The Effect of Aqueous and Hydroalcoholic Extracts of the Mango Fruit on Development of Embryonic Tissues during Pregnancy

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ARTICLE INFO

Keywords:
Mangifera indica
Fetus
Embryo
Teratogen

ABSTRACT

Background: Mango fruit (Mangifera indica) is rich of phytoestrogens, polyphenols, calcium, iron and potassium which play an important role in bone and dental growth and development. The aim of this study was to determine the effect of M. indica on embryos in pregnant mice.

Methods: In an experimental study, 24 pregnant mice were equally divided into 3 groups of those receiving aqueous extract of the mango fruit, its alcoholic extract and the control group receiving just distilled water. Animals were sacrificed at 19th day of pregnancy and embryos were removed and fixed in 10% formalin for histological studies.

Results: No abnormality and teratogenic effects were noticed in bone, cartilage, liver, spleen, kidney, digestive tract and spinal cord tissues.

Conclusion: Aqueous and hydroalcoholic extracts of the mango fruit (M. Indica) can be administered safely during pregnancy without any complications in body tissues an in bone and dental growth and development.

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Received: December 18, 2018
Revised: September 15, 2019
Accepted: September 29, 2019


Introduction

Herbal medicine may have irreparable effects on fetal development (1). Even herbas can have many therapeutic effects for ulcerative colitis (2), diabetes (3), cirrhosis (4) and multiple sclerosis (5), but can have teratogenic and toxic impacts during pregnancy for both the mother and the fetus causing fetal abnormalities (6). Among 69 known species of Mangifera, Mangifera indica L. is a medicinal herbal being consumed in tropical regions by indigenous populations. It is the major plant species being used in Ayurvedic, in addition to indigenous medical systems form 4000 years (7). Mango fruit (Mangifera indica) is a tropical fruit belonging to the family of Anacardiaceae that is found in many countries in especially in warmer regions. Its common names are Amba (Oman), Ambo (France), Manga (Brazil), Bumango (Senegal), Aamp, Aanp, Amp (Nepal), Abricotier de St. Domingue, Pauh (Indonsia), and Embe (Tanzania) (8). Its active ingredients are mainly flavonoids, polyphenolics, triterpenoids, and mangiferin. It also contains alanine, glycine, catechin, protocatechic acid, kinic acid, γ-aminobutyric acid, shikimic acid, and tetracyclic triterpenoids cycloart-24-en-3β,26diol, 3-ketodammar-24 (E)-en-20S,26-diol, C-24 epimers
of cycloart-25 en 3β, 24, 27-triol and cycloartan 3β, 24, 27-triol (8).

From it, mangoleanone, manghopanal, friedelin, indicoside A and B, cycloartan-3β-30-diol and its derivatvants have been isolated (8). Mango fruit contains 0.5-1% protein and fruit pulp mainly contains carbohydrate (glucose, fructose, sucrose) (8). M. indica possess various bioactivities such as hepatoprotective and antioxidant (9), antibacterial (10), antiviral and immunomodulatory (11), antifungal (12), Antiparasitic (13), anti diarrhoeal (14), anticancerous (15), and antidiabetic activities (16). In cholesterol induced hyperlipidemic rats, M. indica was shown to have significant antihyperlipidemic activity (17). Li et al. (1998) found that its aqueous extract had negative effect on bone resorption (18). Ngokere et al. (2014) reported that mango applied in Chinchilla rabbit’s ovaries changed female sex hormones and had the potential to change serum concentration of female sex hormones (19). Therefore, this study was performed to evaluate the effect of mango on embryonic tissues in pregnant mice.

Materials and Methods

To produce the aqueous and hydroalcoholic extracts of mango, its powder form (150 g) was used. To reach the hydroalcoholic extract, the powder was transferred in 1000 ml of 50% ethanol alcohol and then into a perculator device for 3 days and was later filtered. To isolate the solvent and concentrate the solution, a rotary machine was used together with a dessicator for 24 hours to be dried.

