Aloe Vera Protects Fluoride Induced Teratogenic Effects During Pre- and Postnatal Development in Mice

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Research Article

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Abstract

Pregnancy and feto-gestational toxicities on exposure to fluoride (F) and its possible amelioration on co-administration with Aloe-vera were studied in pregnant Swiss albino mice. Once the confirmed pregnancy was tested, animals were equally divided into four groups and were given following treatment. Group I was given no treatment and served as Control, Group II and III were administered sodium fluoride, 100 and 300 ppm respectively while group IV was co-administered with sodium fluoride, 300 ppm and Aloe-vera (300mg/kg) daily for 14 days prior to gestation and continued till the 18th day of gestation. Animals were sacrificed on the 19th day of gestation for prenatal observations. Maternal body weight, the gravid uterine weight, number of corpora lutea in both the ovaries, number of implantations and resorptions, number of live (mature and immature) male and female fetuses as well as number of dead fetuses were examined in each dam. The treatment continued in another set of animals till the completion of weaning period to observe postnatal changes due to test substances on the mother and pups. Sodium fluoride treated animals showed morphometric and skeletal changes which were more pronounced in the high dose group showing significantly decreased body weight gain in pregnant mothers; and dead/immature fetuses. Morphometric changes included open eyelids, limb defects, wrinkles on whole body, anophthalmias, pulmonary edema, enlarged esophagus and decreased body weight of fetuses and pups. Alizarin prepared skeletal structures of fetuses of such female mice showed delayed ossification or bending in number of bones of skull, thoracic and limb regions. However, concomitant exposure to Sodium Fluoride and Aloe-vera treated animals, there was a marked improvement in all the prenatal and postnatal variables. The study suggests that Sodium fluoride at the high concentrations may be teratogenic while co-administration of Aloe-vera during fluoride exposure might be beneficial in reducing these toxic effects. We thus recommend use of aloe vera as preventive agent or as a complimentary agent during fluoride treatment.

Introduction

High Fluoride concentration in groundwater is a common problem in number of countries globally and also a serious environmental health issue in India and in particular the state of Rajasthan, India (Perumal et al., 2013). Increased fluoride concentration in soil and water is known to cause wide array of toxic effects and health issues in human and most common among them are tooth defects, skeletal and visceral malformations, brain injury etc. (Jing et al., 2004). Fluoride induced teratogenic effects are less studied. Developing animals are more susceptible to fluoride as it crosses the placental barriers and enter the fetus causing malformations. Fluoride concentration has been reported in saliva, sweat and milk though, tough at low concentration (Narayanaswami and Basha, 2010).

Infant mortality due to congenital malformation is now ranked second most important cause of death in developing countries. (Madu, 2015). A common teratogen is known to increases the incidence of structural and/or functional abnormalities in offspring, if consumed in higher concentration by parents before conception, the female during its pregnancy period or if it directly reaches the developing organism through exposure. Various stages of the development of the fetus are also a key factor that determine the susceptibility to teratogenic agent because a specific damage occurs readily during the organogenesis phase of embryonic development. (Inamdar et al., 2012).

Antioxidants and chelators are commonly used in minimizing fluoride induced toxic effects and/or stress, besides documenting the function of minerals and vitamins specifically A, D, C, E against fluoride toxicity (Dharani et al., 2011). Herbal or Plant extracts including their constituents have recently been reported to be of immense benefit as preventive agents, or being used as complimentary or as adjuvant in reducing metal induced toxic effects. With minimal side effects, the medicinal value of these extracts have assumed an important dimension in the past few decades owing largely due to being a rich source of antioxidants that combat oxidative stress through their redox active secondary metabolites and the rising concerns about the side effects of synthetic drugs (Flora et al., 2013). These studies have led researchers to screen number of plants and their extracts for their possible antioxidant properties and also to bind fluoride. With high concentration of fluoride reported in groundwater in various regions worldwide, it is considered useful to explore to explore their potential to combat toxicity of fluoride.

