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Cytogenetic and Mutagenic Effects of Levocetirizine and Montelukast alone or in combination on pregnant mice and embryos.

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ABSTRACT

Allergic rhinitis is the most common allergic disease affecting the general population worldwide leading to inflammation of the upper air way mucous membranes. In due course of time, if therapy is not given a chronic state of inflammation can develop leading to asthma. Asthma adversely affects about 8% of all pregnancies. Many studies have shown that proper control of asthma in pregnant women significantly reduces the risk of prenatal adverse outcomes. Levocetirizine is a new third generation antihistamine drug is effective in the treatment of allergic rhinitis. Montelukast, a new leukotriene receptor antagonist is used in the treatment of allergic rhinitis and asthma as a mono therapy or in combination with levocetirizine or in fact, there are no adequate and well controlled studies were been done about the using of the two drugs in pregnant animals and women. To evaluate the cytogenetic and mutagenic effects of levocetirizine and montelukast alone or in combination on pregnant females and embryos during pregnancy. Pregnant female mice were administrated orally with levocetirizine, montelukast and their combination at the recommended human daily doses of (0.002, 0.004 and 0.002 + 0.004) respectively. All female mice were administrated from day (3) to day 17 of pregnancy. On day (18) of pregnancy pregnant female mice were killed. Cytogenetic analysis, micronuclei formation and embryo toxicity were examined. The results showed that levocetirizine caused a significant increase in the frequencies of chromosomal aberrations, fetal toxicity and micronuclei formation in the pregnant females and embryos while, montelukast and its combination with levocetirizine caused a slight increase in the chromosomal aberrations, fetal toxicity and micronuclei formation in the pregnant females and their embryos but this increase was not significant and very close to the limit of control group. Our results indicated that levocetirizine has a mutagenic and embryo toxic effects on the females and their embryos, while montelukast and its combination with levocetirizine has no mutagenic or embryo toxic effects to the female mice and embryos during pregnancy.

Keywords: levocetirizine, montelukast, chromosomal aberrations, mice, embryos, micronuclei.

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INTRODUCTION

Allergic rhinitis is the most a topic disorder affecting 18% to 40% of adults world wide, diagnosed by history, physical exam and objective testing. According to the Allergic Rhinitis and its impact on Asthma (ARIA) document it is classified by chronicity (intermittent or persistent), and severity which is based on symptoms and quality of life (mild, or moderate severe).

The terms "seasonal" and "perennial" allergic rhinitis were previously categorized as allergic rhinitis by the clinically significant aeroallergen. Perennial allergic rhinitis is associated with year round and indoor allergens including mold spores, cockroaches, dust mite fecal particles, animal dander and occupational exposure [1].

Seasonal allergic rhinitis is commonly referred to as "hay fever", developing during a defined pollen season and is usually intermittent as a result of allergic reactions to outdoor aeroallergens including mold spores and pollens of trees, grasses and weeds that depend on wind for cross-pollination, commonly there is an overlap of "perennial" and "seasonal" symptoms in some geographic regions which has resulted in decreased use and confusion regarding these terms.

Usually patients are made aware of the fact that allergic symptoms can be controlled and cure is only limited with fair chance of recurrence.

Treatment of allergic rhinitis depends upon several factors. The first involves avoidance of implicated allergens. Unfortunately, the effort to appropriately reduce levels of indoor allergens is often too difficult for patients to accomplish and even more difficult is the prevention of exposure to outdoor allergens [2].

Drug therapy for allergic rhinitis should be guided by the type and severity of individual patient's symptoms and should reduce nasal congestion, sneezing, and rhino rhea over the course of the entire day and night and physician preferences. Pharmacotherapy includes oral and intranasal H¹ antihistamines, intranasal corticosteroids, oral and intranasal decongestants, intranasal anti cholinergic and leukotriene receptor antagonists.

Antihistamines are effective in reducing purities, sneezing and watery rhino rhea and are a mainstay therapy for allergic rhinitis.

Although first generation antihistamines are generally more effective in controlling rhino rhea compared with second generation antihistamines, their use is markedly limited due to greater ant cholinergic effects.

Second generation antihistamines have shown favourable effect on sleep in patients with allergic rhinitis and are in general recommended for mild to moderate diseases as first line therapy [3].

