Volume 2, Issue 3 – July – December -2016

E C O R F A N

Journal-Republic of Guatemala

ISSN-On line: 2414-8849

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ECORFAN Journal-Republic of Guatemala, Volume 2, Issue 3, July-December 2016, is a journal edited semestral by ECORFAN. Kilometer 16, American Highway, House Terra Alta, House D7 Mixco Zona 1, Republic of Guatemala. WEB: www.ecorfan.org/republicofguatemala/, journal@ecorfan.org. Editor in Chief: RAMOS-ESCAMILLA, María. ISSN On line: 2414-8849. Responsible for the latest update of this number ECORFAN Computer Unit. ESCAMILLA-BOUCHÁN, Imelda, LUNA-SOTO, Vladimir, Kilometer 16, American Highway, House Terra Alta, House D7 Mixco Zona 1, Republic of Guatemala, last updated December 31, 2016.

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In Number 1st presented an article Antibacterial analysis and characterization of endotrachal probe of polyvinyl chloride with silver nanoparticles *by DOMÍNGUEZ-HERRERA, José Ernesto, LUÍS-MENDEZ, Zeferina, MALDONADO-SAAVEDRA, Octavio and PADILLA-FLORES, Juan Manuel, in the next Section an article* Effect of lead on reproductive physiology: The model study rat *by HANDAL-SILVA, Anabella, MORÁN-PERALES, José L. and GARCÍA-SUÁSTEGUI, Wendy A with adscription in Universidad Autónoma de Puebla, in the next Section an article:* Evaluation of factors associated with the development of metabolic syndrome in the university population of huasteca potosina by *ALVARADO-SÁNCHEZ, Brenda, ZÁRATE-PADRÓN, Alejandra, DEL TORO-HERRERA, Juan and REYES-MUNGUÍA, Abigail with adscription in the Universidad Autónoma de San Luis Potosí*, in the next Section an article Morphological identification of phytopathogenic fungi in the guanajuatense shallow *by MARTÍNEZ-SCOTT, Marcia Maribel with adscription in Instituto Tecnológico Superior de Salvatierra.*

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Content

Article **ECORFAN Journal**
December 2016 Vol.2 No.3 1-5

Antibacterial analysis and characterization of endotrachal probe of polyvinyl chloride with silver nanoparticles

DOMÍNGUEZ-HERRERA, José Ernesto*†, LUÍS-MENDEZ, Zeferina, MALDONADO-SAAVEDRA, Octavio and PADILLA-FLORES, Juan Manuel.

Received July 12, 2016; Accepted October 18, 2016

Abstract

This article was performed deposition of silver nanoparticles in an endotracheal tube of polyvinyl chloride (PVC) and was evaluated it antibacterial activity; synthesis of silver nanoparticles was performed by an electrochemical method, for which silver nitrate ($AgNO_3$) was used as precursor agent, glucose ($C_6H_{12}O_6$) as a reducing agent and sodium hydroxide (NaOH) as a stabilizing agent, to deposit nanoparticles PVC probe in a process of functionalization was performed with two coupling agents, 3-mercaptopropyl trimethoxysilane ($C_6H_{16}O_3SSi$) and 3-aminopropyl triethoxysilane ($C_9H_{23}NO_3Si$) incorporating nanoparticles was by direct immersion, the product was characterized by infrared spectrometry Fourier transform (FTIR) confirming the chemical functionalization on the probe by means of Scanning Electron Microscope (SEM) and Atomic Force microscopy (AFM) the presence of nanoparticles was observed and an average size of 25nm and is determined through testing antibacterial where PVC samples among E.Coli were exposed zones of inhibition were observed material.

Silver Nanoparticles, PVC functionalization, antibacterial

Citation: DOMÍNGUEZ-HERRERA, José Ernesto, LUÍS-MENDEZ, Zeferina, MALDONADO-SAAVEDRA, Octavio and PADILLA-FLORES, Juan Manuel. Antibacterial analysis and characterization of endotrachal probe of polyvinyl chloride with silver nanoparticles. ECORFAN Journal-Republic of Guatemala 2016, 2-3: 1-5

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Introduction

Today, in Mexico, it is estimated that the frequency of nosocomial infections in hospital units varies from 2.1 to 15.8% [1], a situation that becomes more aggravating in the area of intensive care, since this site produces 23% Of all such infections [2], this can be attributed to the fact that the patients in this room require endotracheal intubation for mechanical ventilation and airway insulation in order to avoid air loss and entry of non-invasive materials Safe to the lungs, however, the endotracheal PVC probe becomes a reservoir of microorganisms that adhere to its surface by developing a biofilm, which is highly resistant to the effects of antimicrobials and host defense mechanisms. This fact often makes it difficult to treat and eradicate such drug infections [3], causing a negative impact on hospital care and a significant increase in costs, since budgets for these infections exceed $ 160 million per year [4].

In the last decades the Nanosciences, with the study of the phenomena and the manipulation of materials at nanoscale has allowed that the nanotechnology that is the application of Nanosciences allows adequate control of biological systems [5,6] [5] [6]], Developing new materials, methods and techniques that have allowed an intervention on biological structures with molecular and atomic precision, in order to maintain and establish health [7].

The use of silver nanoparticles (NP's Ag) have been widely studied and used in diverse areas thanks to its wide applications, one of the areas of greatest interest is in the medical, since it has been proven that NP's Ag Have a wide bactericidal spectrum, especially to reduce the bacterial activity of Streptococcus mutans, Escherichia coli and Staphylococcus aureus [8,9].

This is why an endotracheal PVC probe was functionalized for the incorporation of Ag NPs, the modified probe was characterized and its antibacterial activity.

Materials and methods

The Tollens method for the synthesis of NP's Ag described by Yin et al [10] was used to carry out the functionalization. The endotracheal tube was cut in approximately 2 cm pieces, which were split in half and expanded In plate form using an electric grill. In addition, small sections of tubes of about 0.5 cm were cut to which no modification was made. For functionalisation of PVC, the surface of the material was modified by adding functional groups using sodium hydroxide (NaOH) and 3-Aminopropyltriethoxysilane (C9H23NO3S) for allowing the anchoring of silver nanoparticles [11]. The incorporation of the nanoparticles into the functionalized PVC tube was by direct immersion in the nanoparticle solution, PVC was characterized by Fourier Transform Infrared Spectrometry (FTIR) and electron microscopy (SEM), the Ag NPs were characterized by Atomic Force Spectroscopy and Inductively Coupled Plasma Optical Emission Spectrometry. (ICP-OES), incorporation was corroborated by SEM and elemental chemical analysis for the antibacterial tests was used the normed method.

Results

Characterization of NP's Ag

In figure 1, the image is shown by atomic force spectroscopy of the synthesis of NP's Ag, in which one can observe semi-agglomerates of silver particles dispersed at nanoscale with semi-spherical shapes, according to the bell of Distribution, it can be defined that the particle size has an average of 30.55 nm according to the literature review the synthesis process was developed correctly, since the particle size is less than 50 nm [12, 13]

ISSN-On line: 2414-8849

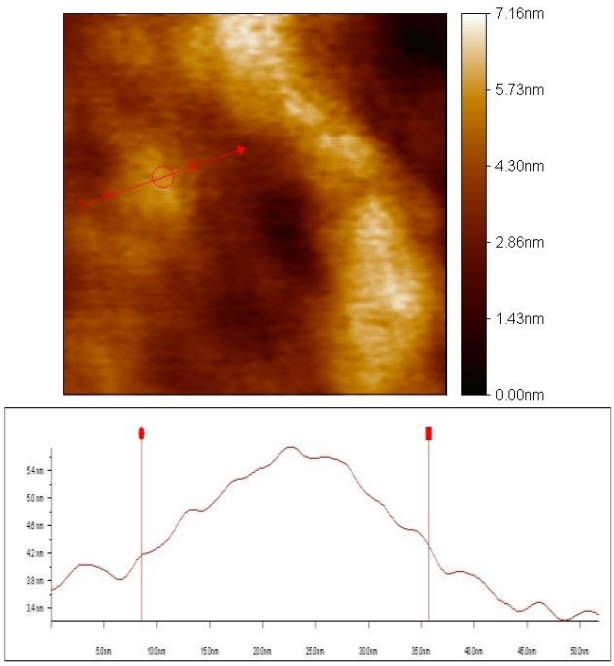

Figure 1 NPs Ag by Atomic Force Spectroscopy

Characterization by ICP-OES

According to the results obtained by ICP-OES, and with a sampling of six solutions, it was obtained that the concentration of silver in the sample is 0.614 mg / L, with a correlation index of 99.94% indicating a high correlation.

Material	Results ICP	Units
NP´s Ag	0.614	Mg/L

Tabla 1 ICP-OES síntesis de NP´s Ag

PVC Functionalization

The characterization of the polyvinyl chloride probe by FTIR allows to appreciate the changes that have been generated by the functionalization, observing that the contact with the solution of Sodium Hydroxide (NaOH) form ester groups (COO-) which will allow the union with Silver ions (Ag +), in addition to the formation of a salt (NaCl).

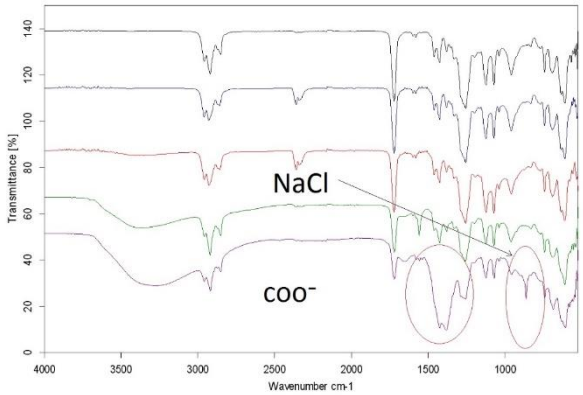

Figure 2 FTIR spectra obtained from PVC probe samples functionalized with NaOH

The SEM characterization visually shows the modification of the polyvinyl chloride probe, and it is verified that the functionalization obtained with 5M Sodium Hydroxide (NaOH) solution has a better distribution on the surface of the material, compared to the hydroxide treatment Of sodium (NaOH) 1N (Figure 3a and 3b). For this reason the anchoring of the silver particles on the surface of the material will be homogeneously dispersed.

