EFFECT OF MATERNAL INTAKE OF FRIED POTATO CHIPS ON THE TONGUE DEVELOPMENT IN THE NEWLY BORN ALBINO RATS: HISTOMORPHOMETRIC, HISTOCHEMICAL AND SCANNING ELECTRON MICROSCOPIC STUDY.

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Introduction:
Fried potato chips contain high amount of acrylamide which predisposes developmental and health complications. The aim of the present study is to evaluate the teratogenic effects of fried potato chips on the tongue development in newly born albino rats.

Materials and Methods:
Fourteen female rats were housed with four male rats for mating. The female rats were divided to 2 equal groups (while the offspring’s tongues were studied). Control group: received normal diet and experimental (chips) group: received diet containing 50% fried potato chips. The tongues of the neonates were measured by weight and size. Histomorphometric analysis and Masson trichrome stain examination in addition to scanning electron microscopic (SEM) evaluation were performed.

Results:
The tongues of the chip group were significantly decreased in size and weight. Histological examination revealed distorted filiform papillae, poorly developed fungiform papillae, slight decrease in the mucosal thickness and minor degeneration in some salivary acini in addition to fibrotic and fatty degenerative changes in the muscle layer. Masson trichrome stained sections showed increased collagen to muscle fiber ratio in the chips group. SEM examination of the chips group presented irregular filiform papillae, depressed fungiform papillae, totally lost circumvallate papillae and almost normal foliate papillae.

Conclusions:
Fried potato chips adversely affect the tongue development of newly born albino rats. The most affected tissue is the muscle layer followed by the lingual papillae. The lingual salivary glands are minimally affected.

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Abstract

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Introduction:-
Fried potato chips are considered one of the most widespread snacks (Clark, 2003), however, frying is generally considered an unhealthy method of cooking (Leitzmann and Kurth, 2012) as some unsaturated fatty acids and vitamins are lost from the fried food due to oxidation triggered by frying procedure (Fillion and Henry, 1998). Moreover, one of the most common hazardous frying byproducts is acrylamide, a low molecular weight monomer. It
is produced when potato is oven cooked, pan fried or heated in microwave at a temperature higher than 120 °C (Tareke et al., 2002; Friedman, 2005; Exon, 2006). Acrylamide amount is not influenced by oil type or frying hours (Santos et al., 2018). On the other hand, boiled potato has not been reported to contain acrylamide (Eriksson, 2005). Acrylamide has also been detected in sugar (Schultzova and Tekel, 1996) and olives (Friedman, 2003). Besides, cigarette smoking produces acrylamide three times more than any dietary product (Olesen et al., 2008).

Excessive intake of fried food rises the hazard of developing type 2 diabetes (Halton et al., 2006; Ylonen et al., 2007; Khosravi et al., 2012), heart failure, obesity and hypertension (Tarakà et al., 2015). In addition, acrylamide is clearly demonstrated to be carcogenic to rodents while no clear evidence of increased risk of human cancer caused by acrylamide or fried potatoes (Pelucchi et al., 2003; Maronpot et al., 2015; Jaya et al., 2018). Several investigations revealed the acrylamide toxicity chiefly toward development and growth. The teratogenicity of potato chips might be due to the ability of acrylamide to cross the placenta, reach significant concentrations and triggers direct developmental effects in rodent neonates (Garey et al., 2005; Annola et al., 2008).

A comparative study conducted on acrylamide and fried potato chips reported teratogenic effects on mice. Both potato chips and acrylamide increased abortion and neonatal mortality rate and decreased the weight as well as size of the offspring before and after birth (El-Sayyad et al., 2011a).

Regarding the oral tissue development, the neonates maternally fed on fried potato chips exhibited palate surface alterations with noticeable impairment of taste buds. Furthermore, atrophied fungiform papillae and significant decline of keratinization in filiform papillae were reported (El-Sayyad et al., 2017). However, the diversity of the tongue structures necessitates more comprehensive work. Hence, the aim of the present work is to evaluate the teratogenic effects of fried potato chips on the rat newborn tongues.

Materials and methods: -
Fourteen fertile female albino rats (Rattus norvegius) weighing around 250 g were used in the present work. Rats were housed in cages with healthy fertile male rats (two males for each 7 females) and maintained in a room with good ventilation. All the experiments were done in compliance with the bio-ethical guidelines of Ain Shams University Animal House. From the first day of mating, the female rats were arranged into two groups (7 rats each) while the tongues of their offspring were considered the samples of the present study:

1-Control group: female rats received regular diet composed of carbohydrates and protein and fresh vegetables till the labor.

2-Chips group: female rats received same regular diet supplied to the control group with addition of fried potato chips with a ratio of 1:1 (Regular diet: potato chips) till the labor (El-Sayyad et al., 2011b).