Aloe vera, a succulent plant and a native of arid places like Rajasthan in India with its thick, fleshy, serrated, lanceolate-shaped leaves of green-greyish color belonging to family Asphodelaceae was selected to determine its ameliorative effect against fluoride induced teratogenicity. Its inner gel used in the present study was collected from the lower leaves of the plant by slicing the leaf open. It is a clear, odorless and tasteless and has been used in traditional and folk medicines for thousands of years to treat and cure variety of diseases because vitamins, minerals, enzymes, polysaccharides, phenolic compounds and organic acids are found in abundance in it. (Nada et al., 2013) Studies have reported that Aloe vera gel possess diverse pharmacological and therapeutic activities viz. anti-inflammatory, antibacterial, antioxidant, hypoglycemic and hypolipidemic etc. The gel has also been found to be useful to neutralize free radicals for maintaining the integrity of the antioxidant status. (Eshun and He, 2004). Acemannan, an acetylated glucosaminan that makes up the major active component of the mucilaginous Aloe vera gel has great impact on bone formation, influencing the osteoblast and osteoclast activities in the developing and wound healing stages. (Godoy et al., 2018). Besides this, it has phytoestrogen components such as beta Sitosterol that can support normal development of fetus (Atik et al., 2019).

Materials And Method

Animals

Adult parous female Swiss albino mice weighing between 25-30 grams were first acclimatized in the animal house of the University (registered with CPCSEA with registration number #1689/PO/Re/S/13/CPCSEA) at controlled temperature and humidity. All animals were provided standard food pellets and water ad libitum. The experiments were carried out in accordance with the guidelines of the Institutional Animal Ethical Committee (IAEC).

Test chemical
The test substance Sodium Fluoride (HiMedia Laboratories Pvt. Ltd.) was used after making a solution in normal potable water (Fluoride content in the water was in the range of 1 – 2 ppm).

**Plant**

Mature, healthy and fresh leaves of Aloe-vera were washed with fresh water, cut transversely into pieces and thick epidermis was selectively removed. The solid gel in the center of the leaf was taken after homogenizing the leaves (Madhusudhan et al., 2009). The water extract of Aloe-vera gel was prepared mechanically and kept in a clean and air-tight bottle. Each time a dose was prepared by taking 0.7g of leaf gel in 10 ml water, which was properly ground.

**Teratological evaluation**

Mice were used for studying the teratogenic and anti-teratogenic evaluation. They were divided into 4 groups of 8 mice in each and kept in an air-conditioned room with 12 h of daylight and 12 h of night. Estrous cycle was monitored for all animals before mating. Two females with one male animal per cage were kept overnight. Next day, in the early hours, the females were examined for signs of mating by flushing the vagina with saline using a pipette, and then examining the obtained solution microscopically. Mating was confirmed by the presence of sperm cell/plugs. The presence of spermatozoa was recorded as day zero of pregnancy, daily increase in weight further confirmed pregnancy (Hussein and Mahmoud, 2013).

**Experimental design**

Animals were randomly divided into four groups with 8 pregnant mice in control group and 24 pregnant mice in treatment groups. Group 1 was given no treatment and served as Control; Group 2 was administered a dose of 100 ppm NaF; Group 3 was administered 300 ppm NaF while, Group 4 was administered 300 ppm Sodium fluoride along-with Aloe-vera. Doses were selected based on its concentration present in many polluted areas and what has been reported in the literature. Doses were given through drinking water ad libitum from 14 days prior to mating and continued till 18th day of gestation.

**Postpartum procedure** - The animals were autopsied one day after the completion of dose. Following laparotomy, the uterus was exteriorized and the number and location of fetuses, resorption, live and dead fetuses and number of corpora lutea in each ovary were noted, then their weight and length were measured. Living fetuses were distinguished from the dead by reflex movement initiated by gently touching the fetus. Live fetuses were weighed and examined by a hand lens, to observe any malformations. Individual fetuses were examined carefully for external anomalies, then 50% fetuses were examined for skeletal malformation; rest 50% fetuses were kept in Bouin’s solution for soft tissue examination.

**Data analysis** – Statistical analysis was performed on parametric values. The values have been expressed as Standard Error of mean (S.E). Maternal weight gain, fetal weight, fetal length and neonatal weights was analyzed statistically using student’s t-test.

**Results And Observations**

**Maternal toxicity**

20% mortality was observed during the treatment with 300 ppm dose of Sodium fluoride. The general body weight gain in mother during the three phases viz: 14 days prior to mating, gestation and lactation periods was significantly reduced at 300 ppm of Sodium fluoride relative to controls; while the maternal body weight in Sodium fluoride+ Aloe vera group increased in all three phases compared to 300 ppm Sodium fluoride exposed group. No mortality was observed in any of the females in this group. Water and food consumption decreased significantly in the animals exposed to 300 ppm Sodium fluoride.