In an experimental study, 24 female mice (3 months old, mean weight of 25-30 g) were provided from Laboratory Animal Center of Shiraz University of Medical Sciences, Shiraz, Iran. They were equally divided into 3 groups. The control group received 200 mg distilled water daily, for the second group, 300 mg/kg of aqueous extract of mango was administered and for the third group, 300 mg/kg of hydroalcoholic extract of the fruit was used. The animals received the mango fruit and the distilled water by a gavage for 19 days beginning from the first day of pregnancy. The mice were kept at 22±1°C under 12 h light/dark cycle, and were free to have access to food and water ad libitum. The diet was standard for feeding the mice. The Animal Care Committee of Jahrom Branch of Islamic Azad University Ethics Committee approved the study protocol (Code: IR-JIAU.REC.88-J-1388).

Under general anesthesia on day 19th of the pregnancy, the abdomen was opened and the uterus was incised to expose the embryos. The survival of the embryos was assessed and then the embryos were transferred into a petri dish containing PBS to wash the embryos and remove the presence of any blood contamination. Later, the embryos were evaluated individually for any macroscopic abnormality in any tissue including brain, spinal cord, skeleton, kidney, liver, spleen, and esophagus. Embryos from each

Figure 1: Normal tissue development in embryos during pregnancy following consumption of mango fruit in A: Bony tissue derived from mesoderm (x20), B: Spinal cord (ectoderm and bone with cartilage; mesoderm) (x20), C: Brian with choroïd plexus derived from ectoderm (x20), D: Kidney with small premature glomeroli, liver and spleen derived from endoderm (x20), and E: Esophagus derived from endoderm and spleen (x20) (H&E).
mouse were fixed in 10% neutral phosphate buffered formalin for further histological investigation.

To do histological assessment, all separated tissues in 10% buffered formaldehyde were taken out and following dehydration by 2 changes of cold ethanol, clearance by 3 changes of cold xylene, and embedding by paraffin at 53°C, a 5 µm tissue section was serially prepared and dried during an hour at 37°C. The slides were further stained by hematoxylin and eosin (H&E) and visualized under a light microscope.

Results
Mango was shown without any histological teratogenic effect. Figure 1A denotes to a normal tissue developing in gastrointestinal tract, liver and pancreatic tissues (H&E, ×100) and Figure 1B demonstrates a normal development in spinal cord and bony tissues (H&E, ×100). A normal ossification was noticed in cartilaginous tissues (Figure 1C, H&E, ×100) and in kidneys, the development was also normal (Figure 1D, H&E, ×400) after consumption of aqueous and hydro alcoholic extracts of the mango fruit during pregnancy. No mortality or mummified fetus was observed. The number of embryos in each pregnant mouse varied from 8 to 10 with no abnormal macroscopic changes in all organs too.

Discussion
No abnormality was noted in embryonic development during pregnancy following consumption of aqueous and hydroalcoholic extracts of mango fruit at dose of 300 mg/kg in pregnant mice macroscopically and histologically in absence of any abnormality or teratogenic effect of the fruit. The growth and development of fetuses were normal denoting to the safe consumption of mango during pregnancy when of the fruit was used. McClain et al.’s study also revealed absence of any embryonic abnormality after consuming mango during pregnancy because of genistein in the fruit (20) that poses positive effect on bone mineralization and normal growth and development of the embryonic tissues (21).

Tousen et al. demonstrated that from 5th day of pregnancy to 13th day of birth, administration of genistein during pregnancy had no negative effect on embryonic tissues and newborns (22). In chickens, it was shown that replacement of 20% of the maize in the diet with boiled mango did not induce any adverse effects on growth and development (23). Batool et al. (2018) denoted to antioxidant, immunomodulatory, antiallergic, and anti-inflammatory properties of Mangifera indica to play an important role for normal growth and development of embryos during pregnancy (9).

It was shown that presence of micronutrients such as selenium and polyphenols such as mangiferin, phenolic acids, phenolic esters, and flavan-3-ols, could account for the powerful positive activity of mango fruit (24) to be effective against phospholipidic peroxidation in rat brains, counteracting DNA damage caused by iron/bleomycin or copper phenantroline exposure (25).

Conclusion
In our study, mango fruit was shown without any teratogenic effect in embryonic tissues when consumed during pregnancy. It did not induce any gross and histological abnormality in growth and development of liver, spleen, kidney, esophagus, ossification, brain and spinal cord.

Acknowledgement
The authors wish to thank Jahrom Islamic Azad University for financial support.

Conflict of Interest
None declared.

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