Montelukast and levocetirizine are the two newly oral medications used in the treatment and control of allergic rhinitis (Perennial and seasonal) and asthma symptoms Montelukast as (montelukast sodium) is part of a group of medications called (leukotrienes) modifiers. Leukotrienes are chemicals produced by the body in response to allergens (substance that cause allergies), in the lungs, they cause swelling and inflammation in the air ways and constriction of the muscles of the respiratory tract, in the most leukotrienes are released after exposure to allergens leading to allergic symptoms.

Montelukast works by blocking receptors, preventing these chemicals from causing allergy or asthma symptoms, also montelukast, as a mono therapy has been effective in improving day time and night time symptoms in patients with allergic rhinitis and in comparison to antihistamines appear to have significantly better improvement in night time symptoms [4].

On the other hand, Levocetirizine (as Levocetirizine dihydrochloride) is a third-generation non-sedative antihistamine, developed from the second-generation antihistamine cetirizine. Chemically, Levocetirizine is the effective enantiomer of cetirizine.

Levocetirizine works by blocking histamine receptors. It does not prevent the actual release of histamine from the mast cells, but prevents its binding to its receptors. This in turn prevents the release of other allergy chemicals and increased blood supply to the area, and provides relief from the typical symptoms of hay fever (seasonal allergic). For the treatment of allergic rhinitis and asthma symptoms some patients are treated with only one of the two medications but most patients are treated with the both montelukast and levocetirizine this combination has shown a significant improvement in the treatment of allergic rhinitis[5].

During pregnancy, montelukast and levocetirizine may be crosses the placenta into the fetus following the oral administration to pregnant women and animals, but in fact there have been no adequate and well-controlled studies in pregnant women to determine the effects of the two medications on the fetus.

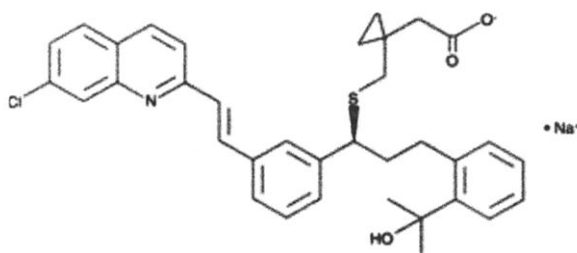
So, the present study was undertaken to determine the effects of montelukast and levocetirizine alone or in combination therapy on the pregnant females and embryos if they taken orally at the recommended doses during pregnancy.

MATERIALS AND METHODS

Chemical drugs:

Singulair as (montelukast sodium)

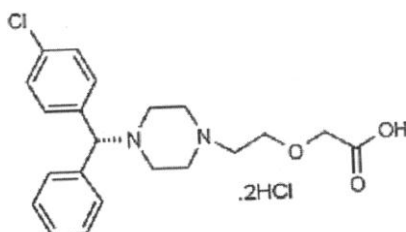
Produced by (Merk) is an oral leukotriene receptor antagonist that is used for the treatment of asthma and seasonal allergic rhinitis (hay fever). The chemical name is 2-[1-[2-[(7-chloro-2-quinoly)]vinyl]phenyl]-3-[2-(1-hydroxy-1-methyl-ethyl)phenyl]-propylsulfanylmethyl]cyclopropyl) acetic acid sodium salt. The structural formula is.



The molecular formula is $C_{35}H_{35}ClNNaO_3S$, and the molecular weight is 608.2. It is available as tablets each tablet contains 5 or 10 mg montelukast sodium and it is a freely soluble in water. The recommended dose for human is 10mg once daily.

Levcet as (levocetirizine dihydrochloride) produced by (Marcyl) is a third-generation non-sedative antihistamine, developed from the second-generation antihistamine cetirizine.

Levcet is prescribed for seasonal allergic rhinitis perennial allergic rhinitis and chronic urticaria. The chemical name is (R)-[2-[4-C₄[chlorophenyl]phenylmethyl]1-piperaziny/L] ethoxy] acetic acid dihydrochloride and the structural formula is:



Levocetirizine dihydrochloride is white powder with a molecular formula of $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ and a molecular weight of 461.82. It is freely soluble in water and partially insoluble in acetone.

The recommended dose of levcet is 5mg once daily.

Animals and treatment:

Dilution of different concentrations was prepared by dissolving all tablets in distilled water. Females weighting (25-28) g were housed in cages with adult males. After one day of mating the females which exhibiting a vaginal plug was considered as the 1st day of gestation. The pregnant females were divided into four groups as following: The first group of 5 pregnant females were administrated orally with a dose equal to the recommended dose of levocetirizine (0.002 mg/kg/day) once daily.