**Fetal toxicity**

The prenatal teratological observations in were recorded after performing autopsy on 19th day of gestation. The first parameter which was assessed was the number of corpora lutea counts in animal under study. The average number of corpora lutea in all the animals were found to be insignificantly different from the values recorded in control. In total 77 live fetuses were observed from all the eight animals in the control group; the number of fetuses obtained from all the eight animals in animals exposed to 100 ppm NaF were reduced (non-significant) compared to the number noted in control group. The value were significantly decreased to 53 in 300 ppm NaF exposed group indicating significant toxic effects during the developmental phase of the animals. The increase in litter size in NaF (100 ppm) + Aloe vera group was close to litter size in control indicating positive effects of Aloe vera co-administration. Table 1 indicates significant toxic effects of 300 ppm NaF where out of 67 implantation sites, only 53 live fetuses were counted but this included 45 mature fetuses while 8 fetuses were found to be immature (Figure 1) (Eii); Number of resorptions and dead fetuses were high in this group of animals. Aloe vera, in view of reported antioxidant effects and other medicinal properties provided significant protective value which were evident from the elevated number of mature fetuses as 65 with just 2 immature fetuses out of the total 67 live fetuses. The resorptions and other parameters were also not significantly on the higher side. The average fetal body weight was recorded at 1.162 ± 0.045 g in control, which was significantly decreased in the 300 ppm NaF (0.716±0.054). In the other experimental groups there was no significant decrease in average fetal body weight and the average placental weight compared to control.

Normal morphometric development could be seen in the fetuses of control group. Sodium fluoride (HD) group fetuses showed anomalies like open eyelids, wrinkles on whole body (Figure 1–D), limb defects (Figure 1–B,C) and stunted fetus (Figure 1 E ii). Body weight got drastically decreased in NaF(HD) group as compared to control group and a low dose group; whereas Aloe-vera group in combination with NaF(HD) showed no specific morphometric changes and no significant decrease in body weight compared to control.

When 50% fetuses of each female were sectioned through head, thorax and abdomen; the sections showed normal structure of various organs in the control group. However, in 300 ppm NaF exposed group, sections in soft tissue depicted anophthalmias, pulmonary edema (enlarged air spaces in lungs) and enlarged esophagus (Figure 2). Structures seemed normal in female fetus which were co-administered Aloe vera with NaF.
The alizarin staining in remaining 50% of the fetus which were processed to observe defects of the skeletal system indicates normal ossification in the skull bones of the control group. The ribs, Sternebrae, vertebrae and limb bones did not show any malformations, the observations were similar in the Aloe vera treated group. Sodium fluoride (300 ppm) fetuses showed Craniofacial disorganization, reduced ossification in skull bones, and widened cranial sutures (Figure 3). The rib defects like wide gaps between ribs, and absence of ribs were not found in low dose of NaF group, whereas in the treated group of NaF (300 ppm) a significant number of fetuses had skeletal abnormalities. In this group highly reduced skull ossification, widened cranial sutures, ribs defects like partially ossified ribs, wide gap between ribs, wavy ribs, and reduced Sternebrae, partial ossification of metacarpals (Mc) and metatarsals (Mt), absence of 13th rib were clearly observed (Figure 4).

The postnatal observations of each mother and pups was recorded following parturition till 21 days of weaning period.

Table 2 shows consistently decreased in the average number of alive pups in NaF (HD) treated group from day 1 through 21 days of weaning period compared to control. (Control 8.40 ± 0.51 on day 1 and 7.60 ± 0.24 on day 21, NaF (300 ppm) - 7.00 ± 0.45 on day 1 and 3.60 ± 0.51*** on day 21) while in NaF (100 ppm) and NaF (300 ppm) + Aloe-vera group there was insignificant difference in number of alive pups compared to control. The trend was the same in viability index and weaning index also. Average pup weight which is also expressed as Growth index was calculated for different postnatal days findings. It was not much affected in NaF (300 ppm) and NaF(300 ppm)+Aloe-vera, compared to control but in NaF (300 ppm) it got significantly affected.