Three newborns (one day old) were collected from each female rat which became pregnant and was healthy till labor. The tongues of the newborns were dissected; one tongue was processed for scanning electron microscopic examination and also served for measuring the weight and dimensions while the other two tongues were prepared for light microscopic examination via hematoxylin and eosin (H&E) stain and Masson trichrome stain to demonstrate the different histological features of the lingual structures.

Gross anatomy measurements: -
Three parameters were evaluated: tongue weight, circumference and outer area. The weights of the tongues were measured by Chyo Digital Balance (Model: MJ-500; weighing-range: 1 mg to 500 grams). While the circumference and area were measured by taking photographs of the tongues held on graph paper using a digital camera. The images were analyzed using Image J software (version: 1.52f Wayne Rasband, National Institute of Health, USA) (fig. 1).

Histomorphometric analysis: -
The thickness of the epithelial linings as well as the underlying connective tissue thickness were measured in the ventral surface of the tongue. Five separate regions were randomly selected in each slide under magnification (x200). Besides, the width of the intrinsic muscle bundles in 3 separate regions of the muscle layers (5 different bundle widths in each region were measured) i.e. 15 different muscle bundles were measured in each sample.
Masson trichrome stain was selected to differentiate between muscle fibers and collagen fibers (muscles were red stained while collagen was blue stained). The area percentages of the collagen fibers were measured in 5 regions in each slide (in constant area: 200x200 microns) under magnification x400. All the histomorphometric measures were implemented by Image J software.

The numerical data (gross anatomy and histomorphometry) were tabulated and statistically analyzed using student’s “t” test as study consisted of 2 groups. The results were considered significant if P value <0.05.

**Scanning electron microscopic examination**

The samples were dried at the critical point using (Autosamdri 815 machine), then coated by gold sputter coater (SPI-module) and examined by scanning electron microscopy (JEOL-JSM-5500LV) by using high vacuum mode at the Regional center of Mycology and Biotechnology, Al Azhar University, Cairo, Egypt. Scanning electron microscopic examination was performed at magnifications (35, 300, 500 and 1100) to illustrate the topographic features of the lingual papillae.

**Results:**

**Gross anatomy measurements:**

**Tongue weight:**

The mean value of the tongue weight of control group newborns was (14.4 mg) while the chips group newborns showed about 50% decrease in the average tongue weight compared to control group.

**Tongue size:**

Regarding the morphometric analysis of the tongue’s circumference and area, slight decrease in both measurements was observed in the chips group relative to the control group.

Table (1) and figure (2) represents the mean and standard deviation (SD) of each of the three studied parameters. The differences between the groups in all these parameters were statistically significant (P value <0.05).

**Histological (Hematoxylin and Eosin stain):**

Examination of the tongues of the control newborns revealed developed filiform papillae which presented two different forms: the conical shaped papillae with backward curvature which predominate normally in the intermolar area (fig. 3A) and the slender shaped papillae at the posterior region of the papillary part (fig. 3B). The filiform papillae showed regular orientation with well-defined keratin layer. Among the filiform papillae, the fungiform papillae were observed with their characteristic mushroom shape (fig. 3C). The foliate papillae were detected with shallow grooves separating the folia (fig.3D), while the circumvallate papillae were not well defined to be represented in the light microscopic sections.

The ventral surface of the tongue showed thin keratinized stratified squamous epithelium with even thickness overlying dense fibrous connective tissue separating the epithelium from the lingual muscles (fig. 4A). The serous salivary acini with their dense basophilic appearance and the mucous tubules with their foamy shape were developed (fig.4B). The muscle layer of the control group was densely packed with muscle bundles which were long and cylindrical. The muscle sarcoplasm was acidophilic with clearly seen cross striations and peripheral elongated nuclei beneath the sarcolemma(fig. 4C).

Examination of the chips group tongues revealed distortion in the filiform papillae in many regions with torn keratin layer(fig. 5A) and atrophied papillae in other regions (fig. 5B) while some samples presented areas of lost filiform papillae (fig. 5C). Fungiform papillae were poorly developed (fig. 5D) While foliate papillae showed almost no observable changes from the control group except for minute tearing in the keratin(fig. 5E). The circumvallate papillae were not detected in any sample of chips group. Regarding the ventral surface of the tongue, many areas displayed apparent decrease in the epithelial and connective tissue thicknesses in addition to minute keratin detachments and degenerative changes in the lamina propria (fig. 5F). The minor salivary glands showed similar features as those of the control group except for occasional epithelial discontinuities in few samples (fig. 6A). The major histological changes were detected in the muscle layer where the muscle bundles were less densely packed compared to those of the control rats with observed areas of sarcoplasm fragmentation as well as fatty degeneration(fig. 6B). There were patchy areas of fibrous tissue infiltrating the muscle layer in many regions with variable sizes (fig. 6C and 6D).
Histochemical examination (Masson trichrome “M.T.” stain):
Masson trichrome stain was used for differentiation between the muscle bundles (red color) and the collagen fibers (blue). The epithelial lining in both groups was red stained while the underlying connective tissue was mostly blue stained due to the predominance of collagen in the lamina propria (fig. 7A and 7C). Regarding the muscle layer, the samples of the control neonates revealed minimal percentage of collagen fibers between the muscle bundles (fig. 7B) while the chips group displayed more collagen fibers occupying the spaces between muscles (fig. 7D).

