Cytogenetic and mutagenic effects of Alvidar and thyroid hormones on female mice and embryos

Hanaa M. Roshdy
Cell Biology Department, National Research Centre -Dokki, Cairo, Egypt
Corresponding author: Hana-amr@hotmail.com

ABSTRACT

Background: Hypothyroidism is one of the most common problems that develop in the thyroid gland which can be happened to females during pregnancy causing many diseases to the pregnant females and their expecting embryos. The two thyroid hormones levothyroxine (T_4) and triiodothyronine (T_3) are synthetic hormones used in the treatment of (hypothyroidism). Also another newly herbal medication called, Alvidar is used for the treatment of hypothyroidism which composed of herbal that help in improving the function of thyroid gland without any chemicals or hormones. In fact no adequate studies were been done in the human and animals concerning the mutagenicity of Alvidar, levothyroxine (T_4) and triiodothyronine (T_3) especially during pregnancy. **Aim of the study** is to evaluate the cytogenetic and mutagenic effect of Alvidar, (T_4) and (T_3) on the pregnant females and their embryos. **Materials and Methods** : Pregnant female mice were administrated orally with a doses (1.3, 0.13) and 0.07 mg/kg/day of Alvidar, T4 and (T_3) respectively from day (1 to day 17 of gestation. Cesarean section were completed on (18) day of gestation, cytogenetic analysis micronuclei formation and developmental toxicity were examined. The results showed that Alvidar and triiodothyronine T_3 caused a significant increase in the frequencies of chromosomal aberrations fetal toxicity micronuclei formation and fetal toxicity in the pregnant females and their embryos and these toxicity were highly increased in the (T_3) group than Alvidar group compared with controls. While in the thyroxine (T_4) group no mutagenic or embryo toxic effects were observed in the mothers and their embryos compared with control group. **Conclusion**: These results indicate that triiodothyronine (T_3) hormone and Alvidar (herbal medication) had a cytogenetic and embryotoxic effects when administered orally during pregnancy. While levothyroxine (T_4) hormone had no cytogenetic or embryotoxic effects during pregnancy. [Report and Opinion 2010;2(4):62-70]. (ISSN: 1553-9873).

Key words: Alvidar, triiodothyronine, (T_3) thyroxine (T_4) micronuclei, chromosomal aberrations, mice, embryos.

Introduction

Thyroid is gland, shaped like a butterfly, located in the lower part of the neck. The function of it is to secrete hormones (triiodothyronine (T3) and levothyroxine

(T4) Hapon (2007). These thyroid hormones deliver energy to cells of the body.

Levothyroxine is synthesized by the follicular cells from free tyrosine and on the tyrosine residues of the brain called thymoglobulin (TG). Thyroid hormones that are secreted from the gland is about 90% T4 and about 10% T3.

Cells of the brain are a major target for thyroid hormones; also thyroid hormones play particularly an important role in brain development during pregnancy.

The most common problems that develop in the thyroid gland are Hypothyroidism (lack of hormone production) and Hyperthyroidism (over active thyroid). Hypothyroidism leads to adverse effect on fertility as it decrease ovulation, milk production and also affects the development of the embryos during pregnancy. Bunevicouse,(2002)

Hypothyroidism can affect pregnancy in many-ways, it can cause infertility in woman as it prevents the egg production and also pregnant lady is at higher risk for miscarriage. Hypothyroidism when is left untreated during the entire period of pregnancy, the lady is likely to develop high blood pressure and premature delivery. On the other hand, baby produced by untreated or partially treated mother may not reach its intellectual potential. Haddow et al (1999) stating that women with untreated thyroid deficiency during pregnancy are four times more likely to have abnormal children.

So treatment of hypothyroidism during pregnancy is necessary by either thyroid hormone replacement therapy (T_3 and T_4) or by natural supplements for hypothyroidism such as (Avidar) which is natural food supplement that contains ingredients can improve and sustain normal thyroid function. Alvidar does not try to replace deficiency of thyroid hormones like synthetic hormones thyroxin (T_4) and triiodothyronine (T_3) but, it works by helping the body to increase the production of thyroid gland on its own. Alvidar contains ingredients that help to repair, improve, and re-program

thyroid gland to work correctly, without any chemicals choi et al., (1995).