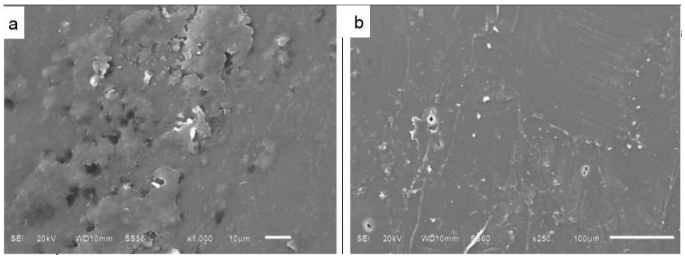

Figure 3 Functionalization by 5M NaOH (a) and 1N NaOH (b)

NP's Ag incorporation

The presence of NPs Ag in the form of flakes on the surface of the PVC can be observed through scanning electron microscopy (SEM), echo corroborated by the elemental chemical analysis (EDS) showing the presence of silver in the analyzed area (Figure 4).

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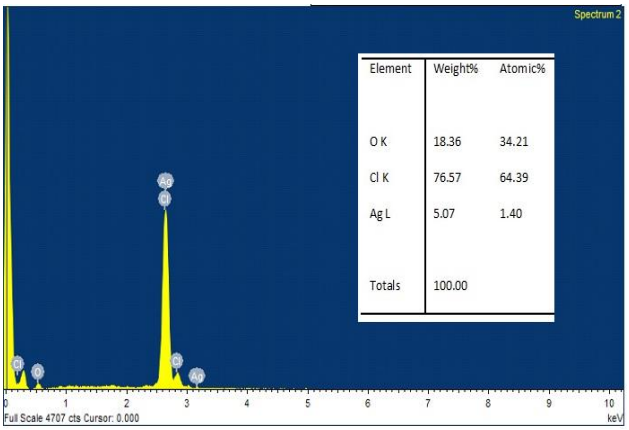

Figure 4 PVC Probe EDS with NPs Ag

The functionalization of PVC by Sodium Hydroxide solution (NaOH) generates esters groups, allowing the silver (Ag +) ions to be anchored, this anchoring is done in greater proportion in the 5M solution of NaOH due to the greater surface change of the material of support.

Antibacterial test

The antibiograms facilitate the determination of a material with better antibacterial property, so in the comparison of inhibition halos generated by the samples presented in table 6, it reflects that the sample 7 and 22 has better antibacterial properties being the solution samples of However, there are also inhibition halos in the 1N solution at a lower radius, these radii can be observed in Figure 5, according to the extended or oval shape of the PVC tubes there is no difference in the adhesion of the Nanoparticles on the PVC support.

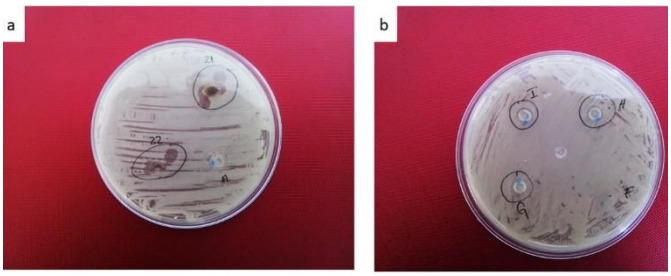

Figure 5 Inhibition Halo 5M NaOH solution (a) and 1N NaOH solution (b)

#	Shape	Radius	#	Shape	Radius
1	Circular	1mm	19	Circular	1mm
2	Circular	1mm	21	Circular	1mm
3	Circular	1mm	22	BiCircular	4 y 3 mm
4	Circular	1mm	23	Circular	1mm
5	Circular	1mm	24	Circular	1mm
6	Circular	1mm	25	Circular	1mm
7	Cyst	4 y 3 mm	26	Circular	1mm
8	Circular	1mm	28	Circular	1mm
12	Circular	1mm	30	Circular	1mm
18	Circular	1mm		Shape	

Table 2 Form and radius of NP inhibition Ag

Conclusions

The synthesis of NP's Ag by the Tollens method is a method that allows to obtain particle sizes between 8 and 36 nm with a concentration of 0.614 mg / L.

The formation of the ester groups (COO-) on the surface of the PVC probe confirms its functionalization, allowing it to function as a support for the nanoparticles.

The direct immersion as a method of incorporation of NP's Ag in the functionalized probe of PVC allows the adhesion of up to 5% in the surface of the support

The tests with the highest inhibition halo correspond to the samples functionalized by 5M NaOH solution with a radius of up to 5mm in its outer part, whereas the tests with 1N NaOH solution have a smaller inhibition halo with a radius of 1mm .

The incorporation of the NP's Ag is not linked to the extended or oval shape of the PVC pipe.

ISSN-On line: 2414-8849

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ISSN-On line: 2414-8849

Article **ECORFAN Journal**
December 2016 Vol.2 No.3 6-18

Effect of lead on reproductive physiology: The model study rat

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Received July 21, 2016; Accepted September 20, 2016

Abstract

AIM: to determine the lead (Pb)-induced reproductive disturbances in CII-ZV rats. MATERIALS AND METHODS: 40 female CII-ZV rats were divided into 4 groups of 10 rats each one, a control group and three treatment groups that received graded doses of lead acetate 0.003, 0.03 and 0.6 g/L via oral route for 30 days. We determined blood lead (Pb) concentrations by anodic stripping voltammetry (ASV), progesterone and 17 estradiol by radioimmunoassay. RESULTS: We found a direct relation between the concentrations of Pb administered and determined in blood. Pb administration induced morphological and physiological alterations in the ovary, changes in development and maturation of follicles as well as on steroid hormone secretion. CONCLUSION: Pb induced reproductive disturbances in CII-ZV rats altering the homeostasis of Hypothalamic-pituitary gonadal axis.

Effect, reproductive physiology, model

Citation: HANDAL-SILVA, Anabella, MORÁN-PERALES, José L. and GARCÍA-SUÁSTEGUI, Wendy A. Effect of lead on reproductive physiology: The model study rat. ECORFAN Journal-Republic of Guatemala 2016, 2-3: 6-18

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† Researcher contributing first author.

Introduction

Lead is an important environmental pollutant present in nature due to natural and anthropogenic sources (ATSDR 2005).

Exposure to lead occurs mainly through feeding, ingestion of water or inhalation and its deposition is evident in several tissues, such as kidney, liver, brain and bones (Bresslery Goldstein, 1991; Russell Moser, 1995; Michael J. Et al., 1999). Studies in both animals (Anwer et al., 1988, Antonio and Leret 2000, Burger J. et al., 2005, Yara M. Müller et al, 2008) and in humans (Al-Saleh 1994; Fraser et al., 2006). It has also been studied the effect of heavy metals on the reproductive system and the toxicity of Cd and Pb in human reproduction (X. Wang et al., 2004; LW Jackson et al., 2008; LW Jackson et al., 2011; Al-Saleh et al., 2008).

It is well documented that lead exerts a wide range of adverse biological effects on the reproductive system (Murakami, K. et al, 1993; Zhigang, D. L. et al, 1997). Lead crosses the placental barrier and accumulates in fetal tissue during the gestational period (Barraclough, C.A, 1982, 1983). The effect of Pb on the female reproductive system causes a reduction in luteinizing hormone (LH) binding and follicle-stimulating hormone (FSH) binding, altered in vitro steroidogenesis in granulosa cells isolated from rats (PN Priya et al. , 2004). Studies conducted by Katalin Paksy et al. 2011, showed that lead levels in ovarian follicular fluid do not represent a danger for the secretion of progesterone in the ovary. There was also a decrease in serum gonadotropin levels (Pillai A. et al, 2003) and serum progesterone (Gupta S. et al, 2002) by simultaneous exposure to Pb and cadmium and it was shown that in vitro exposure of cells From granulosa to Pb and Cd, cause a decrease in the production of gonadotropins and binding steroids. (Priya P. et al., 2004).

Also Laxmipriya P. Nampoothiri and Sarita Gupta (2006) demonstrated that lead and cadmium cause significant reduction in gonadotropin binding, which alters the androgenic enzymatic activity of granulosa cells.

Lead can interfere with steroidogenesis (Wiebe, JP, et al, 1983), and may affect androgen receptors (MacLean, FC et al., 1961) and inhibit Leydig cell testosterone production in vitro (Caffey, J. 1961). Research conducted by Neeta Adhikari et al. (2000) and Derbrand, B.C. et al. (1974) demonstrated that lead induces damage in spermatogenesis, decreases sperm production. The investigations of Sokol R. Z. et al. 1994; Laskey, J.W., and Phelps, P.V. 1991; Masser, 1995; Hoyer, P. et al., 2001) reported that lead in vivo causes suppression of the hypothalamic-pituitary-testicular axis. Studies by Rebeca Z. 1994 and Blazka, M. et al., 1994 showed that the toxic effects of lead on reproductive hormones in the male rat are reversible. The present investigation will allow to obtain approximate information on the health risk of people exposed to lead with doses higher than the determined average of blood lead in the general population, with the objective of applying control measures in environmental health.

Methodology.

Experimental design

The mean blood lead level in the human population of Santiago Xalizintla (Municipality of San Nicolás de los Ranchos in the State of Puebla) was 9 □g / dL value that is within the limits permissible according to NOM-199-SSA1-2000, ENVIRONMENTAL HEALTH. The lead concentration to be evaluated was 9 □g / dL lead in blood, which is approximately 0.03g / L, taking into account the average weight and volume ratio of human and rat.

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The rats were provided by Claude Bernard Bioterio and were treated according to the rules of the Mexican Council on Care and Use of Experimental Animals based on the NOM-062-ZOO 1999 standard and the current CICUAL-BUAP parameters. Female rats of the newborn CII-ZV strain were used with a light / dark cycle controlled 12/12 hrs, with free access to the mother until the age of weaning (21 days), food (Labdiet 5008) and water ad Libitum until the day of the sacrifice. Three experimental groups and a control group of 10 rats were formed each group.

After the day of weaning (26 days) the rats were given lead acetate in the drinking water. The doses to be studied were four higher and one lower than the average dose of blood lead: 0.0 (control), 0.003, 0.03 and 0.6 g / L (three experimental doses) administered during 18 consecutive estrus cycles. On each of the four days of the third estrous cycle (estrus, diestrus I, distro II and proestrus) groups of 2 rats were weighed and randomly sacrificed simultaneously with 2 specimens from the control group. The autopsy was dissected and the ovaries were weighed. From each animal blood was obtained from the trunk in two vacutainer tubes with heparin, and the lead concentration was determined by the "ese" lead analyzer. To the second tube the blood was allowed to coagulate for 30 minutes, centrifuged and the serum removed, which was stored at -20 ° C until the quantification of progesterone, 17 / □-estradiol. On each day of the estral cycle, the control group and each experimental concentration, 2 rats.

Histological analysis

After weighing the ovaries; the histological analysis was performed, the sections were stained with hematoxylin - eosin and analyzed in the Axioplan II Confocal motorized microscope.

Radioimmunoassay

Quantification of steroid hormones.