Discussion

Epidemiological studies conducted in the areas with high levels of naturally fluoridated water shows increase in birth defects placing fluoride into the same category as toxic metals like lead, mercury, methyl mercury etc. (Guth et al., 2020). Exposure to high fluoride concentration in drinking water during in utero development may result in skeletal fluorosis which becomes evident in childhood. Adverse effects on reproduction have been reported in animals exposed to 100 ppm or greater concentrations of fluoride in their diet or through drinking water. Number of previously reported animal studies suggest that exposure to low concentrations of fluoride do not result in impaired fertility and reproductive capabilities. The present study is an effort to give reason to and correlate the findings obtained through experiments with the various mechanisms by which Sodium fluoride reaches the tissues of the body and influences the normal physiological function of the body; thereby causing developmental defects during the pregnancy period. The study also attempts to find the possible reason / mechanism for averting the teratogenic effect of fluoride by using Aloe vera gel which is taken as a supplement during the early development of a fetus.

It has been reported that Sodium fluoride and other Soluble fluorides in groundwater are absorbed from the gastrointestinal tract into the blood (Barbier et al. 2010; EFSA 2005), attaining the peak plasma levels within 20–60 min after oral ingestion (EFSA2005; Whittford et al. 2008). Fluorides is also able to cross biological membranes by diffusion as the non-ionic hydrogen fluoride (HF) (Gutknecht and Walter 1981) and remains retained in tissues for a significant period of time. The largest amount of absorbed fluoride is retained in bone and teeth, where about 99% of the total fluoride is detected (Ekstrand et al. 1977). Animal studies have reported that fluoride crosses the placenta and gets incorporated in fetal tissues; increasing its concentration in fetal blood two folds. Fetus is not protected against fluoride that circulates in the maternal blood. The study further reports that fluoride gets transferred to the infant through mother's milk. Thus, the fetus and new born pups are much vulnerable to fluoride (Caldera et al. 1988; Shen and Taves 1974). The skeletal deformities and other morphometric changes observed in the present study can be explained on the basis of such above mentioned findings (Guth et al., 2020). Not only this, fluoride also interacts with proteins, particularly enzymes, and usually inhibits/ stimulates enzyme activity at the concentrations in the millimolar range (Barbier et al. 2010; Mendoza-Schulz et al. 2009) thereby disrupting normal physiology of the body. Fluoride hampers cell functions through generation of superoxide anions (Garcia- Montalvo et al. 2009; Izquierdo-Vega et al. 2008); mitochondrial toxicity, e.g., opening of the transition pore (Anuradha et al. 2001); release of cytochrome c from mitochondria and induction of apoptosis (Chubek et al. 2003; Lee et al. 2008). The previous study also suggest that by interfering with calcium metabolism and enzyme mechanisms, fluorides act as direct cell poisons (Guth et al., 2020). It has also been reported that Fluoride interacts with hydrochloric acid in the stomach to form a powerful corrosive hydrouoric acid. Fluoride ions also combine with serum cations after absorption, especially calcium and magnesium leading to hypocalcaemia and hypomagnesaemia. (Narayanswami et al., 2010). Bones are more susceptible to its effect primarily because fluoride replaces hydroxides and itself gets deposited in bones. The chronic effects of which combine with serum cations after absorption, especially calcium and magnesium leading to hypocalcaemia and hypomagnesaemia. (Narayanswami et al., 2010). Bones are more susceptible to its effect primarily because fluoride replaces hydroxides and itself gets deposited in bones. The chronic effects of which may result in skeletal fluorosis which becomes evident in childhood. Adverse effects on reproduction have been reported in animals exposed to 100 ppm or greater concentrations of fluoride in their diet or through drinking water. Number of previously reported animal studies suggest that exposure to low concentrations of fluoride do not result in impaired fertility and reproductive capabilities. The present study is an effort to give reason to and correlate the findings obtained through experiments with the various mechanisms by which Sodium fluoride reaches the tissues of the body and influences the normal physiological function of the body; thereby causing developmental defects during the pregnancy period. The study also attempts to find the possible reason / mechanism for averting the teratogenic effect of fluoride by using Aloe vera gel which is taken as a supplement during the early development of a fetus.

