have been found to possess biological features like cytotoxic, antiviral, and anti-diabetic effects. For pharmacological study, these chemicals and the plants that carry them are becoming more and more attractive. One of the grain types on which study has been done and the mechanisms of its benefits are aimed for clarity is quinoa seed, which contains a variety of phytochemicals and antioxidant compounds. (Okumus and Temiz, 2021). Aloe vera has recently become a novel and valuable product in the food, cosmetics, and pharmaceutical industries all over the world (Boghaniet al., 2012). Aloe gel and leaf exudates have anti-inflammatory, antioxidant, antifungal, antibacterial, antiarthritic, antirheumatoid, anticancer (Tabolacciet al., 2010), cytoprotective, heart stimulating, and immunomodulatory properties

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(Kouidhi et al., 2012). It aids in the healing of wounds, treats a number of skin problems, and prevents gastrointestinal ulceration. The mucilaginous pulp at the centre of the aloe plant's stiff, lance-shaped, grayish-green leaves is filled with a clear gel. The pharmacologically active components are abundant in both the gel and the rind of Aloe vera leaves, according to clinical assessments. (Salem et al., 2014)

2. Material and methods:

2.1 Animal model

This study made use of the Swiss albino mouse strain. Individually ventilated cages (IVCs) were utilised to house the animals, and care and management followed the standards established by the Committee for the Purpose of Control and Supervision of Experiments on Animals. All biological tests were performed using mature albino mice, both male and female (weighing 25 to 30 g).IVCs were furnished with aspen wood chip bedding and kept at a temperature of 22°C 2°C and 30%-70% humidity for the duration of the trial. Feed and water were available ad libitum. The water that the animals drank was dosed with sodium fluoride, and the feed was given to them as pellets once a day. Starting two weeks before to mating and continuing until the day of the caesarean section (CS), the groups that had been treated with sodium fluoride were given a therapeutic diet in accordance with the appropriate dosage. The dams in the control group underwent CS and were mated on the same day as the dams receiving the medication treatment.

2.2 Test chemical

The test substance Sodium Fluoride(HiMedia Laboratories Pvt.Ltd.) was used after making a solution in normal potable water. The powdered seeds of Quinoa were given orally with water to mice at a dose of 250 mg/kg body weight (Saxenaet al., 2017) and the gel of Aloe-vera leaf at a dose of 300 mg/kg body weight given to mice by intubution method using gavage pipes. (Narayanaswamiet al., 2010).

2.3 Experimental design

In the present investigation female albino mice were divided in four groups. I group consideras control administered distilled water. II group treated with sodium fluoride (300 ppm) and III and IV group was given Quinoa and Aloe-vera with Sodium fluoride at the dose of 250mg/kg body weight and 300mg/kg bodyweight respectively from 14 days prior of gestation, throughout gestation and whole lactation period. Autopsy done on Gestation day (GD) 18 and foetuses were examined all teratological parameters. In spontaneous delivery the offspring were examined for morphological and teratological alterations and were comparable to respective control animals in all treated animals. In the initial experiment, gestational day GD0 was designated because two female mice and one male mouse were housed together in IVC on this day. 75 ppm sodium fluoride was given to the mice along with of water ad libitum. Male and female mice were kept together for 4-5 days before the male mouse was separated. The female mice were separated into individual cages when the vaginal plug and rise in body weight of the pregnant ones were seen. One mouse was autopsied on GD18, and one mouse gave birth to the pups on GD21. Eight viable foetuses were retrieved from mouse 1, and 10 pups were born from mouse 2. To check for the occurrence of any anomalies, all of the foetuses and pups from both strains of mice were morphologically inspected and photographed. All pups' and foetuses' weights were recorded. No abnormalities were reported in any of the foetuses or pups. The preparation and administration of therapeutic feed can now be standardised with the help of this early experiment. (water with Sodium Fluoride ad libitum).

2.4 Standardization of dose of Sodium Fluoride

The two female mice received a conventional dose of 100 ppm of sodium fluoride, yet neither the foetus nor the pups displayed birth to three and one pups, respectively. As a result of the high dose of 300 ppm sodium fluoride that had been given, the weight and size of the litter of both mice who gave birth were significantly less than those in the previous trial. We were able to standardise the greatest dose of sodium fluoride to be given during this experiment in order to conduct this teratology study.