The effects of Alvidar, Triiodothyronine and levothyroxine on mothers and their embryos during pregnancy have not been adequately studied. There fore, the present study was under taken to determine the cytogentic and developmental toxicity of Alvidar thyroxin (T_4) and triiodothyronine (T_3) when they taken for 17 consecutive days during pregnancy and compared their effects with each other and with the controls.

2-Material and methods

2. 1- Materials:

2.1.1.Test substances:

2.1.1.1 Alvidar produced by Selmedica pharmaceutical Company in form of capsules each contains 500mg of herbal ingredients which improve and sustain normal thyroid function. These ingredients are Laminariae stipites, ducus vesiculosus, serenoa Rupens. Gentianae Rodix, Capsicum frutescents, Echinacea Purpurea ananous officinale, comosus, allium sativum, Ilex parayvariensis, biloba, panax, Fili pendula ulmaria. Hypericum perforatum and Valeriana officianalis. The recommended dose for human is 500 mg once daily.

2.1.1.2. Eltroxine (T4) produced by (Galaxosmith kline pharmaceutical Company, USA) contains the active ingredient levothyroxine sodium (alike the naturally produced). The recommended dose for human is 50 mg once daily.

2.1.1.3. Cytomel (T_3) produced by (Galaxosmith kline pharmaceutical Company, USA) contains the active ingredient Triiodothyronine sodium (T3) which alike the naturally produced. The recommended dose for human is 25mg once daily.

2.1.2. Animals and treatments:

Dilution of different concentrations was prepared by dissolving all tablets in distilled water. Forty females were housed in cages with adult males. After on day of mating the females which exhibiting vaginal plugs were considered as pregnant. The day of the appearance of the vaginal plug was considered as the 1st day of pregnancy.

The pregnant females were divided into four groups as following:

The first group (10 pregnant females) was administrated orally by a single dose of Alvidar (1.3 mg) in the first morning once daily.

The second group (10 pregnant females) were administrated orally by a single dose of Eltroxine (T_4) (0.13mg) in the first morning once daily.

The third group (10 pregnant females) were administrated orally by a single dose of Cytomel (T_3) (0.07mg) in the first morning once daily.

The fourth group (10 pregnant females) severed as a control group administrated distilled water orally .

Doses of Alvidar, T_4 and T_3 1.3, 0.13 and 0.06 mg/kg/day respectively are the recommended doses for human after modified to suit the small weight of albino mice (30g) according to Pagat and Barnes (1964).

All the pregnant females were administrated orally a single dose of (Alvidar, Eltroxine and Cytomel) form the day 1 to the day (17) of pregnancy and on the day (18) of pregnancy the pregnant females were sacrificed by cerfical dislocation for studding the developmental and cytogenetic effects of hormones on females and their embryos.

2.2. Methods:

2.2.1. Development toxicity:

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

2.2.2. Chromosomal aberrations assays:

2.2.2.1. Bone marrow cells (in pregnant females):

Chromosome preparations were made by the method of Yosida et al. (1971). Mice were injected with colchicine (2.5 mg/kg/b.w.i.p./3h 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 5 min. 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.

2.2.2.2. Chromosome preparations (in embryos):

At day (18) of gestations, embryos were prepared cytogenetically according to the method of Evans et al (1972) with minor modifications .Embryonic livers were incubated in T.C.M. media containing 0.1 mg/ml colcemid for 90 min at 37°C and centrifuged at 1000 rpm 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 or three drops of cells 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 (Periploid and polyploidy).

2.2.3. Micronucleus tests:

2.2.3.1. In females:

The females were sacrificed by cervical dislocation on day (18) of gestation. For each treatment five females were used in different groups. Bone marrow smears and staining were done following the method of Schmid (1976). 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 resuspended 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).

2.2.3.2. In embryos:

Embryos were taken on day (18) of gestation. Blood smears were taken from each embryo according to the method of Schmid (1976) Briefly, blood smears were taken from the Tail of embryo 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 and stained with 5% Giemsa stain, 500 erythrocytes were analyzed for the presence of micronuctei (MN).