The second group of (5) pregnant females were administrated orally with a dose equal to the recommended dose of montelukast (0.004 mg/kg/day) once daily.

The third group of (5) pregnant females were administrated orally with two doses of levocetirizine and montelukast (0.002 + 0.004mg/kg/day) equal to the recommended doses of montelukast and levocetirizine once daily.

The fourth group of (5) pregnant females served as control were administrated orally with distilled water.

These doses of montelukast and levocetirizine are the recommended doses for human after modified to suit the small weight of albino mice (28g) according to pagat and Barnes (1964).

All the pregnant females were administrated orally from day (3) to day (17) of pregnancy and on day (18) of pregnancy the pregnant females were sacrificed by cervical dislocation and the uterus were opened for studying the developmental and cytogenetic effects of the two drugs on the females and embryos.

Methods:

Developmental toxicity:

On day (18) of gestation, the females were sacrificed by decapitation the uterus contents were evaluated for the number of implantation sites, dead and live embryos.

chromosomal aberrations assays:

Bone marrow cells (in pregnant females):

Chromosome preparations were made by the method of Yosida et al[7]. Mice were injected with colchicines (2.5 mg/kg/b.w.i.). 3 hours prior. Females were killed by cervical dislocation the bone marrow cells were aspirated in phosphate buffer solution (P.H. 7.2) centrifuged at 1000 r.p.m. for 5min. The pellets obtained were mixed in aqueous solution of KCl (0.56%) and left for 30 min at 37°C. Cells were re-centrifuged, fixed in (3:1) methyl: glacial acetic acid.

Finally slides were-dried and stained with 10% Giemsa stain for 20 minutes.

Embryonic cells (in embryos)

At day (18) of gestations, embryos were prepared cytogenetically according to the method of Evans *et al.* [8] with minor modifications embryonic livers were incubated in T.C.M. media containing 0.1 mg/mL colchicines for 90min at 37°C and centrifuged at 1000 r.p.m. for 5 minutes after centrifugation 5ml of hypotonic solution of (0.56%) KCl was added to the pellet at 37°C and incubated for 15 minutes. Then 5ml fixative (3:1) (methyl:glacial acetic acid) was added gently to the cells drop by drop. Two on three drops of cell suspension were dropped on a clean slide and stained with 5% Giemsa stain for 15 minutes.

About 50 metaphases were scored for each female and embryo. Aberrations were divided into (structural aberrations) includes (breaks, deletions, endomitosis and centromeric attenuation) and numerical aberrations includes (periploidy and polyploidy).

Micronucleus tests:

In females:

The females were sacrificed by cervical dislocation on day (18) of gestation. Bone marrow smears and staining were done following the method of Schmid [9]. Briefly, both the femora were removed and the bone marrow was flushed out into a centrifuge tube with 1% sodium citrate solution. The bone marrow cells were dispersed by gentle pipetting and centrifuged. The cell pellet was re-suspended in a small volume of 5% fetal calf serum. A drop of this suspension was smeared on a clean slide air-dried, fixed in absolute methanol for 15 min and stained with 5% Giemsa stain. 500 erythrocytes were analyzed for the presence of micronuclei (MN).

In embryos:

Embryos were taken on day (18) of gestation. Blood smears were taken from each embryo according to the method of Schmid [9]. Briefly, blood smears were taken from the tail embryos and the blood was re-suspended in a small volume of 5% fetal calf serum. A drop of suspension was smeared on a clean slide; air dried, fixed in absolute methanol for 15 min and stained with 5% Giemsa stain. 500 erythrocytes were analyzed for the presence of micronuclei (MN).

Statistical analysis:

The incidences of implantation, live and dead fetuses between experimental and control values were calculated non parametrically using Wilcoxon's rank sum test Siegal [10].

The data of chromosomal aberrations in the females and embryos were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran [11] least significant differences were used to compare between means of treatments according to Waller and Duncan [12] at probability 5%. The data of micronucleus tests are expressed as percentage.