2.5 Gross histopathological screening

After Standardization of doses and mode of administration of doses, we selected eight female albino mice each into four groups: first group received Sodium Fluoride at 300 ppm dose, second group received Quinoa at 250 mg/kg bw, third group received Aloe-vera at 300 mg/kg bw and fourth group was taken as the control group. External and interior organs were examined after the dams were put to death by cervical dislocation and CS on GD18. Ovaries, uteri, and placentas were taken out of each female mouse and inspected. The number of live or dead foetuses, the pregnancy status, and the weights of individual foetuses were all recorded. At CS, a visual external inspection of foetal pups was done to look for any obvious structural flaws brought on by exposure to the tested drug while they were developing. Each foetus was also looked at using a dissecting microscope to look for any flaws or changes to the exterior or interior morphology.

2.6 Statistical analysis

The mean and standard deviation of the differences between the control and treatment groups were reported. The birth-related variables were compared between the two groups using an unpaired t-test. Differences were considered statistically significant at $P< 0.05$.

3. Results

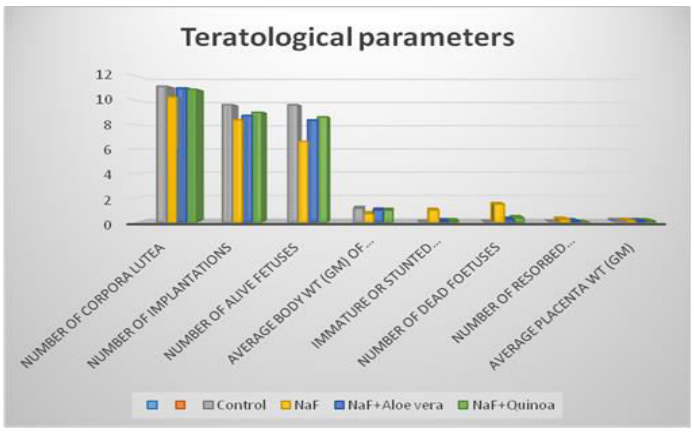
The following observations have been put in tabular and graphical form after conduction of experiments. The experiment was conducted in two phases;

1. Comparison between Control and other experimental groups (NaF, NaF+Aloe-vera and NaF+Quinoa)
2. Comparison between NaF and other experimental (protective) groups (NaF+Aloe-vera and NaF+Quinoa)

Table 1: Comparison of various Teratological parameters amongst Control, NaF, and NaF+ different phytodrug groups respectively.

Group	Number of corpora lutea	Number of implantations	Number of alive Fetuses	Average body wt (gm) of foetuses	Sex Ratio M:F (%)	Immature or Stunted Foetuses	Number of dead Foetuses	Number of resorbed Foetuses	Average Placenta Wt (gm)
Control	11.13±0.30	9.63±0.46	9.63±0.46	1.16±0.04	46.00:53.00	0.00	0.00	0.00	0.15±0.006
NaF	10.25±0.59	8.38±0.75	6.63±0.73**	0.716±0.054**	44.00:55.00	1.00±0.42**	1.50±0.46**	0.25±0.25	0.152±0.010
NaF+ Aloe-vera	11.00±0.38	8.75±0.37	8.38±0.37	1.01±0.035	48.00:51.00	0.13±0.42	0.25±0.16	0.13±0.13	0.133±0.005
NaF+ Quinoa	10.88±0.30	9.00±0.46	8.63±0.53	1.008±0.041	51.51:48.48	0.13±0.13	0.38±0.26	0.00	0.13±0.0046

*Values represent Mean ± SD; Statistically significantly different from vehicle control at *Significant difference(P<0.05), **Highly significant difference (P<0.01), ***Extremely Significant difference (P<0.001); Comparisons were done followed by Student's t-test using IBM SPSS statistics 22 software.*



Graph 1. Comparison of various Teratological parameters amongst Control, NaF, and NaF+ different phytodrug groups respectively.