Quantification of progesterone and 17β-estradiol was performed by the solid-phase radioimmunoassay method, with a Coat-A-Count Kit.

Preparation of the standard curve of progesterone and 17β-estradiolProgesterona

The standard curve was performed in duplicate, using calibrators of 0.1, 0.5, 2.0, 10, 20 and 40 ng / mL.

Estradiol

The standard curve was performed in duplicate, 0.0, 20, 50, 150, 500, 1800 and 3600 pg / mL calibrators were used and dilutions were performed to obtain standards of 0.0, 5.0, 10, 20, 50, 75, 150 and 250 pg / ML.

Statistic analysis

The results obtained were analyzed by the Kruskal-Wallis test followed by the Dunn test and ANOVA followed by Tukey.

Results

Morphometry

The body weight of the rats was similar in all experimental groups relative to the control group (Graph 1).Results related to lead concentration in blood showed a general tendency to increase and was significant in concentration 0.6 g / L. At the concentration 0.03 g / L, there was a decrease in blood lead concentration compared to 0.003 g / L and control (Graph 2). Comparing the weight of the right and left ovaries between the treated and control groups was similar, and decreased in the concentration 0.6 g / L, in relation to the control group (Graph 3).

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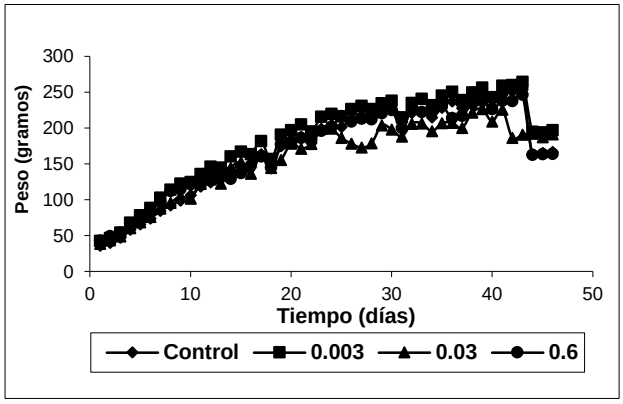
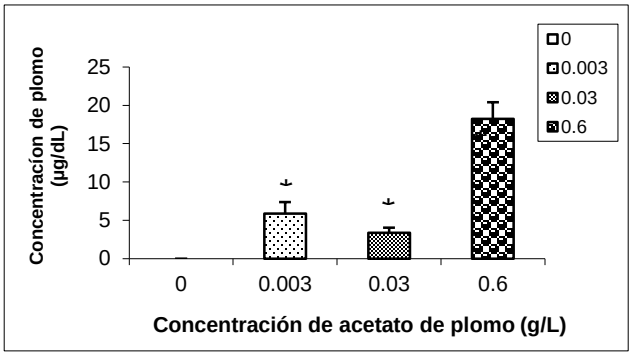
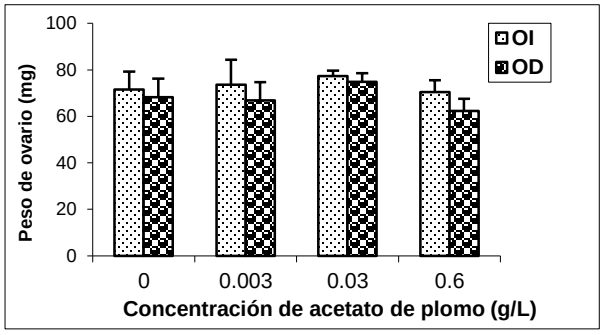
In figure 4 it was observed that as the blood lead concentration increased, the total number of follicles decreased and in the concentration 0.003 g / L significantly decreased.



Graphic 1 Mean ± ha of the ovarian weight of the control group and the groups treated at different concentrations of lead acetate □*P<0.005 vs control (ANOVA followed by Tukey).

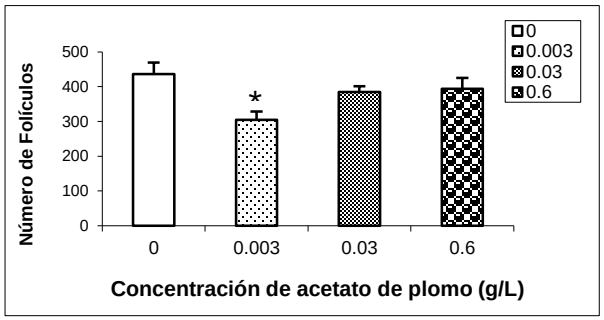


Graphic 2 Mean Â ± SEM of the blood lead concentration of the control group and the groups treated with lead acetate. * P <0.0001 vs control (ANDEVA followed by Tukey).

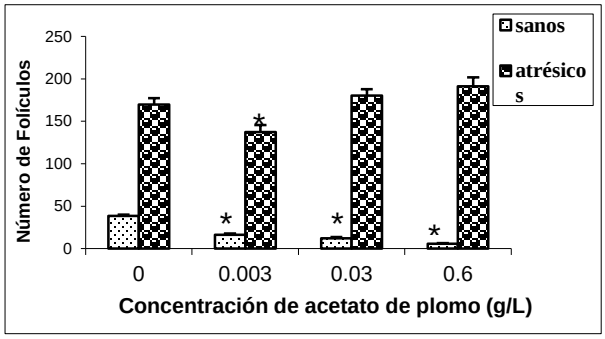


Graphic 3 Media ±e.e.m. Graph 2. Mean ± SEM of ovarian mass (both ovaries) of the control group and groups treated with lead acetate. * P <0.05 vs control, (ANOVA, followed by the TUKEY test).

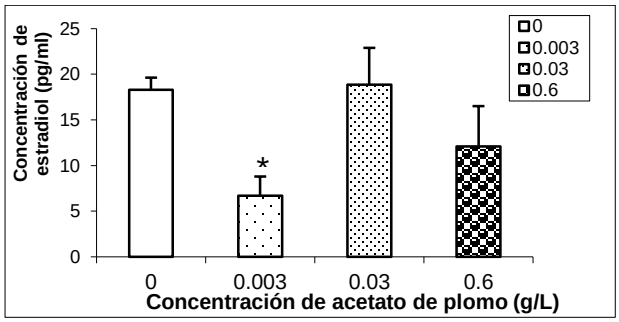
In Figure 5 a significant decrease in the number of healthy follicles was observed compared to the control group. At concentrations of 0.03 and 0.6 g / L the number of atresic follicles is similar to the control group and significantly lower in the 0.003 g / L group.



Graphic 4 Media ±e.e.m. del Graph 2. Mean æem of the total number of follicles in the Control group and the groups treated with lead acetate. * P <0.05 vs control (Kruskall - Wallis followed by the Dunn test).



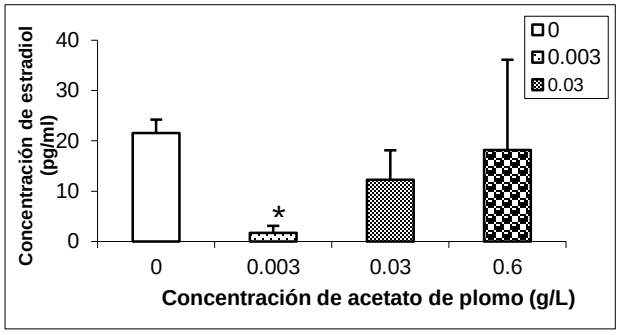
Graphic 5 Media ±e.e.m. del número de folículos Graph 2. Mean ± eem of the total number of healthy and atresic follicles of the control group and those treated with lead acetate. * P <0.0001 vs control (Kruskall - Wallis followed by the Dunn test).



Graphic 6 Media ± eem Graph 2. Mean ± SEM of the total estradiol concentration of the control group and the groups treated with lead acetate. * P <0.05 vs control, (ANOVA, followed by the TUKEY test).

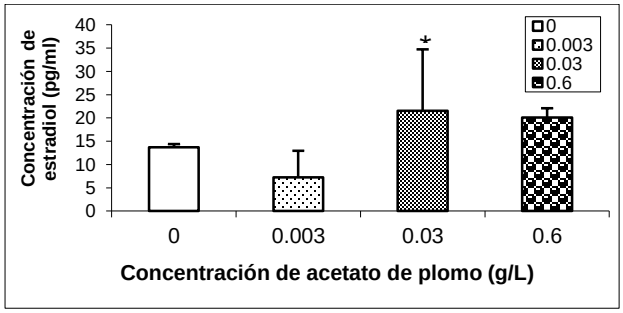
The total estradiol concentration decreased in the concentrations 0.003 and 0.6 g / L and in the concentration 0.03 g / L remained similar to the control (Graph 6).

Plasma levels of oestradiol in proestrus at all concentrations were lower than controls and in the concentration 0.003 g / L the estradiol level was significantly lower (Graph 7).

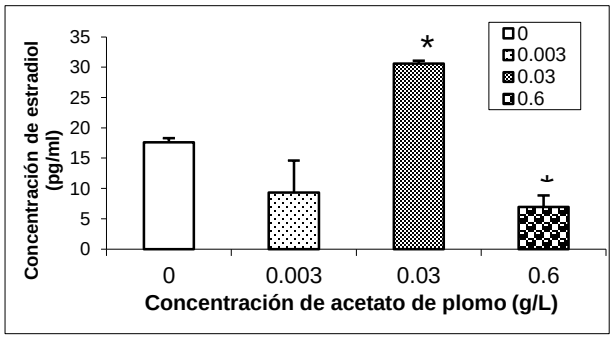


Graphic 7 Media ± eem de la Graph 2. Mean æem of the estradiol concentration in Proest of the control group and the groups treated with lead acetate. * P <0.05 vs control, (ANOVA, followed by the TUKEY test).

Plasma levels of oestradiol estrus increased in relation to control and were significantly lower in the concentration 0.003 g / L (Graph 8).



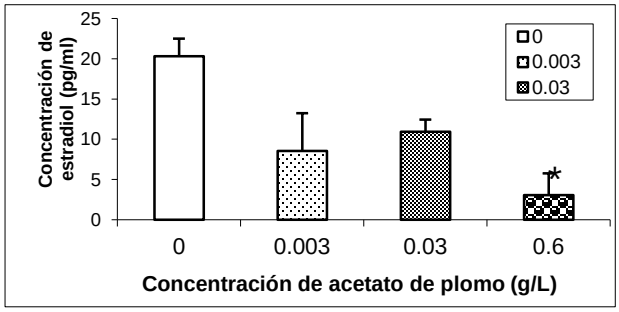
Graphic 8 Mean ± SEM of the estradiol concentration in estrus of the control group and the groups treated with lead acetate. * P <0.05 vs control, (ANOVA, followed by the TUKEY test).