2.2.4. Statistical analysis:

The incidences of implantation, live and dead fetuses between experimental and control values were calculated on Parameterically using wilcoxon's rank sum test (Siegal, 1956).

The data of chromosomal aberrations in the females and embryos were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1990) least significant differences were used to compare between means of treatments according to Waller and Ducan (1969) at probability 5%. The data of micronucleus tests were expressed as percentage.

3. Results

3.1. Embryo toxic effects:

There were treatment related effects on the number of implantations. The number of live embryos and the number of dead embryos (Table 1).There was an increase in the incidence of dead embryos in all treated groups compared with the control.

However, the total number of dead embryos where highly increased in the embryos treated with cytomel triiodothyronine (T_3) compared with the other treated groups and control. Also, there was an increase in the incidences of dead embryos in the group of embryos treated with Alvidar if compared with Eltroxin group (T_4) and control. On the other hand the group of embryos treated with (Elttroxin T_4) showed a slight increase in the incidence of dead embryos but this increase in the limit of control.

Also, there was a significant change in the average fetal body weight in all treated groups compared with the control.

However, the mean fetal body weight increased in the embryos treated with triiodothyronine and Alvidar compared with (T_4) and control groups. The increases in the body weight resulted of the embryo swollen and not increase in the body volume of embryo.

In the other hand the mean fetal body weight of embryos treated with (T_4) hormone were slightly change but within the same limit of controlembryos.

3.2. Micronucleus test:

Results of micronucleus in pregnant females and embryos are given in Table (2). Generally, the group of pregnant females and embryos treated with Cytomel (T_3) showed a highly significant increase in the total number of micronucleated cells compared with the other treated groups of Alvidar and (T_4) and with control.

Also the group of pregnant females and embryos treated with Alvidar showed a significant increase in the total number of micronucleated cells compared with Eltroxin (T_4) and control group. However, the group of pregnant females and embryos treated with Eltroxin (T_4) showed a slight increase in the total number of micronucleated cells and this increase was within the limit of control. In addition, the increase in the total number of micronucleated cells was higher in the treated female groups than in the treated embryo groups.

The results of the total number of chromosomal aberrations and their types in treated females, their embryos and control group are illustrated in Table (3) and (4). The total number of chromosomal aberrations (structural and numerical) was increased.

3.3. Chromosomal aberrations (females and embryos):

Significantly in all treated groups (females and embryos) compared with the control. The most highly significant among the treated groups is the group (females and embryos) treated with cytomel (T_3) at the recommended dose for human and the second group of significant is the group of (females and embryos) treated with (Alvidar) at the recommended dose for human. The last group of less significant is the group of (females and embryos) treated with (Eltroxin T_4**)** at the recommended dose for human.

Table (1): Reproductive and fetal effects.

Dose treatment	Structural aberrations							Numerical aberrations			
	Chromatid gaps	Chromatid breaks	Deletions	Fragments	Centromerie attenuations	Endo-melosis	T.S.A	>40	<40	Poly-ploidy	T.N.A.
Control	$2.33^{c} \pm 0.58$	$0.67^{c} \pm 0.58$	$0.67^{c} \pm 0.58$	$0.67^{c} \pm 0.58$	$2.00^{c} \pm 0.00$	$2.33^{c} \pm 0.58$	$8.67^{c} \pm 0.58$	$2.33^{c} \pm 0.58$	$3.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$	$5.33^{c} \pm 0.58$
Alvidar 1.3	$6.00^{b} \pm 1.00$	$4.67^{b} \pm 0.58$	$5.33^{b} \pm 0.58$	$4.00^{b} \pm 0.00$	$4.33^{b} \pm 0.58$	$5.00^{b} \pm 0.00$	$29.33^{b} \pm 1.53$	$4.00^{b} \pm 1.00$	$4.33^{b} \pm 0.58$	$1,53^{b} \pm 0.58$	$9.67^{b} \pm 0.58$
Eltroxin T_4 0.13	$2.67^{c} \pm 0.58$	$0.67^{c} \pm 0.58$	$1.00^{c} \pm 0.58$	$1.33^{c} \pm 0.()0$	$2.67^{c} \pm 0.58$	$2.33^{c} \pm 0.58$	$10.67^{c} \pm 0.58$	$3.00^{bc} \pm 0.00$	$2.67^{c} \pm 0.58$	$0.67^{bc} \pm 0.58$	$6.33^{c} \pm 0.58$
Cytomel T_3 0.07	$7.33^{a} \pm 0.58$	$6.60^{a} \pm 0.58$	$7.00^{a} \pm 0.00$	$5.67^{a} \pm 0.58$	$6.00^{a} \pm 1.00$	$7.00^{a} \pm 0.00$	$39.67^{a} \pm 0.58$	$6.00^{a} \pm l .00$	$6.33^{a} \pm 0.58$	$3.00^{a} \pm 0.00$	$15.33^{a} \pm 0.58$
L.S.D. at 0.05	1.331	1.087	0.941	0.769	1.21.6	0.769	1.71.8	1.438	0.941	0.769	1.087