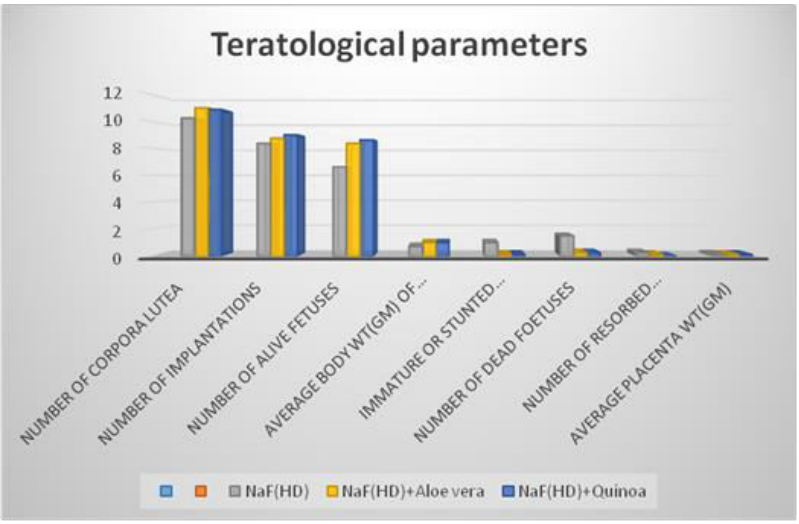
The following observations were made from Table and graph 1- The teratological observation of each mother was recorded after autopsy on 18th day of gestation. The average number of corpora lutea per mice was 11.13±0.30 in control, which are not significantly different from other experimental groups (NaF - 10.25±0.59, NaF+Aloevera - 11.00±0.38, NaF+Quinoa - 10.88±0.30).The average number of implantation sites per mice was 9.63±0.46 in control, whereas in experimental groups there was no significant difference in implantation sites (NaF - 8.38±0.75, NaF + Aloe vera - 8.75±0.37 and NaF+ Quinoa - 9.00±0.46).The number of alive fetuses was also recorded as 9.63±0.46 in control, whereas in other experimental groups there was no significant decrease in number of alive fetuses (In NaF + Aloe vera - 8.38±0.37, NaF+ Quinoa - 8.63±0.53) but in NaF experimental group there was a significant decrease in alive fetuses (6.63±0.73** when compare to control.

The average fetal body weight was 1.162±0.045gm in control, which was found to be decreased in the NaFas 0.716±0.054** that was statistically significant compared to control, whereas in other experimental groups there was no significant decrease in average fetal body weight (NaF + Aloevera - 1.013±0.035, NaF + Quinoa -1.008±0.041). No fetus was found stunted, dead or resorbed in control, whereas in NaF – 11.94% fetuses were stunted, 17.91% were dead fetuses and 2.98% fetuses were resorbed, in NaF + Aloe vera 1.42% fetuses were stunted, 2.85% were dead fetuses and 1.42% fetuses were resorbed and in NaF + Quinoa 0.13±0.13% fetuses was stunted, 0.25% were dead fetuses and 0.0% fetuses were resorbed.In control the average placental weight was 0.155±0.006 gm. There was no significant difference in all experimental groups when compared with control. The ratio of male and female fetuses was 46%:53% in control, 44%:55% in NaF, 48%:51% in NaF+Aloevera and 51.51%:48.48% in NaF+Quinoa respectively. Many bone defects were seen in NaF treated group by Alizarin preparation.

Table 2. Comparison of various Teratological parameters amongst NaF, and NaF+ different phytodrug groups respectively.

Groups	Number of corpora lutea	Number of implantations	Number of alive Fetuses	Average body wt(gm) of foetuses	Sex Ratio M:F (%)	Immature or Stunted Fetuses	Number of dead Foetuses	Number of resorbed Foetuses	Average Placenta Wt(gm)
NaF	10.25±0.59	8.38±0.75	6.63±0.73	0.716±0.054	44.00:55.00	1.00±0.42	1.50±0.46	0.25±0.25	0.152±0.010
NaF+ Aloevera	11.00±0.38	8.75±0.37	8.38±0.37**	1.013±0.035**	48.00:51.00	0.13±0.13**	0.25±0.16**	0.13±0.13	0.133±0.005
NaF+Quinoa	10.88±0.30	9.00±0.46	8.63±0.53**	1.008±0.041**	51.51:48.48	0.13±0.13**	0.25±0.25**	0.00±0.00	0.13±0.004