Graphic 9 Mean ± SEM of the estradiol concentration in right-handed 1 of the control group and the groups treated with lead acetate. * P <0.05 vs control, (ANOVA, followed by the TUKEY test).

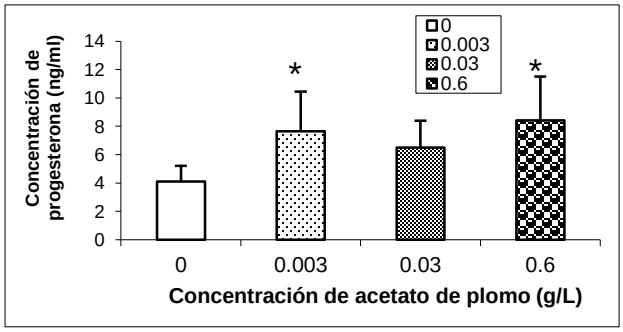
Plasma levels of estradiol in diestrus 1 decreased, being significant in the concentration 0.6 g / L. At the 0.03 g / L concentration the estradiol level increased significantly compared to the control (Graph 9).

Plasma levels of estradiol, at right ventricle 2, decreased and was significant at the concentration 0.6 g / L compared to control and other experimental groups (Graph 10).



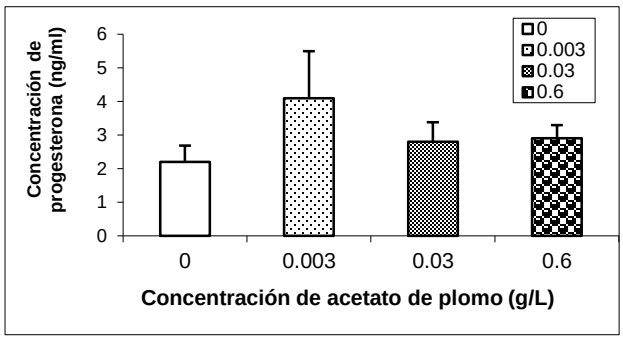
Graphic 10 Mean ± SEM of the estradiol concentration in right-handed 2 of the control group and the groups reated with lead acetate. * P <0.05 vs control, (ANOVA, followed by the TUKEY test).

Plasma progesterone levels increased in the 0.003 and 0.6 g / L concentrations and were significantly higher in the control group (Graph 11).



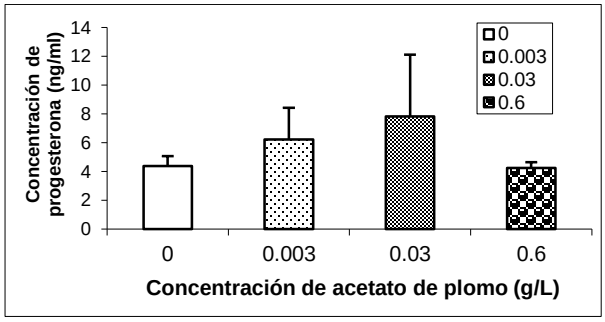
Graphic 11 Mean ± eem of the progesterone concentration of the control group and the groups treated with lead acetate. * P <0.05 vs control, (ANOVA, followed by the TUKEY test).

Plasma levels of progesterone in proestrus increased and were significant in the concentration 0.03g / L in relation to the control (Graph 12).



Graphic 12 Mean ± eem of the progesterone concentration of the control group and the groups treated with lead acetate. * P <0.05 vs control, (ANOVA, followed by the TUKEY test).

Plasma levels of progesterone in estrus increased at concentrations of 0.003 and 0.03 g / L and in the concentration 0.6 g / L was similar to the control group (Graph 13).



Graphic 13 Mean ± SEM of the estrus progesterone concentration in the control group and the groups treated with lead acetate. * P <0.0007 vs control, (ANOVA, followed by the TUKEY test).

Plasma levels of progesterone in diestrus 1 increased by 0.003 and 0.3 g / L relative to control and decreased significantly in the concentration 0.6 g / L (Graph 14).

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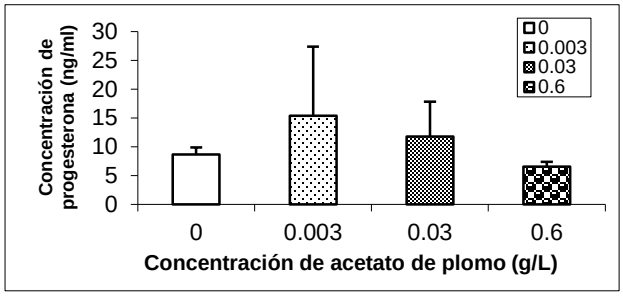
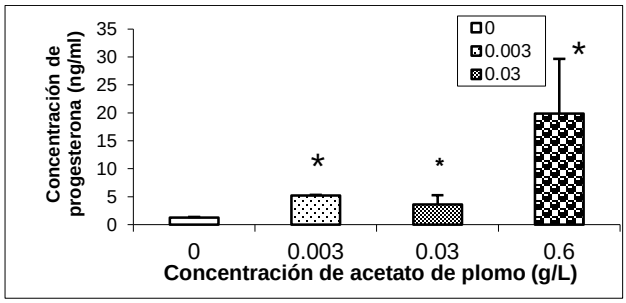


Graphic 14 Mean ± ha of the progesterone concentration in right-handed 1 of the control group and the groups treated with lead acetate. * P <0.0007 vs control, (ANOVA, followed by the TUKEY test).



Graphic 15 Mean ± SEM of the progesterone concentration in right-handed 2 of the control group and the groups treated with lead acetate. * P <0.0007 vs control, (ANOVA, followed by the TUKEY test).

Plasma levels of progesterone in diestrus were significantly increased in all treated groups compared to the control group and were very high at 0.6 g / L (Graph 15).

Pathology

Similar morphological alterations were observed in the development of proliferative and cytological phenomena and were more aggressive and invasive in the 0.6 g / L concentration of lead acetate.

Figure 1 shows granulosa cells that do not have a nucleus, these cells are also located in the antrum in the follicular fluid.

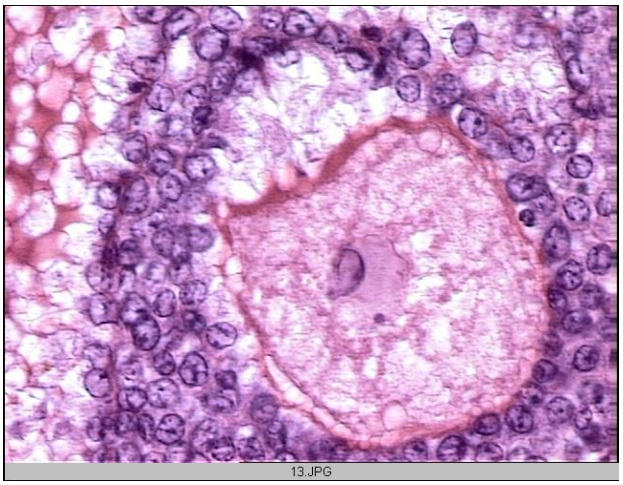


Figure 1 Transverse section of ovary. The oocyte presents nucleus and nucleolus and is atresic, stained with Hematoxillin-eosin 40x.

The follicle oocyte is atretic is surrounded by anucleated cells, has lost its shape and there are some pycnotic cells in the crown, rupture of the pellucid membrane is observed and the follicle begins to luteinize.

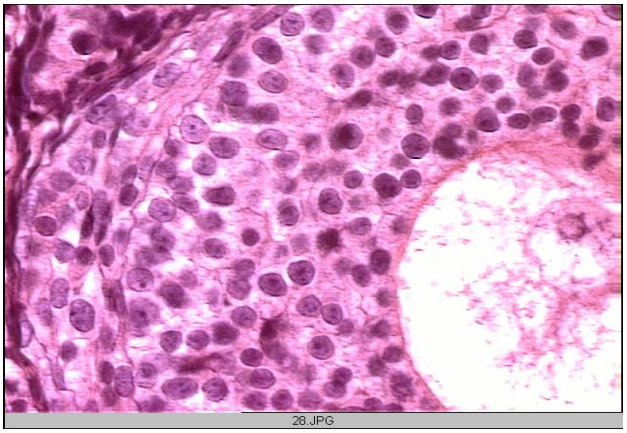


Figure 2 Transverse section of ovary. Primary follicle with atresic oocyte and internal teak thickening. Tinted with Hematoxillin-eosin 40x.

Figure 2 shows an atresic follicle, presenting an oocyte with its nucleus, nucleolus and zona pelucida; there are presence of surrounding anucleated cells, granulosa cells with picnosis, desquamation and large intercellular spaces.

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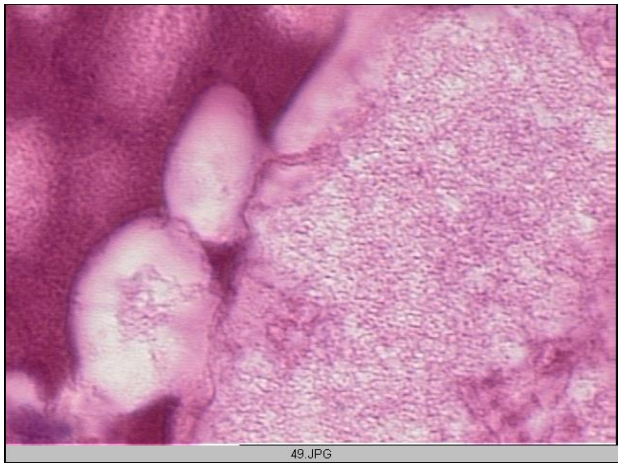

Figure 3 Transverse section of ovary. Infiltration by anucleated cells into the oocyte. Tissue with Hematoxillin-eosin 100x.

Figure 3 shows how channels form between the pelucidal zone and the oocyte membrane through which the contents of the anucleated cells, formed in the radiated corona, can be directed into the oocyte and the degradation of the pellucida and oocyte membrane.

Discussion

Lead acetate has been shown to reduce the weight of some organs of the reproductive system depending on the dose, duration and age of the animal (Sokol, R. Z et al, 1991; Nahan, E et al., 1992, Correa et al., 2004, Yara M. R et al, 2008). Based on the results obtained on body weight and ovarian mass in most of the experimental groups, it was observed that lead does not affect the body weight of the rat, however, in the concentration 0.6g / L the weight of the ovary decreased (Figure 1).

Research by Palminger et al. (1991) showed that most lead in blood is fixed in erythrocytes (Lorentzo AV et al, 1977) and that blood lead levels remain constant despite continued exposure to Lead, in this situation, the body has to maintain the homeostatic balance by accumulating surplus lead in bone and other tissues (Fei Yu et al., 2008).