RESULTS

Maternal observations:

Table 1: The effect of Levocetirizine, Montelukast and their combination on the fertility and off spring development

Parameters	Treatment groups (mg/kg/day)			
	Control	Levocetirizine 0.002	Montelukast 0.004	Levocetirizine + montelukast (0.002 + 0.004)
No of females	5	5	5	5
No of pregnant females	5	5	5	5
Total number of implantations	49	48	47	46
Total number of dead fetuses	2	3	2	2
%	4.1%	6.3%	4.3%	4.4%
Total number of live fetuses	47	45	45	44
%	95.9%	93.8%	95.7%	95.6%
Mean maternal body weight (gm)	30.50	27.82	28.55	28.00
Mean fetal body weight (gm)	3.85	3.65	3.82	3.72

Pregnant females administered with recommended doses of levocetirizine, montelukast and their combinations from day (3) to day (17) of gestation showed no signs of ill-health or abnormal behaviour and appeared normal on gross observation. The animals exposed to levocetirizine, montelukast and (levo + montelukast) did not exhibit any toxicity during pregnancy. On the other hand, there was a decrease in the mean number of maternal body weight in all treated groups but this decrease was more frequent in the levocetirizine group compared with the other treated groups and compared with control (Table 1).

Embryo toxic observations:

There were treatment related effects on the number of implantations, the percentage number of live embryos and the percentage number of dead embryos (Table 1).

The percentage number of dead embryos were highly increased in the embryos treated with levocetirizine compared with the other treated groups and control.

However, the percentage number of dead embryos in montelukast and (levo+montelukast) groups was slightly increased but this increase was very close to the control group.

Also, the percentage number of live embryos were decreased in all treated groups but these decreases were more frequent in the embryos treated with levocetirizine compared with the other treated groups and control. However, the percentage number of live embryos in (Montelukast) and (levo + Montelukast) were slightly decrease but these decrease in the same limit of control.

Also, there was a slight decrease in the mean fetal weight in all treated groups but this decrease in the same limit of control.

Chromosomal aberrations:

In Bone marrow cells:

The results of chromosomal aberrations are given in the table (2).

The administration of levocetirizine to the pregnant females from day 3 to 17 of pregnancy caused a significant increase in the total number of chromosomal aberrations (structural and numerical) compared with the other treated groups and control.

However, the administration of montelukast alone or in combination with levocetirizine showed no significant increase in the total number of chromosomal aberrations (structural and numerical) compared with control. The total structural and numerical aberrations in levocetirizine, montelukast and (levo + montelukast) were (29, 10.33, 23,7 and 24, 8) respectively compared with control (21 and 6.67).

In embryonic cells:

The results of chromosomal aberrations are given in table (3).

The total number of chromosomal aberrations (structural and numerical) were increased significantly in embryos treated with levocetirizine compared with the other treated groups and control.

However, the total number of chromosomal aberrations (structural and numerical) in embryos treated with montelukast and (levo + montelukast) showed no significant effect compared with control embryos.

Table 2: Cytogenetic effects of levocetizine, montelukast and levo + montelukast on pregnant females on at day (18) of gestation

Treatment groups	Structural aberrations						Numerical aberrations					
	Chromatid gaps	Chromosomal gaps	Chromatid breaks	Deletion	Fragments	Endo-metosis	Centro-meric attenuation	T.S. A	<40	>40	Poly-ploidy	T.N. A
Control	3.67 ^a ± 0.577	3.33 ^a ± 0.577	1.33 ^a ± 0.577	2.00 ^a ± 1.00	2.33 ^{ab} ± 0.577	4.33 ^a ± 0.577	3.67 ^a ± 0.577	21.00 ^a ± 1.00	3.00 ^a ± 0.00	3.00 ^a ± 0.00	0.67 ^a ± 0.577	6.67 ^a ± 0.577
Levo-cetizine	5.67 ^c ± 0.577	4.00 ^a ± 1.00	3.33 ^b ± 0.577	3.33 ^a ± 0.57	3.00 ^b ± 0.00	5.67 ^b ± 0.577	4.67 ^b ± 0.577	29.00 ^b ± 2.00	5.00 ^b ± 1.00	3.67 ^a ± 0.577	1.67 ^a ± 0.577	10.33 ^b ± 2.082
Montelukast	4.67 ^b ± 0.577	3.33 ^a ± 0.577	2.67 ^b ± 0.577	2.33 ^a ± 0.577	2.33 ^{ab} ± 0.577	4.33 ^a ± 0.577	3.33 ^a ± 0.577	23.00 ^a ± 1.00	3.33 ^a ± 0.577	2.67 ^a ± 0.574	1.00 ^a ± 0.00	7.00 ^a ± 0.00
Levo cetizine + montelukast	5.00 ^{bc} ± 0.00	3.67 ^a ± 0.577	2.33 ^{ab} ± 0.577	2.67 ^a ± 0.577	2.00 ^a ± 0.00	4.67 ^{ab} ± 0.577	4.00 ^{ab} ± 0.00	24.33 ^a ± 0.577	3.67 ^a ± 0.577	3.00 ^a ± 1.00	1.33 ^a ± 0.577	8.00 ^a ± 7.00