*Values represent Mean ± SD.; Statistically significantly different from vehicle control at *Significant difference(P<0.05), **Highly significant difference (P<0.01), ***Extremely Significant difference (P<0.001); Comparisons were done followed by Student's t-test using IBM SPSS statistics 22 software.*



The following observations were made from Table and graph 2- The average number of corporalutea in NaF was 10.25±0.59. In phytodrugs groups there was no significant difference in coroporalutea when compared with NaF (HD). The value of corpora lutea in NaF + Aloe vera 11.00±0.38 and in NaF + Quinoa 10.88±0.30. The average number of implantation sites per mice was 8.38±0.75 in NaF, whereas in other experimental groups there was no significant difference in implantation sites (NaF + Aloe vera - 8.75±0.37 and NaF + Quinoa - 9.00±0.46). The average number of alive fetuses was also recorded as 6.63±0.73 in NaF, whereas in other experimental groups there was significant increase in number of alive feuses (NaF+ Aloe vera - 8.38±0.37** and NaF + Quinoa -8.63±0.53**). The average fetal body weight was 0.716±0.054 gm in NaF, whereas in other experimental groups there was a significant increase in average fetal body weight (NaF + Aloe vera - 1.013±0.035** and NaF+ Quinoa -1.008±0.041**) when compared with NaF. In NaF – 11.94% fetuses were stunted, 17.91% were dead fetuses and 2.98% fetuses were resorbed, in NaF + Aloe vera 1.42% fetuses were stunted, 2.85% were dead fetuses and 1.42% fetuses were resorbed and in NaF + Quinoa 0.13±0.13 fetuses were stunted, 0.25% were dead fetuses and 0.0% fetuses were resorbed that was significant decrease compared with NaF. In NaFthe average placental weight was 0.152±0.010 gm. There was no significant difference in all experimental groups when compared with NaF. The ratio of male and femalefetuses was 44%:55% in NaF. In NaF + Aloe vera 48.00%:51.00% and in NaF + Quinoa 51.51%:48.48%.

Discussion

In the present study developmental and reproductive effects of fluoride and phytodrugs (Quinoa seeds and Aloe-vera) were studied during whole period of gestation and weaning period. The early 1990s reviewed of the NaF research that had already been conducted (NTP, 1990), and investigations of the effects on reproduction were deemed insufficient to identify any potential risks to development or reproduction. The effects of fluoride on rat embryo development have since been the subject of numerous investigations. (Collins et al., 1995) and rabbits (Heindelet al., 1996).Fluoride metabolism showed that the majority of fluoride is absorbed from the stomach and small intestine into the blood stream after fluoride consumption, such as drinking a glass of properly fluoridated water. The amount of fluoride in the blood temporarily rises as a result of this. Within 20 to 60 minutes, the fluoride levels peak after a rapid increase. In contrast, because fluoride is absorbed by hard tissue and is effectively excreted by the kidneys, the concentration falls off quickly, typically within 3-6 hours after the highest levels. (Dhar and Bhatnagar, (2009). Oxidative damage may be the cause of an increase in resorptions and dead foetuses. Gupta et al. (2009), Tao et al. (2006), Oncuet al. (2007). People who live in locations where fluorosis is common experience cell damage as a result of high fluoride levels because they accumulate significant amounts of free radicals and peroxides and superoxide dismutase and glutathione peroxidase activities are inhibited. Singh et al. (2013). Fluoride inhibits catalase, glutathione peroxidase, and superoxide dismutase in the ovary and increases lipid

peroxidation, which damages tissue. It primarily results in increased cell membrane permeability, denaturation of proteins, and peroxidation of membrane lipids. (Shubramaniamet al., 1994).