This criterion coincides with the results, which showed that the administration of 0.6 g / L lead acetate reflected a concentration of 19 □g / dl lead in blood (see graph 2), suggesting that there is a limit of Transport of lead in blood and that circulating lead surplus is redistributed and accumulated in bone and other organs (Fei Yu et al., 2008); In this case we can suggest that one of the affected organs could be the ovary due to the morphological alterations observed during the follicular development. This criterion agrees with the results obtained by Flower et al. 1994; Khan-Dawood et al .; Wilson C.A. Et al. 1992, Fei Yu et al., 2008, A. Pollack et al, 2011; C.M. Gallagher et al, 2010; E.F. Krieg Jr., H.A. Feng 2011; K. Paksy et al, 1997; L.W. Jackson et al, 2011.

It is known that granulosa and teak cells are sensitive or vulnerable to heavy metals, by research conducted by Krinitz et al (1978); Petrusz et al (1979); Vermande-Van Eck et al. (1960), showed that lead salts cause follicular atresia, inhibit follicular development, ovulation does not occur and puberty is delayed. This suggests that lead crosses the granulosa layer and interferes with the process of steroidogenesis (P.N. Priya, A. Pillai, S. Gupta, 2004). This information coincides with the results obtained in the concentration of 0.6g / L lead acetate, a reduction in the number of secondary and tertiary follicles was observed and follicular atresia increased (Graph 5). These results coincide with the work of (Petrusz, P et al, 1979; Maxim Khotimchenko et al 2006). We can infer that as the concentration of lead in blood increases, the number of healthy follicles decreases and the atresics increase (Graphs 4 and 5).

There is evidence that lead exerts its toxic effects on the hypothalamic-pituitary-gonad axis produced by inhibition in the synthesis and release of gonadotropins. In this sense the works carried out by Ronis and col (1998).

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They showed that in prepubertal females exposed to lead they had delayed vaginal opening and the estrous cycle was interrupted. By in vitro studies, lead blocks the secretion of GnRH in the middle eminence, an event associated with low PGE2 secretion. We also found low levels of IGF-1 in the hypothalamus required to activate the GnRH / LH release systems (P.S. Christensen et al, 2016). Therefore it can not be ruled out that lead exposure disrupts the circulatory development of GnRH within the hypothalamus and the mean eminence.

The results showed that, by administering minimal concentrations of lead acetate, estradiol concentrations decreased and progesterone increased proportionally as the administered lead acetate concentration increased (L. W. Jackson et al., 2011) (see graphs 6-15).

These results demonstrate that exposure to lead is associated with increased DNA and RNA and protein synthesis; so we can infer that there is a relationship between the concentration of lead in the nucleus and the subsequent alterations in cell division.

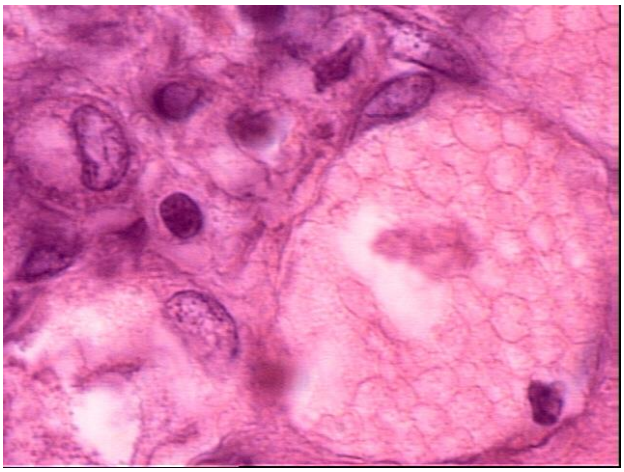

Figure 4 Transverse section of ovary. Formation of irregular bodies in the corpus luteum. Dyed with Hematoxillin-eosin 100x.

A characteristic sign of lead poisoning is intracellular inclusion bodies. These were located in granulosa cells in the process of desquamation and in the corpus luteum, these results coincide with those of Terry D. Oberley et al. (1995). (Figure 4).

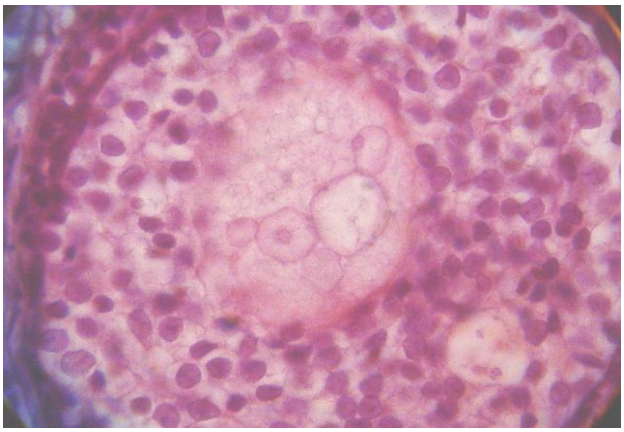

Figure 5 Transverse section of ovary. Presence in some follicles of multiple and multinucleated oocytes. Tissue with Hematoxillin-eosin 100x.

Another characteristic alteration was the presence of multiple oocytes within an ovarian follicle, some oocytes presented several nuclei (Figure 5).

The antecedents and results obtained can be inferred that lead delays the vaginal opening, alters the estrous cycle, and interferes with the secretion of FSH, LH, in the production of progesterone and estradiol in the follicle, producing an increase in the atresia of healthy follicles.

These results support the hypothesis that as the concentration of lead acetate in the bloodstream increased, the greater the morphological alteration in the ovary and the physiology in controlling the secretion of ovarian hormones, growth and Gonadal maturation (A. Pollack et al, 2011, CM Gallagher et al, 2010, EF Krieg Jr., HA Feng 2011, K. Paksy et al, 1997; LW Jackson et al, 2011).

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It is concluded that the administration of lead acetate alters the normal functioning of the hypothalamic-pituitary-gonadal axis and directly influences the ovary physiology. There is a direct relationship between the concentration of lead administered and that determined in blood. Alterations in the secretion of ovarian hormones caused morphological alterations and changes in follicular development and maturation characteristic of the concentration 0.6g / L.

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ISSN-On line: 2414-8849

Article **ECORFAN Journal**
December 2016 Vol.2 No.3 19-26

Evaluation of factors associated with the development of metabolic syndrome in the university population of huasteca potosina

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Received July 15, 2016; Accepted November 16, 2016

Abstract

Metabolic syndrome (MS) has been classified as one of the most common causes of risk for a heart attack, taking factors such as diabetes (Diab), prediabetes (Pre-D), dyslipidemia and hypertension. The objective of this study was to evaluate the presence of factors associated with the development of MS in a population of young adults. For this, various parameters related to the development of MS in young adults of university to Huasteca Potosina were evaluated, using clinical measures: blood pressure (BP), anthropometric weight, height and waist circumference (CCIN) and biochemical determinations: glucose (Glu), cholesterol (Chol) and triglycerides (TGL). The results show that the prevalence of hyperglycemia (Glu> 105 mg / dL) for the study population was 20.8%, the prevalence of hypercholesterolemia (> 200 mg / dL) and hypertriglyceridemia (> 150 mg / dL) is 5.0% and 13.8% respectively. These results reveal that the study population is in a vulnerable state, which can lead to suffer chronic degenerative diseases like diabetes mellitus type 2 or cardio-vascular diseases and therefore SM.

Diabetes, hyperglycemia, obesity, dyslipidemia, overweight

Citation: ALVARADO-SÁNCHEZ, Brenda, ZÁRATE-PADRÓN, Alejandra, DEL TORO-HERRERA, Juan and REYES-MUNGUÍA, Abigail. Evaluation of factors associated with the development of metabolic syndrome in the university population of huasteca potosina. ECORFAN Journal-Republic of Guatemala 2016, 2-3: 19-26

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Introduction

Metabolic syndrome is classified by the International Diabetes Federation (DFI) as one of the most frequent causes of risk of heart attack, including diabetes (Diab), prediabetes (Pre-D), dyslipidemia and hypertension (IFD, 2005). Globally, it is estimated that 20-25% of the adult population suffers from this syndrome, which makes it twice as likely to die and three times as likely to suffer from cardiovascular accidents, compared with people who do not suffer from it (Zimmet et. Al., 2005).

In Mexico, by 2012 it was estimated that 45% of the population had this syndrome (Salas, et al, 2014); At present, these figures coupled with reports on the prevalence of obesity and overweight have increased up to 50% in the last ten years; About 31% of women of reproductive age are overweight and 22% are obese (Monrreal et al, 2010).

Obesity as a risk factor is associated with increased blood lipid levels and their fractions, such as HDL and LDL cholesterol or triglycerides; High concentrations of these in the blood are associated with a high risk of atherosclerosis, coronary morbidity and some other cardiovascular diseases, especially in the middle-aged population, dyslipidemias, on the other hand, has a different prognostic effect, according to age: The younger the person, the greater the negative impact on life expectancy (Monrreal et al, 2010).

Cardiovascular diseases currently account for 30% of all deaths in the world and reduce 10% of healthy life years, affects about 13 million American citizens and is the most important cause of death in Latin America (Munguia et al.

In Mexico, studies have been carried out in which some of the components of MS in adults are analyzed.

However, there are few who value the overall. The scarce data available do not allow us to relate the magnitude of the problem, since the cut-off criteria of the different studies do not agree with each other.

Therefore, it was considered important to study the prevalence of factors that have previously been associated with the development of MS in a young adult population. All the information collected and processed can be useful to demonstrate the effects of overweight and obesity that currently affect the young Mexican adult population.

Methodology

Study population

In order to carry out this study, 400 new students from the Autonomous University of San Luis Potosí - Multidisciplinary Academic Unit Huasteca Area in Ciudad Valles San Luis Potosí, belonging to the school year 2015-2016, from rural areas (20.25 %) And urban (79.75%), of which 175 (43.75%) are men and 225 (56.25%) are women.

Clinical measures

Blood pressure

Blood pressure is defined as the force exerted by the blood on the arterial walls, which can be expressed as systolic pressure, diastolic pressure or mean pressure (Conyer et al., 2002). To obtain the BP measurement, the protocol suggested by NOM-030-SSA2-2009 was used, for which the patient was asked to sit for five minutes in a seat that supported the back. Discover the left arm and place it flexed at the level of the heart.

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The bracelet was placed 2 cm above the fold of the elbow, with the help of the stethoscope the humeral artery was located and it was left there, the handle of the baumanómetro was filled until the pulse disappeared and the air was slowly released by means of the Valve until hearing the loud and clear beat, there took the first reading of PA, then continued to release the air until the beat was stopped listening and at the point where this happened took the second reading. PA values were classified according to Table 1.