Means of different letters (a, b, c d) in the same column are significantly different.
The column without letters is not significant.
50 metaphase were examined from each animals.

Parameters	Group Treatments			
	Control 0	Alvidar 1.3	Eltroxin (T_4) 0.13	Cytomel (T_3) 0.07
No of females	10	10	10	10
No of pregnant females	10	10	10	10
No of implantations	85	80	84	79
No of live fetuses	84	76	82	74
%	98.8%	95%	97.6%	93.6%
No of dead fetuses	1	4	2	5
%	1.2%	5%	2.4%	6.3%
Mean fetal weight (gm)	3.8	4.2	3.7	4.4

Table (2): The effect of oral administration of hypothyroid hormones on pregnant females bone marrow cells.

Table (3): The effect of hypothyroid hormones on embryos at day (18) of gestation

Test substance	Dose mg/kg/day	Number of assessed PCE	Total Number of micronuclei	percentage of micronucleated per 500 cells
Mother				
Control	0	500	180	36%
Cytomel T_3	0.07	500	230	46%
Alvidar	1.3	500	200	40%
Eltroxin	0.13	500	187	37.4%
Embryos				
Control	0	500	165	33%
Cytomel T_3	0.07	500	196	39.2%
Alvidar	1.3	500	182	36.4%
Eltroxin T_4	0.13	500	170	34%

Table (4): Results of micronucleus in mothers and embryos aftermaternal oral administrations with (Eltroxin, Alvidar and Cytomel).

Dose treatment	Structural aberrations							Numerical aberrations			
	Chromatid gaps	Chromatid breaks	Deletions	Fragments	Centromeric attenuations	Endo-melosis	T.S.A	>40	<40	Poly-ploidy	T.N.A.
Control 0	$1.67^{c} \pm 0.58$	$0.33^{c} \pm 0.58$	$0.67^{c} \pm 0.58$	$1.00^{c} \pm 0.00$	$1.67^{b} \pm 0.58$	$2.00^{c} \pm 0.00$	$7.33^{c} \pm 1.15$	$1.67^{c} \pm 0.58$	$2.33^{c} \pm 0.58$	0.00 ± 0.00	$4.00^{c} \pm 0.00$
Alvidar 1.3	$4.33^{b} \pm 0.58$	$3.33^{b} \pm 0.38$	$4.00^{b} \pm 1.00$	$3.00^{b} \pm 1.00$	$3.33^{a} \pm 0.58$	$3.33^{b} \pm 0.58$	$31.33^{b} \pm 1.53$	$3.33^{b} \pm 0.58$	$4.00^{bn} \pm 0.00$	0.33 ± 0.58	$7.67^{b} \pm 0.58$
Eltroxin T4 0.13	$2.33^{c} \pm 0.58$	$1.00^{c} \pm 1.00$	$1.33^{c} \pm 0.58$	$1.33^{c} \pm 0.58$	$1.67^{b} \pm 0.58$	$1.67^{c} \pm 0.58$	$9.33^{c} \pm 1.15$	$2.67^{b} \pm 0.00$	$2.33^{c} \pm 0.58$	0.33 ± 0.58	$5.33^{c} \pm 0.00$
Cytomel T3 0.07	$3.67^{a} \pm 0.58$	$5.33^{a} \pm 0.58$	$6.00^{a} \pm 0.00$	$5.00^{a} \pm 0.00$	$4.33^{a} \pm 0.58$	$4.67^{a} \pm 0.58$	$31.00^{a} \pm 1.00$	$6.00^{a} \pm 0.00$	$5.32^{a} \pm 0.58$	0.67 ± 0.58	$12.00^{a} \pm 1.00$
L.S.D. at 0.05	1.087	1.331	1.216	1.087	1.087	0.941	2.306	0.769	0.941	N.S	1.087

50 metaphase were examined from each animals.
Means of different letters (a, b, c d) in the same column are significantly different.
The column without letters is not significant.