Means of different letters (a,b,c,d) in the same column are significantly different.
 The column with the same letters is not significant
 50 metaphase were examined from each animals ± S.D. at (P < 0.05)

Table 3: Cytogenetic effects of levocetizine, montelukast and (Levocetizine + Montelukast) on embryos at day (18) of gestation.

Treatment groups	Structural aberrations						Numerical aberrations					
	Chromatid gaps	Chromosomal gaps	Chromatid breaks	Deletion	Fragments	Endo-metosis	Centro-meric attenuation	T.S. A	<40	>40	Poly-ploidy	T.N. A
Control	3.67 ^a ± 0.577	2.67 ^a ± 0.577	1.67 ^a ± 0.577	2.00 ^a ± 1.000	1.67 ^a ± 0.577	2.67 ^a ± 0.577	2.33 ^a ± 0.577	15.67 ^a ± 2.082	3.00 ^a ± 0.00	2.67 ^a ± 0.577	0.00 ^a ± 0.000	5.67 ^a ± 0.577
Levo-cetizine	5.67 ^c ± 0.577	4.67 ^b ± 0.577	3.00 ^b ± 1.000	3.00 ^a ± 0.00	4.00 ^b ± 1.000	3.67 ^a ± 0.577	4.00 ^a ± 1.00	22.33 ^b ± 6.65	4.33 ^b ± 0.577	3.33 ^a ± 0.577	1.33 ^b ± 0.577	8.67 ^b ± 0.577
Montelukast	4.67 ^b ± 0.577	3.33 ^a ± 0.577	2.00 ^{ab} ± 0.00	2.00 ^a ± 0.00	1.67 ^a ± 0.577	3.33 ^a ± 0.577	2.33 ^a ± 1.155	17.67 ^a ± 1.755	2.67 ^a ± 0.577	2.33 ^a ± 0.577	0.33 ^a ± 0.577	5.33 ^a ± 1.528
Levo cetizine + montelukast	5.00 ^{bc} ± 0.00	3.67 ^{ab} ± 0.577	2.67 ^{ab} ± 0.577	2.00 ^a ± 1.00	2.33 ^a ± 0.577	3.00 ^a ± 1.000	3.67 ^a ± 0.577	19.67 ^a ± 2.082	3.33 ^a ± 0.577	3.00 ^a ± 1.000	0.67 ^{ab} ± 0.577	6.33 ^a ± 1.528

Means of different letters (a,b,c,d) in the same column are significantly different.
 The column with the same letters is not significant
 50 metaphase were examined from each animals ± S.D. at (P < 0.05)

Table 4: Results of micronucleus tests in mothers and embryos after maternal oral administrations with (levocetizine, montelukast and their combination)

Parameters	Dose mg/kg/day	Number of assessed PCE	Total number of MN	No. of cells with			Percentage of micronuclei Per 500 cells
				1MN	2MN	3MN	
Mothers							
Control	0	500	180	95	70	15	36%
Levo	0.002	500	200	100	80	20	40%
Montel	0.004	500	185	95	75	15	37%
Levo + Montelukast	0.002+0.004	500	193	98	78	17	38.6%
Embryos							
Control	0	500	150	85	65	0	30%
Levo	0.002	500	170	95	75	0	34%
Montelukast	0.004	500	155	90	60	0	31%
Levo + Montelukast	0.002+0.004	500	162	95	68	0	32.4%

The total number of (structural and numerical) aberrations in levocetizine, montelukast and their combination were (22.33, 8.67, 17.67, 5.33 and 19.67, 6.33) respectively compared with control (15.67 and 5.67).

Micronucleus observations

Results of micronucleus in pregnant females and embryos are given in table (4).

Generally, the group of pregnant females and embryos treated with levocetirizine showed highly increase in the total number of micronucleated cells compared with montelukast and (levo + montelukast) groups and with control.

However, the groups of pregnant females and embryos treated with montelukast and (levo + montelukast) showed a slight increase in the total number of micro nucleated cells compared with the control but this increase is very close to control group.