Blood Pressure	Reference Value
Great	< 120/80 mm Hg
Normal	120-129/80-84 mm Hg
High normal	130-139/85-89 mm Hg

Table 1 Type of blood pressure according to the reference value. (Source: Undersecretariat for Prevention and Protection of Health, 2002.

Anthropometric measurements

Weight

Weight is an anthropometric measure determined by body mass, which is expressed in kilograms. This determination was carried out by using a scale in which the protocol was suggested by the SSA, where the participant was asked to remove the shoes and stand on the scale with the feet in parallel And without moving, the reading was taken once the weight indicating hand was kept fixed (Undersecretariat for Prevention and Protection of Health, 2002).

Size. Size is a measure used to determine the height of an individual, this result is expressed in meters. In order to take this measure, the patient was asked to remove his shoes, caps and in the case of the girls, the hair was loose, the position was erected, once the patient had placed the correct position, the height was measured Undersecretariat of Prevention and Protection of Health, 2002).

Determination of obesity

The determination of obesity was performed by two methods, the first by calculation of Body Mass Index (BMI) and the second by waist circumference (CCin).

Body Mass Index. BMI is defined by the World Health Organization as an indicator of the relationship between weight and height of an individual. It is used in the identification of overweight or obesity and is used more frequently in adults than in infants. This was calculated by equation 1:

$$IMC = \frac{weight\ (kg)}{\text{height}^2\ (m)} \quad (1)$$

For the interpretation of the result obtained from the calculation, the classification proposed by the WHO was used (Table 2).

Classification	***Value***
Malnutrition	**<18.50**
Severe thinness	<16.00
Moderate thinness	16.00 – 16.99
Light thinness	17.00- 18.49
Normal	**18.5 – 24.99**
Overweight	**≥25.00**
Pre-obese	25.00 – 29.99
Obesity	**≥30.00**
Mild obesity	30.00 -34.99
Average Obesity	35.00 – 39.99
Morbid obesity	≥40.00

Table 2 Classification of obesity according to WHO criteria (Source: WHO, 2015)

For the population of low stature (men <1.60 m and women <1.50 m), the cut-off point is between> 23.00 and> 25.00 for overweight and obesity respectively (NOM-008-SSA3-2010).

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Waist Circumference (CCin)

CCin is an anthropometric measure in which the abdominal diameter is determined with the help of a tape measure, based on the protocol marked in NOM-043-SSA2-2012, which indicates that to carry out this measurement The lower point of the last rib and the upper point of the iliac crest must be located correctly, the measuring tape is placed so that it does not tighten or tighten the abdomen of the person and the data obtained at the end of the expiration is taken Of the patient. If the patient is overweight already diagnosed, this measurement should be performed on the widest part of the abdomen. This measure is useful in the diagnosis of central or visceral obesity, which is significantly related to the presence of elevated serum cholesterol and triglycerides (McCarthy et al, 2003).

Gender	Reference value
Male	≤ 90 cm
Female	≤ 80 cm

Table 3 Reference for CCin measurement. (Source: NOM-043-SSA2-2012).

Biochemical determinations

For the accomplishment of the biochemical determinations, a sample of peripheral blood was collected by means of venipuncture, in a tube without anticoagulant with particles of silicone (BD Vacutainer®). The blood sample was collected in the morning, with a fasting not greater than 12 hours, as stipulated in NOM-037-SSA2-2012. Serum was then obtained by centrifugation of the sample at 3500 revolutions per minute (rpm) for 15 minutes in a centrifuge (Thermotec®).

Once the serum was separated from the globular package, the sample was processed in an automated equipment (MINDRAY® BS-120) for the quantification of Glucose (Glu), Cholesterol (Col) and Triglycerides (Tgl) using the spectrophotometry method, with reagents SpinReact® brand, taking as normal values of Glu up to 100 mg / dL, as pre-diabetes values> 100 and <125 mg / dL and as diabetes> 126 mg / dL (NOM-015-SSA2-1994). Col values were <200 mg / dL and for Tgl <150 mg / dL (NOM-037-SSA2-2002).

Statistic analysis

Data analysis included descriptive statistics with means quantification and standard deviation for continuous variables. For the analysis of statistical significance, the statistical program GraphPad Prism V 7 was used; For data from a normal distribution, ANOVA and Pearson correlation analysis were used, whereas for data not coming from a normal distribution, a Kruskal-Wallis test and a Pearson correlation analysis were performed, taking One $p \leq 0.05$ as statistically significant.

Results

Table 4 shows the characterization of the population according to each of the determinations that were performed to the patients, it can be observed that the average values presented in each one of the determinations, are within the range of reference used By NOM 015, 037 and 030.

As far as lipid concentrations are concerned, the findings of this study are in agreement with Barquera et. In 2007 (144.6 + 35.4 mg / dL) as regards cholesterol concentration, they also indicate that the prevalence of hypercholesterolemia was more frequent in men than in women (186.6 vs 181.1 mg / dL) respectively, which coincides in the same way with the result obtained.

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For the case of triglycerides, the values presented greater difference between those reported by the same author vs those obtained in the present study; however, they show the same trend of higher values for men than women.

Determination	Total n=400 (100%)	Male n=175 (43.75%)	Female n=225 (56.25%)
Biochemistry			
Glu (mg/dL)	94.35 ±7.22	95.98 ±6.57	93.09 ±7.47
Col (mg/dL)	152.12 ±27.55	153.92 ± 26.69	150.72 ±28.18
Tgl (mg/dL)	101.26 ±63.93	108.97 ±75.07	95.26 ±53.11
Clinical Measure			
P.A. (mm Hg)	109.89/71.22 ±12.08	116.70/74.42 ±12.0	104.75/68.74 ±10.60
Obesity Index			
CCin	78.86 ±12.20	85.08 ±12.20	74.03 ±9.66
IMC	24.10 ±4.95	25.46 ±5.18	23.04 ±4.51

Average values + SD

Table 4 Characterization of the population.

Table 5 shows the characterization of the population according to indicators of obesity, which were BMI and PC, also having as a variable the locality of origin (urban or rural), it can be observed that patients from urban areas present (31.0%) and OP (15.4%), which means that approximately 2 out of 10 young adults suffer from this type of disorder, in terms of values considered normal, it is observed that the population From rural areas (58.0%) have a higher percentage of normal BMI than those from urban areas (53.6%), this could be attributed to a healthier lifestyle.

In the report given by ENSANUT in 2006, it is mentioned that 39.3% of the population analyzed by locality shows rural areas, while 39.6% have urban areas; An increase of approximately 0.3%, which is in line with what was reported in this research, 22.2% and 22.6%, respectively.

As for IMC nor, ENSANUT in the same year reports that in the rural localities the percentage is 34.1% while in the urban localities, the percentage found is 27.7%, whereas in the present study we found values of BMI Nor Of 53.6% and 58% in urban and rural populations, respectively. For the case of PO, ENSANUT reports that for rural areas, 24.8% of the study population presents this condition, whereas in urban communities, 31.3% suffer from it. When comparing the results obtained in the present research with the figures obtained with those reported in ENSANUT, it is observed that the percentage of OP in both localities (R = 13.6% vs U = 15.4%) which may differ by the n used in each One of the studies.

Obesity index		U n=319 (79.8%)	R n=81 (20.3%)	T n=400 (100%)
IMC	Dn	n=27 (8.4%)	n=5 (6.2%)	n=32 (8.0%)
	Nor	n=171 (53.6%)	n=47 (58.0%)	n=218 (54.5%)
	Sp	n=72 (22.6)	n=18 (22.2%)	n=90 (22.5%)
	OP	n=49 (15.4%)	n=11 (13.6%)	n=60 (15.0%)
PC	OC	n=99 (31.0%)	n=20 (24.7%)	n=119 (29.8%)
	Nor	n=220 (69.0%)	n=61 (75.3%)	n=281 (70.3%)

U: urban. A: rural. T: total

Table 5 Characterization of the population by indicators of obesity.

Table 6 shows the prevalence of hyperglycemia and dyslipidemia, according to different criteria: Health Secretariat NOM-015-SSA2-1994, American Diabetes Association (ADA) and World Health Organization (WHO). Section A) of Table 6 shows the comparison between the reference values used by the different organizations. According to the limits marked by NOM-015-SSA2-1994, the 20.8% population of the study presented has Pre-D status, compared to that reported by Monreal et. In 2009, the prevalence of Pre-D was 4.2% for the population of aspirants to the same house of studies, which is indicative of a change in the lifestyle of the families with the passage of the years, provoking an increase in the percentage of young people with elevated glucose levels.

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When comparing each of the criteria used for the determination of hyperglycemia in patients, it is observed that the use of the Pre-D marking by the NOM covers the largest number of people with the possibility of developing diabetes, The diagnosis of Diab is performed until the patient shows values higher than 126 mg / dL, so that the entire population, at least of this study would be without preventive treatment for the development of this disease, with the subsequent effects that this entails . As for the values given by the AAD, the percentage of patients with hyperglycemia is 5.5%, using this criterion the early detection of the disease is achieved in only 22 patients of the 83 who are identified by the Pre-D criterion using the NO M. In the classification criteria given by the WHO, only 1.5% of the population studied presented hyperglycemia, a figure well below that observed when using the NOM. Unfortunately the percentage of subjects diagnosed with diabetes using the NOM criterion drops to 0% for this population. Therefore a recommendation would be to sensitize the population (doctors and patients) for the use of AAD values as a cutoff point for diagnosis or to give sufficient relevance for the proper management of the Pre-D patient.

In section B), the comparison between the cutoff points marked by the NOM and by the WHO is shown, in this case the values of both coincide, which shows that 5% of the population presents a hypercholesterolemia problem, being More frequent in women than in men, contrary to what Barquera et al. Al, 2007, this being possible because of the size of the population that was studied in both cases.

Section C) refers to the prevalence of hypertriglyceridemia based on the criteria given by NOM-037-SSA2-2012 and WHO in 2015, when evaluating both criteria, it is observed that the NOM with the cut-off points that are used to make this diagnosis, 13.8% of the study population presented with this condition.

While using the reference value indicated by the WHO is only diagnosed at 6.5%, corresponding to the half of the population identified by NOM.