Acemannan, an acetylated glucosamannan that makes up the major active component of the mucilaginous Aloe vera possess multifunctional properties and therefore acts as a bioactive molecule, exerting an immunostimulatory effect by activating macrophages, (Callaghan et al., 2012) and possessing wound healing actions. (Osefo et al., 2009, Hull and Beale, 1985). It also stimulates fibroblast proliferation, (Lee et al., 2017) tissue regeneration (Rauws et al, 1990) and bone marrow stromal cell proliferation and differentiation in vitro. (Hull and Beale, 1985). Thus, it is an interesting preventive agent for tissue repair. Aloe vera sap has also been found to have favorable effects on estrogen synthesis due to its phytoestrogen components such as beta sitosterol that can increase the estrogen level, thus bringing hormones to support normal development of fetus (Atik et al., 2019). More detailed studies are required to validate and explain the action mechanism of acemannan in bone building.

It is also an established fact that nutritional supplements such as vitamins and antioxidants prevent the toxicity that is induced by fluoride because these supplements reduce the oxidative stress, decreases the lipid peroxidation, and augments the activity of antioxidant enzymes. (Elisham et al., 2018). One of
the strategies to protect the body from oxidative injury and to prevent from many disorders is to increase the levels of antioxidant enzymes by increasing the dietary intake of supplements rich in antioxidant enzymes (Godoy et al., 2018). Aloe vera has very strong antioxidant nutrients. Glutathione peroxide activity, superoxide dismutase enzymes, and a phenolic antioxidant have been reported to be present in Aloe vera gel, which may be responsible for these antioxidant effects (Atik et al. 2019). Aloe vera also has unique ability to improve the absorption of natural antioxidants like vitamin C and vitamin E that in turn enhances antioxidant status to alleviate fluoride induced ROS (Vinson et al., 2005). Vitamin E which is an essential component of Aloe vera must have exerted antioxidant effects by scavenging lipid peroxyl radicals that are free radicals in vivo as well as in vitro (Niki, 2014). Ayurveda also mentions that Aloe-vera can been taken as a Naimittika Rasayanas during pregnancy; because, it being rich in nutritional contents and iron supplements that are very essential to promote the health of the pregnant women and facilitates full growth and development of progeny in the womb (Shastry,1999; Singh, 2000).

The findings in the present study suggest the multifaceted qualities of Aloe vera. The present study also suggests marked efficacy of Aloe vera in reducing fluoride induced teratogenic effects. However, still more detailed studies are required using variable doses of Aloe vera post flouride exposure

Conclusion

The present study suggest that pregnant mice exposed to Sodium fluoride may be at the risk of congenital malformations. Aloe vera, a promising herbal product with its various clinical applications in medicine could be useful if co-administered during fluoride to minimize fluoride induced toxic effects including teratogenicity.

Declarations

Ethics approval: Institutional Ethics committee gave its approval to work on the given experimental model (vide SI 2-18-5-18).

Consent to Participate:

Authors have given consent to participate in the given research work

Consent to publish:

Authors have given consent to publish the work which includes figures and tables in between the text in the journal.

Conflict of interest

The authors declare that there is no conflict of interest related to this study.

Availability of data and materials: Not applicable

Author's funding

The author(s) received no specific funding for this work.

Authors' contribution

PM conceptualized the experimental plan, supervised the experiment, prepared the first draft of the manuscript and the main author., SC performed the experiment, collected data and analyzed. PB prepared the final draft, participated in the discussion during the experiment, and overall supervision of the experiments.

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**Tables**

**Table 1**: Pre-natal observations in mice after administration of test substances (n=8 in each group)

| Groups          | Average no. of corpora lutea | Total no. of implants | Total no. of live fetuses | Average body weight of fetus (g.) | Total resorptions | Total immature fetuses | Total dead fetuses | Average Placental weight (g.) |
|-----------------|-----------------------------|-----------------------|---------------------------|----------------------------------|-------------------|------------------------|-------------------|-------------------------------|
| Control         | 11.13±0.30                  | 77                    | 77                        | 1.16±0.04                        | 0.0               | 0.0                    | 0.0               | 0.15±0.006                    |
| NaF, 100 ppm    | 11.25±0.41                  | 75                    | 68                        | 0.93±0.05                         | 0.0               | 5                      | 7                 | 0.14±0.008                    |
| NaF, 300 ppm    | 10.25±0.59                  | 67                    | 53                        | 0.716±0.054**                     | 2                 | 8                      | 12                | 0.15±0.01                    |
| NaF, 300 ppm + Aloe-vera | 11.00±0.38                 | 70                    | 67                        | 1.01±0.035                        | 1                 | 2                      | 2                 | 0.13±0.005                    |

Values are mean ± SD; ***p<0.001, **p<0.01, *p<0.05 compared to control as evaluated by Student’s ‘t’ test using IBM SPSS statistics 22 software.