The distribution of micronuclei were different between mothers and embryos, in the mothers the majority of cells containing one, two and three micronuclei but in the embryos the majority of cells were containing only one and two micronuclei.

DISCUSSION

Rhinitis and asthma are often associated and the two disorders interact at various levels. Rhinitis typically precedes the development of asthma and can contribute to unsatisfactory asthma control. The presence and type of asthma is influenced by sensitizations and the duration and severity of allergic rhinitis.

Allergic rhinitis and asthma adversely affects up to 8% of all pregnancies. Untreated asthma leads to increased risk of preterm delivery vaginal hemorrhage and pregnancy induced hypertension. Many studies have shown that proper control of asthma in pregnant asthmatic women significantly reduces the risk of prenatal adverse outcomes [13].

Second generations antihistamines (Levocetirizine) are effective in reducing majority of symptoms of allergic rhinitis, but are ineffective for nasal congestion and night time symptoms. Montelukast serves a role in helping reduce symptoms of allergic rhinitis and asthma that are not controlled with antihistamines alone. In fact in pregnant women and animals no available data are found that illustrates the safety use of levocetirizine alone or in continuation with montelukast during pregnancy.

In the present study the administration of (levocetirizine) to the pregnant females during. Pregnancy at a recommended dose caused a significant increase in the incidence of chromosomal aberrations and in the percentage of micro nucleated cells in the pregnant females and embryos and also caused embryo toxic effects (increase in the number of dead embryos, decrease in the number of live fetuses and decreased in the maternal and fetal body weight). These finding was agreement with Kallen and otterbald [14] who observed that when levocetirizine used during pregnancy it crosses the placenta into the fetus following oral administration to animals and caused embryo toxic effects. Also, similar results was observed by Roger et al [15] who found than in a 2 year carcinogenicity study in mice, cetirizine caused an increased in incidence of hepatic tumors in males at a dietary dose of (6 mg/kg/b.w.). However, these finding was in agreement with Weber and Schaefer [16] who observed that in rats and rabbits, levocetirizine was not teratogenic approximately 320 times the recommended dose.

Similar results were reported by So et al [17] who observed that levocetirizine was not mutagenic in the Ames test and not clastogenic in the in vivo micronucleus test in mice.

In the fertility and general reproductive performance Schatz et al [18] observed that in mice, cetirizine did not impair at an oral dose of 64 mg/kg.

In our study the administration of montelukast to pregnant females during pregnancy caused a slight increase but not significant in the embryonic toxicity, chromosomal aberrations and micronuclei in maternal bone marrow cells and in the embryonic cells when compared with the control group. These finding was agreement with Sarkar et al [19] who observed that montelukast had no evidence of mutagenic or clastogenic activity in the chromosomal aberration assay in Chinese hamster ovary cells, and in the in vivo mouse bone marrow chromosomal aberration assay.

Also, similar result was observed by Bakhireva et al [20] who found that exposure to montelukast during pregnancy did not appear to increase the risk of major malformations above the control.

However, negative result was observed by Tamasil et al [21] who reported that the administration of montelukast during pregnancy caused a lower in the maternal and fetal body weight, shorter gestational age and a little number of embryos with major malformation in pregnant women.

Also, in the present study we found that when pregnant females, were administrated with a combination of montelukast and levocetirizine at a recommended dose for human during pregnancy caused a slight increase in the total number of chromosomal aberrations, in micro nucleated cells and in the embryonic toxicity but this increase is not significant and very dose to control group. These finding was agreement with Vipin and Prithpal[22] who reported that the combination of montelukast and levocetirizine is effective and safe in the patients of allergic rhinitis when administrated with a recommended dose.

CONCLUSION

In conclusion our results indicated that levocetirizine had mutagenic and cytotoxic effects on both mothers and their embryos when administrated orally at a recommended dose during pregnancy. This may be as are a result that levocetirizine can cross female placenta causing cytotoxic effects to the mothers and embryos. However, the treatment with montelukast and its combination with levocetirizine (levo+montel_) at recommended doses during pregnancy did not exhibit significant mutagenic effects or cytotoxic effects on pregnant females and their embryos. This may be as a result that montelukast had a safety profile when used alone or in combination with antihistamine drug. So from the above results we concluded that levocetirizine should be avoided during pregnancy while, montelukast and its combination with levocetirizine can be used during pregnancy.

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