4. Discussion

Thyroid hormones are critical for development of the fetal and neonatal brain, as well as for many other aspects of fetal growth. Hypothyroidism in either the mother or fetus frequently results in fetal diseases in humans including high incidence of mental retardation.

The fetus has two potential sources of thyroid hormones; its own thyroid and his mother´ thyroid. Human fetuses acquire the ability to synthesize thyroid hormones at 10 to 12 weeks of gestation. There are three types of thyroid deficiency states known impact on fetal development:

a) Isolated maternal hypothyroidism, when the pregnancy occurs in the mothers have hypothyroidism there is increased risk of intrauterine fetal death.
b) Isolated fetal hypothyroidism this occur due to the failure of the fetal thyroid gland to produce adequate amounts of thyroid hormone but most fetuses with this disorder are normal at birth because maternal thyroid hormones are transported across the placenta during gestation, c) Iodine deficiency combined maternal and fetal hypothyroidism. Iodine deficiency is the most common preventable cause of mental retardation in the fetuses.

Therefore, females with hypothyroidism should take thyroid hormone replacement during pregnancy Bunevicous (2002).

Alvidar is a natural herbal medication which helps in improving the function of thyroid gland.

In the other hand the two thyroid hormones levothyroxine (T_4) and triiodothyronine (T_3) are synthetic hormones used for the treatment of hypothyroidism in the human. These thyroid hormones are indicated as replacement therapy in the treatment of thyroid hormone deficiency (hypothyroidism) but in general levothyroxine (T_4) is the preferable thyroid hormone for use in the treatment of hypothyroidism than triiodothyronine (T_3) because of the absence of variability and the ease in monitoring of plasma concentrations.

No adequate studies were done in the human and animals about the mutagenicity of Alvidar as a natural medication used in the treatment of hypothyroidism and also about the mutagenicity of levothyroxine (T_4) and triiodothyronine (T_3) especially if administered during the pregnancy in this study, we aimed at defining the safty use of Alvidar and thyroid hormones during pregnancy for the mothers and their embryos.

Results of our study showed that the treatment with levothyroxin (T_4) from 1 day to 17 day of gestation with a dose of (0.13) mg/kg/day which equivalent to (50) mg/kg/day the therapeutic dose for human caused as slight increase but not significant in developmental toxicity, chromosomal aberrations and micronuclei in maternal bone marrow cells and embryos cells when compared with the control group.

However, the treatment with Alvidar (natural Herbs) with a dose of 1.3 mg/kg/day which equivalent to (500 mg/kg/day) which is the therapeutic dose for human caused a significant increase in the chromosomal aberrations, developmental toxicity and micronuclei in the maternal bone marrow cells and embryonic cells. While the treatment with triiodothyronine (T_3**)** with a dose of (0.07) mg/kg/day which equivalent to therapeutic dose for human (25mg/kg/day) caused highly significant increase in the frequencies of chromosomal aberrations development toxicity and micronuclei in the maternal bone marrow cells and in the embryonic cells.

These results were in-agreement with Rosato (1998) who observed that the triiodothyronine (T_3) when administrated in the rat during pregnancy no genotoxic effects found in the rats and their embryos.

Also, negative results were obtained by Hapon et al, (2003) who observed that the administration of thyroid hormones (T_4) and (T_3) to the pregnant rats during pregnancy caused no toxic effects to the rats and their embryos.

However, positive results were observed by Rosato et al (1992) who found that in rats treated with 1mg/kg thyroxin (T_4) before and during pregnancy did not show any evidence of genotoxicity in rats and their embryos.