Defects in bones of the developing embryo in alizarin preparation are more prominent in high dose of NaF. The finding of the present work show similarity with the findings of Collins et al. 1995. They conducted embryo toxicity study on different strains such as Balb c, Swiss white Rhodeless mice, Rats at different doses of NaF ranging from 0, 10, 25, 100, 175 and 250 g/ml during the complete gestation period of the animals. Then observations were that NaF at lower doses did not make significant changes in bone structure however it did effect significant at higher dose 250 g/ml. Fluoride retention is more in the bone in earlier stages of skeletal development. The finding by Whitford 1990 says that fluoride accumulation is more in younger stages than in adult forms. Gedelia (1998) also came up with the same findings and has mentioned in one of his paper. Although it would be affected by significant variations from the typical amounts of fluoride in the diet, the amount of fluoride accumulated by the skeleton is strongly tied to the concentration of fluoride in drinking water. Numerous studies have highlighted the mechanism of fluoride retention, which suggests that fluoride replaces hydroxyl or bicarbonate groups often associated with hydroxyapatite structures in the bone, increasing the crystallinity of the apatite. Bone becomes more brittle yet has a more stable crystal structure by fluoride.
Poor nutrition seems to be an important cause of osteo defects in a high fluoride environment but increasing dietary energy, calcium, protein and vitamins help its prevention in pregnant and nursing women and in children. (Zanget al., 1996).

Treatment with NaF in the present study resulted decrease in weight of mice as compared to control (Table-1), while there was no changes on weight in NaF + Quinoa treated animals. The observations were more significant with high dose of NaF treated animals. Several other observations like weight of pups, placental weight, implantations and resorptions etc. were less seen in quinoa supplemented animals. The findings are in concurrence with the findings of Panchal and Verma, 2014 and Madhusudhanet al., 2009 wherein there is a dose dependent relationship between NaF exposure and embryonic growth retardation in mice. However, if supplemented with vitamin and antioxidants rich substances, the effect become mild.

Quinoa a wonder plant, has ample of nutritive values, it is rich in proteins and antioxidants prevent cell damage caused by free radicals.Its rich reservoir of bioflavonoids, namely Quercetin 5 found in seeds exerts biological effects like free radical – scavenging activity (Pietta, 2000), protects DNA from oxidative damage caused by hydroxyl radicals and peroxides (Galaris and Evangelou, 2002). Moreover, polyphenols, minerals, vitamins and amino acids present in this superfood (Park et al., 2017), together enhances the antioxidant capacity, a report mentioned by Paskoet al., (2010), in which quinoa seeds have shown protective effect against fructose induced changes in rat.The findings are similar to the observations in the present study, where quinoa has fulfilled all the nutritional requirements has boosted the antioxidant property and has averted the impact of teratogenic effect of Sodium fluoride. Aloe vera gel has a great potential to improve the absorption of vitamin C and vitamin E (Vinson and Andreoli, 2005). Choi and Chung (2003) and Eshun and He (2004) thus faceted multi biological properties of aloevera are due to the variety of its chemical components present in it, namely anthraquinones, glycoproteins, polysaccharides, vitamins and enzymes. Other than this, the three Aloe derivatives namely isorabaichromone, feruoylaloesin, and p- coumaroylaloesin are potent free radical and superoxide anion-scavenging substances (Yagi et al., 2003).

Besides this the flavonoids in the aloe vera gel have also been found to protect ascorbate from degradation in the intestinal tract (Lee et al., 2000). According to Vinson et al. (2005), aloe also has a special potential to increase the absorption of natural antioxidants like vitamin C and vitamin E, which improves antioxidant status and reduces fluoride-mediated ROS. The antioxidant properties of vitamin E, a crucial component of aloe vera gel, must have been achieved by scavenging lipid peroxyl radicals, which are free radicals in both in vitro and in vivo systems. (Niki, 2014). The results of this study show evidence of the many properties of aloe gel. The results show a marked rise towards normally in all the parameters studied in the mention work. (Mathur et al., 2022) revealed that sodium fluoride at high concentrations may be teratogenic while co-administration of aloe vera during fluoride exposure might be beneficial in reducing these toxic effects. The use of aloe vera as a preventive agent or as a complimentary agent is thus recommended following fluoride exposure through the oral route.

4. Conclusion

The aqueous seed extracts of Quinoa and Aloe-vera gel extract have strong protective action against sodium fluoride-induced teratogenicity, according to an evaluation of the protective effects based on prenatal and postnatal parameters and skeletal assessment. The presence of secondary metabolites in plant extracts may contribute to the overall antioxidant activities of the chosen medicinal plant extracts, providing protective benefits against teratogenicity in Swiss albino mice.

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