**Table 2**: Postnatal observations of various treatment groups at different days of the 21 days long weaning period in Swiss albino mice.

| Groups          | Day 1 | Day 4 | Day 7 | Day 14 | Day 21 |
|-----------------|-------|-------|-------|--------|--------|
|                 | No. of alive pups | Avg. pup weight (Growth index) | Viability index (%) | No. of alive pups | Avg. pup weight (Growth index) | Viability index (%) | No. of alive pups | Avg. pup weight (Growth index) | Viability index (%) | No. of Alive pups |
| Control         | 8.40±0.51 | 1.55±0.14 | 100 | 8.20±0.20 | 2.56 | 97.61 | 8.00±0.32 | 4.12 | 95.23 | 7.80 | 6.16 | 7.60 |
| NaF, 100 ppm    | 8.40±0.05 | 1.70±0.05 | 100 | 8.00±0.32 | 2.72 | 95.23 | 8.00±0.00 | 3.82 | 95.23 | 7.40 | 68 | 7.20 |
| NaF, 300 ppm    | 7.00±0.45 | 1.68±0.05 | 100 | 5.00±0.05 | 2.48 | 71.42 | 4.60 | 4.00 | 65.71 | 4.60 | 3.60 |
| NaF, 300 ppm + Aloe-vera | 7.60±0.51 | 1.62±0.11 | 100 | 7.20±0.12 | 2.44 | 94.73 | 7.00 | 3.66 | 92.12 | 6.80 | 6.60 |

Values are mean ± SD; ***p<0.001, **p<0.01, *p<0.05 compared to control as evaluated by Student’s ‘t’ test using IBM SPSS statistics 22 software.
Figures

Figure 1

Figures showing fetuses of treated pregnant female mice on the 18th day of gestation after treatment with Sodium Fluoride (HD). (A) Wrinkles (Wr) on body and eye defect (Ed) (B,C) Limb defects (Lm) in fetuses (D) Open eye (Op) and wrinkles on the whole body (Ei) Healthy fetus (He) of Sodium fluoride + Aloe-vera treated group (Eii) Stunted fetus (St) in Sodium fluoride (300 ppm) treated group

Figure 2

Free hand sections through head, thorax and abdominal regions of a fetus on the 18th day of gestation of Sodium Fluoride (HD) group showing (A) Anophthalmia (absence of eye) (B) Pulmonary edema (enlarged air spaces in lungs) (C) Enlarged esophagus (L – Lungs, E – esophagus, S – Spine, G - Gap) Free hand sections through head, thorax and abdominal regions of a fetus on the 18th day of gestation of Sodium Fluoride (300 ppm) + Aloe-vera treated group showing (D) Normal eye development (E and F) Normal lower and upper thoracic regions respectively showing N - Nasal cavity, O - Olfactory brain, R - Retina, E - Eye
Figures showing lateral view of skull of fetuses on 18th day of gestation stained in alizarin red stain. A. Complete ossification of frontal (F), parietal (P), interparental (Ip), supraoccipital (So), squamosal (Sq), maxilla (Mx), mandible (Mn), nasal (N) bones in control group. B. Craniofacial disorganization, reduced ossification of parietal, interparental and widened cranial sutures (Cs) in skull bones of Sodium Fluoride (300 ppm) treated group. C. Complete ossification of frontal (F), parietal (P) and other bones but reduced ossification of supraoccipital (So) bone in Sodium fluoride (300 ppm) + Aloe-vera treated group.

Figures showing whole body skeletal deformities (dorsal view) in fetuses (on 18th day of gestation) stained in alizarin red stain. A. Complete ossification of mandible (M), skull (S), phalanges (P), ribs (R), sternum (St) and vertebrae (V) in control group. B. Wavy ribs and C. Widened rib gaps in Sodium fluoride (300 ppm) treated group. D) Partial ossification of metacarpals (Mc) and metatarsals (Mt), poorly ossified Sternebrae(S), in Sodium fluoride (300 ppm) treated group. E) Foetus (Ventral view) showing prominent wavy rib appearance but complete ossification of tibia, fibula, femur, metacarpals, metatarsals and sternbrae in Sodium fluoride (300 ppm) + Aloe vera treated group.