A)	NOM Pre-D./Diab (≥100 mg/dL)	AAD (>105 mg/dL)	OMS (>110 mg/dL)
T n=400 (100.0%)	n=83 (20.8%)	n=22 (5.5%)	n=6 (1.5%)
H n=175 (43.75%)	n=44 (25.1%)	n=13 (7.4%)	n=2 (1.1%)
M n=225 (79.75%)	n=39 (17.3%)	n=9 (4.0%)	n=4 (1.8%)
B)	**NOM** (≥ 200 mg/dL)		**OMS** (≥ 200 mg/dL)
T n=400 (100.0%)	n=20 (5.0%)		n=20 (5.0%)
H n=175 (43.75%)	n=9 (5.1%)		n=9 (5.1%)
M n=225 (79.75%)	n=11 (4.9%)		n=11 (4.9%)
C)	**NOM** (≥ 150 mg/dL)		**OMS** (≥ 200 mg/dL)
T n=400 (100.0%)	n=65 (13.8%)		n=26 (6.5%)
H n=175 (43.75%)	n=35 (20.0%)		n=14 (8.0%)
M n=225 (79.75%)	n=30 (13.3%)		n=12 (5.3%)

TO). Glucose. B) Cholesterol. C) Triglycerides.
T: Total. H: Man. M: Woman.
Table 6 Prevalence of hyperglycemia and dyslipidemias by different classification criteria.

Table 7 shows the prevalence of hyperglycemia (reference value given by AAD) and dyslipidemias each classified by the obesity indicator. In the case of IMC Nor, the prevalence of hyperglycemia was 27.3%, hypercholesterolemia 35.0% and hypertriglyceridemia 39.7%, SP 31.8%, 30.0% and 28.6%, respectively; For Ob 36.4%, 35.0% and 31.7%, respectively. The data obtained for PC showed that for OC the prevalence of hyperglycemia was 63.6%, hypercholesterolemia 60% and hypertriglyceridemia was 71.4%, all these percentages based on the population that presented some biochemical alteration.

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Comparing the prevalence of biochemical disorders between BMI Ob and PC Oc, it is observed that the population classified as Oc using PC seems to be a better indicator of alterations at the biochemical level. Therefore it is recommended its use as an early indicator for the diagnosis timely treatment of metabolic disorders.

Obesity index		Glu >105 mg/dL n= 22 (5.5%)	Col ≥200 mg/dL n= 20 (5.0%)	Tgl ≥150 mg/dL n=63 (15.8%)
IMC	Dn	n=1 (4.5%)	n=0 (0.0%)	n=2 (3.2%)
	Nor	n=6 (27.3%)	n=7 (35.0%)	n=25 (39.7%)
	Sp	n=7 (31.8%)	n=6 (30.0%)	n=18 (28.6%)
	Ob	n=8 (36.4%)	n=7 (35.0%)	n=20 (31.7%)
PC	Oc	n=14 (63.6%)	n=12 (60.0%)	n=45 (71.4%)
	Nor	n=8 (36.4%)	n=8 (40.0%)	n=18 (28.6%)

Table 7 Prevalence of hyperglycemia and dyslipidemias classified by BMI and PC.

A Pearson correlation analysis was performed for the BMI and PC data, regarding the serum Glu, Col and Tgl values for each study subject. The results showed a directly proportional correlation between Glu and PC ($r = 0.1620$, $p = 0.0012$), and between Glu and BMI ($r = 0.1024$, $p = 0.0406$). For Col, a positive correlation was found with CP ($r = 0.2214$, $p = 0.0001$), and with BMI ($r = 0.2435$, $p < 0.0001$). As for Tgl, a directly proportional correlation between Tgl and PC concentrations ($r = 0.3561$, $p < 0.0001$) and for Tgl and BMI were also found ($r = 0.3569$, $p < 0.0001$). These results show that for this study population, the anthropometric measurements of CP and BMI could serve as external indicators of the levels of Glu, Col and Tgl, which can be found in serum. These results and those shown in Table 7, confirm the use of PC with a better indicator of metabolic disorders on the use of BMI.

Aknowledgement

A thank you to the Bq. Marlen Rodríguez Espino and the Mtro. Juan Del Toro Herrera staff of the Biomedical Research Laboratory for the technical support provided to carry out this work.

Conclusions

It is possible to observe the presence of risk factors for the development of metabolic syndrome in the study population. Since the metabolic syndrome is a multifactorial disease, the change in lifestyle toward the sedentary lifestyle, poor eating habits and stress typical of today's university life would favor the increase of risk, becoming increasingly evident problems of dyslipidemia, hypertension, intolerance to Glucose, obesity, etc. Among the young adult population. Therefore, the intentional search for chronic degenerative diseases in this population is evident for the diagnosis and timely treatment. This research aims to raise awareness in the authorities in charge to create programs that contribute to improve the health and quality of life of students. Therefore it is hoped to implement and consolidate health, culture and sport programs as part of the integral formation of the student and shape the lifestyle towards good health habits.

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ISSN-On line: 2414-8849

Article **ECORFAN Journal**
December 2016 Vol.2 No.3 27-32

Morphological identification of phytopathogenic fungi in the guanajuatense shallow

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Received July 18, 2016; Accepted October 27, 2016

Abstract

This research was conducted in the guanajuatense shallows during 2014-2016 and consisted of plant pathogenic fungi isolate and analyze samples from 254 crops (tomatoes, peppers, onion, cucumber, broccoli, lettuce, carrots, strawberries, raspberries, spinach, kale and horseradish) and of germinating trays and irrigation water. The identification of microorganisms is performed through the morphological characteristics of the colonies, as well as reproductive structures of pathogens, which depending on their nutritional requirements were placed in various culture media for identification, thereby were determined the causal agents of most common diseases that occur in the guanajuatense shallows. One of the contributions sought for this study was encouraging producers to implement preventive and control strategies to reduce the damage caused by these pathogens. 107 samples were analyzed from cultures (plants), 63 soil, seed 75, six of germinating trays and three irrigation water. A total of 275 pathogens were obtained in 130 samples grouped into 10 genera and 14 species. Strawberry three main pathogens were *Rhizoctonia solani*, *Verticillium albo-atrum* and *Fusarium oxysporum* were presented. Tomato was identified *Alternaria solani* and *Phytophthora* sp. According to the results, the fungus *Rhizoctonia solani* was the pathogen most frequently found in both plants and seed and soil samples. In the germinating trays it was primarily identified *Fusarium oxysporum* and irrigation water to *Pythium* sp and *Trichoderma* sp. The rest of the samples tested negative for fungi.

Fungi, phytosanitary diagnosis, vegetables

Citation: MARTÍNEZ-SCOTT, Marcia Maribel. Morphological identification of phytopathogenic fungi in the guanajuatense shallow. ECORFAN Journal-Republic of Guatemala 2016, 2-3: 27-32

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Introduction

The importance of fungi in nature can hardly be overestimated, essentially because more than 100,000 species are known, most of which are saprophytes; However, about 50 species produce diseases to man and approximately 8,000 directly affect plants, having the capacity to attack and infect wild and cultivated species, causing damages that can be slight until the crops are lost in their totality (However, However, damage caused by phytopathogenic fungi is not only economical, but also disrupts ecosystems, limiting cultivation areas rendering them unusable by the formation of survival structures such as sclerotia and chlamydospores that ensure their permanence in the soil for long periods of time. Time and by their ability to produce pectolytic enzymes that degrade the cells' mean lamella (Harman and Shores, 2007). Infected plants suffer a series of alterations, which can affect the morphology or external appearance of the plant, with the appearance of spots, chlorosis, destruction of tissues, or organs or of the whole plant, decay, chancres, gills and descending death Or they may be internal, such as histological alterations, located in cells and tissues and physiological alterations that cause an increase in perspiration, immobilize nutrients and reduce the photosynthetic rate (Benítez, 2012).

In some cases, it is relatively easy to identify the causal agent of a fungal disease when the symptoms are characteristic and unique to it, reducing the possibility of error (Monroy, 2013). However, for diseases with similar symptoms, the identification can be complex; Thus, phytosanitary diagnoses provide alternatives for the timely identification of phytopathogens, avoiding production losses and economic losses (Sampietro et al., 2010).

The identification of an infectious agent through phytosanitary diagnoses is an irreplaceable tool for the elaboration of integrated disease management programs, resulting in a practical and simple action that allows to build the knowledge of the producers as a fundamental base in the search for alternatives of Solution to their phytopathological problems (Moreno et al., 2008). It is for this reason that this research consisted in analyzing samples from different cultures to determine the causal agents of the main fungus diseases that are presented in the Guanajuato shallow area, seeking to determine some strategies of timely prevention and control that the producers can implement before establishing their crops.

Methodology

Samples from plants. Samples from leaves, stems or roots of plants were disinfected with a solution of commercial sodium hypochlorite 3% and rinsed in triplicate, then placed in a humid chamber for the growth and development of pathogens. With a dissecting needle samples of already sporulated fungi were taken on the vegetal material, being placed under observation under the microscope; A portion of the plant containing typical lesions of the disease was further disinfected and allowed to dry and part of the tissue was deposited in Petri dishes containing Bioxon's Potato Dextrose Agar (PDA), Carrot-Agar (Z. AGAR) 200 g Carrot, prepared with 15 g Agar-Agar acidified with 14 ml of 10% tartaric acid, pH 5.6 ± 0.2; Agar with juice V8 (PARN) 80 ml juice V8, 15 g Agar, 3 g Calcium carbonate CaCO3, 0.27 g Ampicillin, 1.4 g tartaric acid, and 0.10 g Rivazan (PCNB), and Diacid Agar Pentachloronitrobenzene (PCNB) 20 G Agar agar, 1 g of potassium phosphate monobasic (KH2 PO4), 0.5 g of magnesium sulphate heptahydrate, 1 g of PCNB (Rizavan) and 1 g of streptomycin sulphate (Donald et al., 1996).

ISSN-On line: 2414-8849

The boxes were incubated for 96 hours at a temperature of 22 ± 1 ° C for optimum growth of the pathogens (Tsao, 1970).

Soil samples. 41 soil samples were obtained with established culture and 22 samples of bare soil, which were dried in the shade for 48 hours, crushing the lumps with a roller. Ten subsamples of 10 g of soil were then weighed and placed into 250 ml flasks, adding 150 ml of water, and shaking for 45 min, then 100 µL aliquots were taken and seeded in different culture media. For soils with previous plantings and to establish grasses, the following were used: Peat Agar (PPA): 15 g Peptone, 1 g Potassium phosphate monobasic, 0.5 g Magnesium sulphate heptahydrate, 1 g PCNB and 1 g Streptomycin sulphate; 20 g Dextrose, 5 g Potassium phosphate KH2 PO4, 2 g NaNO3, 0.5 g Magnesium sulphate MgSO4 7H2O, 1 g Yeast extract, 0.2 g at 1% FeSO 4 .7H 2 O , 20 g Agar, 1 g Streptomycin Sulfate and 0.0065 g of Dichloran, and for Dichloran Agar-Agar (DCPA) medium: 15 g. Agar agar, Bacto peptone, 1 g Dibasic Potassium Phosphate, 0.5 g Magnesium Sulphate Heptahydrate, 2 mg Botran (Dichloran) and 0.2 g Chloramphenicol. The PARN medium described above was also used.