Also results obtained by Hapon (2007) showed that the administration of thyroid hormones (T_4) and (T_3) to the pregnant rats during pregnancy caused no toxic effects to the rats and their embryos.

Positive results in goats given long-term treatment with (T_3) and (T_3) thyroid hormones during pregnancy were observed by Forsyth et al (1985) who observed that the treatments with (T_3) hormone during pregnancy caused increase in the genotoxic in goats and their embryos. On the other hand, the treatment with (T_4) hormone did not show any genotoxic effects in the pregnant goats and their embryos.

Choi et al. (1995) and Predy et al (2005) observed that Ginseng alone or in combination with other herbs such as garlic and ginger (herbs found in alvidar), can increases the risk of uncontrolled bleeding and hemorrhage if they administered by pregnant females

In conclusion our results indicated that cytomel (T_3) triiodothyronine sodium had a highly significant mutagenic and cytogenic effects on both mothers and their embryos when administrated orally to the pregnant female from day (1), to day (17) of pregnancy at the recommended dose (0.07) mg/kg/day. Also,

treatment of pregnant females with Alvidar from day (1) to day (17) of pregnancy with the recommended dose (1.3 mg/day) caused a significant mutagenic and embryotoxic effects on mother and their embryos. This may be as a result that Alvidar contains Ginseng that has an estrogenic effect which may cause bleeding or hemorrhage when taken alone or combined with other herbs such as garlic and ginger which are found also in the content of Alvidar. Eltroxin (T_4) levothyronine sodium had no significant mutagenic or embryotoxic effects on pregnant females and their embryos when administrated orally to the pregnant females with a recommended dose (0.13) mg / kg/day.

5-References

Bunevicous, R, *el al.* (2002). Thyroxine plus triiodothyronine interment of hypothyroidism after Endocrine, 18: 129-133.

Choi HK *el al.* (1995). Clinical Efficacy of Korean red ginseng for erectile dysfunction. International Journal of impotence. Research 73: 181-6.

Evans, E, Burten Shaw, M.D and ford, EC (1972). Chromosomes of mouse embryos and newborn young: preparations from membranes and tail tips- stain technol. 47:229-234.

Forsyth 1A, Byatt JC, Iley S (1985). Hormone concentrations, mammary development and milk yield in goats given Long-term thyroid hormones during pregnancy. J. Endoxinol 104, (1): 77-85.

Haddow IE, Paloniaki GE, Allan WC, ***et al***. **(1999).** Maternal thyroid deficiency during pregnancy and subsequent near psychological development of the child. New Eng J Med 341: 549-555.

Hapon, MP, Alicia, B, and et al (2007). Hypothyroidism prolongs corpus lustrum function in the pregnant rat. Reproduction. 133:197-205.

Hapon, MP, Simoncini, M et al (2003). Effects of hypothyroidism on pregnancy and lactation in the rat. Reproduction 126:371-382.

Pagat and Barnes (1964). Evaluation of drug activities, Vol. 1 Academic press.

Predy GN, *et al.* (2005). Efficacy of an extract of worth American ginseng containing poly-furanosly-pyranosyl-saccharides for preventing upper respiratory tract infections: a randomized controlled trial CMA.1, 173, 179: 043-8.

Rosato, RR, Gimenez, MS and et al (1992). Effects of chronic thyroid hormone administration on pregnancy, lactogenesis and lactation in the rat. Acta Endocrinologics 127: 547-554.

Rosato, RR, Jamnes, H et al (1999). Effects chronic thyroxine treatment on pregnancy in rats. Endocrine Research 24:269-284.

Schmid, W (1976). The micronucleus test for cytogenetic analysis, Mut. Res. 4:31-53.

Siegal, S, (1956). Non parametric statistics for the Behavioural sciences. MC graw-Hill, New York, p.p 116-127.

Snedecor, GW, and Cochran, WG (1990). statistical methods, 9th. Lowa state Univ.. press, Lova, USA.

Waller, A and Duncean, DB (1969). Multiple Range for multiple test 1969. Bionetries, 11:1-24.

Yosida, TH, Truchiya, K, Moriwaki, K (1971). Frequency of chromosome polymorphism of Ruttus rattus collected in Japan chromosoma, 33:30-37.

16/4/2010