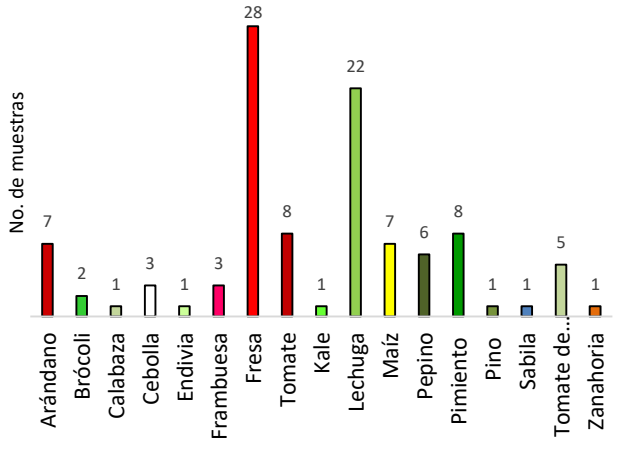
In soils for the establishment of Solanáceas, Brasicáceas or of horticultural families. PPA, DCPA and PARN media were used which were incubated for 96-120 hours at a temperature of 22 ± 1 ° C for optimum growth of the pathogens.

Water Samples. Irrigation water samples were seeded directly into boxes with PDA and Sabouraud culture medium containing 50 mg of streptomycin. The water was placed in 100 ml flasks, which were kept under agitation for 15 minutes, taking 100 µL aliquots by sprinkling in the culture media DCPA, PPA PARN and Z AGAR.

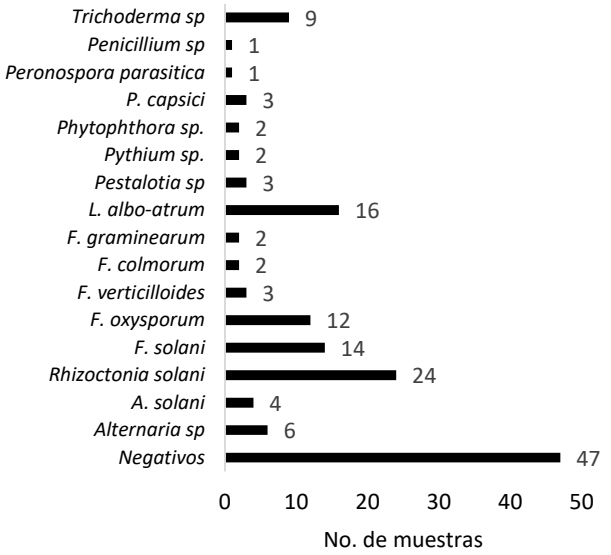
The morphological identification was made through observations under the microscope of the characteristics of shape, color, size and arrangement of spores, absence or presence of sporozoites, sclerotia or stroma in different media, mycelium and colony growth rate, as well A phytopathogenic fungi reference manual from CESAVEG (2011) was used.

Results

Sample from plants. 107 plants from 17 crops were analyzed (Graph 1). The strawberry was the crop with the highest number of samples, followed by Lettuce. R. solani was the most frequently identified pathogen in 24 samples obtained from six broccoli, onion, strawberry, raspberry, lettuce and maize cultures, Lecanicillium albo-atrum was isolated from 16 strawberry, pine and raspberry samples, F. solani Of 14 samples of broccoli, onion, strawberry, raspberry, lettuce and pepper, F. oxysporum of 12 samples of onion, raspberry, tomato, cucumber, pepper and pear tomato. However, Trichoderma sp not considered a pathogen was isolated from 9 samples from strawberry and lettuce (Graph 2).

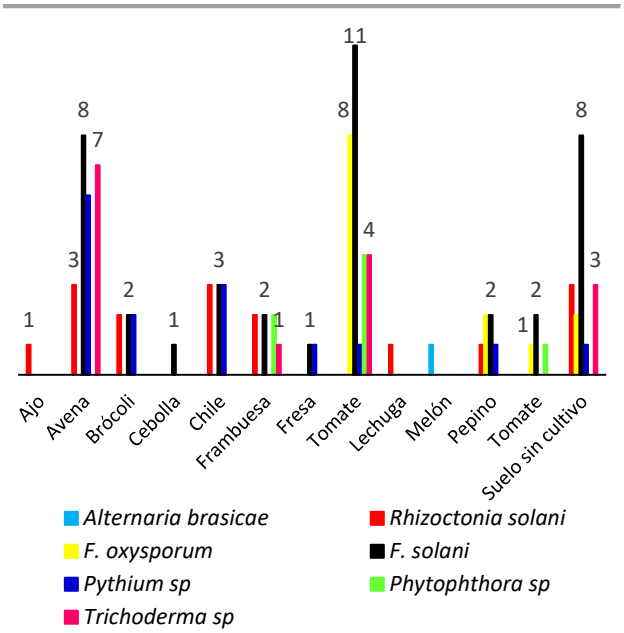


Graphic 1 Cultures analyzed for the identification of phytopathogenic fungi**.**



Graphic 2 Frequency of phytopathogenic fungi in 107 samples from plants.

Samples from soils. Fusarium oxysporum (13), Phytophthora sp (7), Trichoderma sp (15), Trichoderma sp. (15), and Rhizoctonia solani And Alternaria solani (1). The soils with the most susceptible crops were tomato and oats; however, on plant soil, phytopathological problems were also present (Graph 3).

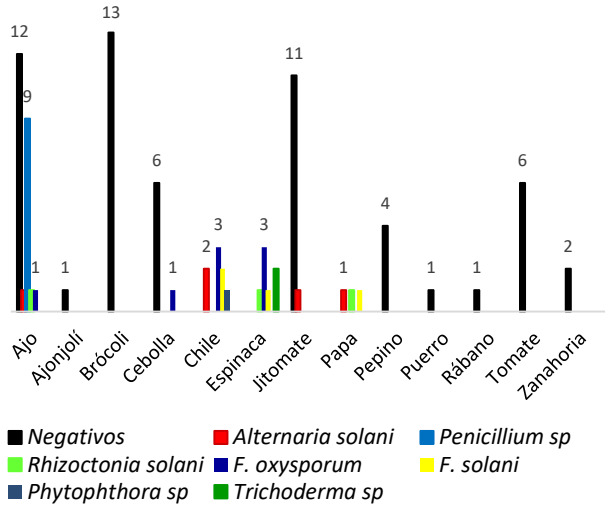


Graphic 3 Presence of fungi in soil samples.

Seed samples. A total of 75 samples from different crops such as garlic, sesame, broccoli, onion, tomato, chili, etc. were analyzed. Of which 57 were negative while, in 18 of them, frequent pathogens such as Penicillium sp (9) and Fusarium oxysporum (8) were found, and to a lesser extent the presence of Alternaria solani, Rhizoctonia solani, F. solani, Phytophthora sp and Trichoderma sp (Chart 4).

The literature reports that about 1,500 organisms have been found in seed lots of approximately 600 plant genera (Donald, et al., 1996), so it can be concluded that most of the pathogens associated with seed can be transported by itself, however, not all microorganisms found in it are causing disease. The effectiveness of the transport of pathogens and the transmission of diseases by the seed depend on a series of biotic and abiotic factors. In general, the causes of pathogen transmission increase when the inoculum is within the seed. The main genera associated with it are R. solani, F. oxysporum, F. solani, V. dahliae, Alternaria solani, Pythium sp, Macrophomina sp., Penicillium sp. And Aspergillus sp. (Agrios, 2008).

Also, the complex of diseases commonly known as Dampin off (Pythium sp., Phytophthora sp., Rhizoctonia sp., Sclerotium sp.) Can be disseminated through infected seed (Jayalakshmi, et al., 2009), so a practice Recommended is to analyze the seed before sowing for both seedling production and direct sowing, it is also recommended to immerse the seed in a solution of 108 conidia of Trichoderma harzianum, which is an effective antagonist deuteromycetes for the control of phytopathogenic fungi Of seed, plant and soil (López et al., 2010).



Graphic 4 Mushrooms found inside the seed.

In the six tray samples only the presence of Fusarium oxysporum was found in one of them and in the three samples of irrigation water Pythium was found in one of them.

It is important to mention that in order to ensure a good harvest, microbiological analyzes of soil, seed, plant and water must be carried out before establishing the crop as a preventive measure in the onset of diseases, which can occur both in developing crops As in the middle of the crop, care must be taken in the spread of pathogens within the farm, because these can remain for long periods in the soil through resistance structures such as chlamydiospores and sclerotia (Martínez-Scott, 2008) . It has sometimes been observed that when soils are infested by Fusarium oxysporum, the structures are carried by the irrigation water or leached to the aquifers and the water is contaminated, causing the plants watered with the same to wilt and stop growing (Ramírez et al., 2009). This research was of great help to the producers of the region of the guanajuatense bajío, because from the results it was possible to take preventive measures and to execute corrective actions to avoid economic losses.

Conclusions

Of the 245 samples analyzed, a total of 275 pathogens were obtained in 130 samples grouped into 10 genera and 14 species. Strawberry cultivation showed the highest number of pathogens such as Rhizoctonia solani, Verticillium albo-atrum and fusarium oxysporum, and Alternaria solani and Phytophthora sp. According to the results obtained, the fungus Rhizoctonia solani was the pathogen that was most frequently found in both plants and seed and soil samples. In germination trays samples were mainly identified to Fusarium oxysporum and in irrigation water to Pythium sp and Trichoderma sp., the rest gave negative to fungi.

Aknowledgement

Thanks to the participation of producers from the region of Salvatierra, Santiago Maravatio and Tarimoro, as well as to the management of ITESS.

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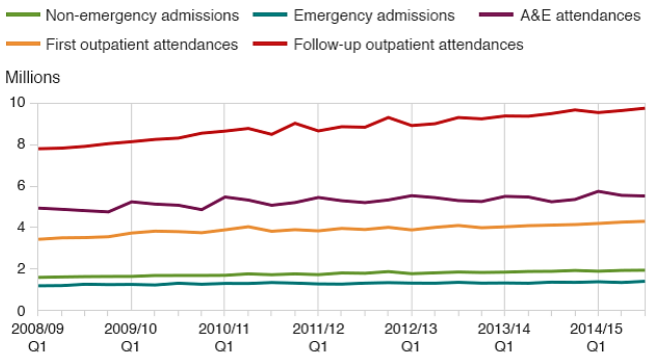

Graph 1 Hospital Activity

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