Histomorphometric results
a-Mucosal thickness:
The averages of epithelium and connective tissue thicknesses were measured in the ventral surface of the tongue which was selected due to the regular and even thickness compared to the dorsal surface. Chips group showed non-significant decrease in both epithelial and connective tissue thickness P value >0.05 (table 2 and fig. 8).

Muscle bundles width:
was measured in 5 muscle bundles in each studied slide. Mucosal thickness and muscle bundle size are measured in magnification x200. Chips group showed non-significant decrease muscle bundle width (table 2 and figure 8). Chips group showed significant increase in the collagen content P value <0.05 (table 2 and fig. 9).

Scanning electron microscopic results:
The topographic features of the dorsal surface of the tongue papillary part were clearly investigated by SEM. The control group newborn’s tongues showed velvet shaped regular surface (fig. 10A) indicating the keratinized densely packed non-distorted filiform papillae with two forms: conical type with convex and concave sides (fig. 10B) and slender (fig. 10C). Fungiform papillae were scattered between filiform papillae (fig. 10D) with regular bulging dorsum. The circumvallate papillae were easily seen with oval central papillary part surrounded by elevated flanking papillary structure from behind with a horseshoe like profile. The outer end of the circumvallate furrow was detected between the central papillary part and the surrounding flanking (fig. 10E). The foliate papillae were detected on the lateral borders posteriorly with parallel grooves separating the folia (fig. 10F).

The chips group newborn’s tongues had a distorted body with many irregularities (fig. 11A). There were many signs of distortion in filiform and fungiform papillae. Filiform papillae were irregularly oriented, rough surfaced and their keratin was degenerated in many regions (fig. 11B). Fungiform papillae showed a depressed dorsal surface (fig. 11C) and degenerated surface layers (fig. 11D). The foliate papillae were almost like those of the control group (fig. 11E) while the circumvallate papillae were not detected in any sample. All the main morphometric, histomorphometric and SEM findings were summarized in Table (3).

Discussion:
The current study aimed to assess the possible teratogenic effects of the fried potato chips which are considered a common source of acrylamide (Zhang and Zhang, 2007). We suggested that most of the reported teratogenic effects of the potato chips in the present work is mainly due to the acrylamide content as potato had enormously high measured levels of acrylamide (Becalski et al., 2003; Foot et al., 2007). Thus, regular intake of fried potato chips has been clearly demonstrated to produce collective amounts of acrylamide in the body, thereby silently increasing the risk for various diseases (Ouhtit et al., 2014). Moreover, El-Sayyad et al., (2011a) reported that the observed congenital malformations in the fried potato chips treated rats were higher than those in the acrylamide treated rats.

The tongue development was selected due to the diversity of tissues included: muscles, mucosa and salivary glands to shed a global light on different categories of tissues. In the present work, potato chips were supplied among the daily diet reliant on that absorption of acrylamide following oral administration is virtually complete in mammals, including humans (Dybing et al., 2005).

In this work, the weight and size of the tongues were markedly decreased in the neonates maternally fed with potato chips in comparison to those of control neonates. This finding might be due to the acrylamide content of fried potato chips which causes significant decrease in body weight (Maronpot et al., 2015). The monomeric nature of acrylamide could be the cause of weight loss which coincides with LoPachin et al., (2002 and 2004) who stated that monomeric form products cause skeletal muscle weakness and weight loss.
In the herein study, filiform papillae displayed distorted outline and poorly developed keratin as well as deformation in fungiform papillae. These findings were detected in both histological and SEM examination. This is in accordance to El-Sayyad et al., (2017) who reported reduction of lingual keratinization especially in filiform papillae. In addition to atrophy of fungiform papillae in the offspring of females subjected to potato chips. The epithelial damage could be explained by the ability of acrylamide in potato chips to disrupt the epithelial intermediate filaments as reported by Arocena, (2006).

The circumvallate papillae of chips group in the present study were not detected neither in histological nor scanning electron microscopic examination indicating incomplete or failure of their development. This might be an indirect effect of fried potato neurotoxicity on the offspring which has been reported by El-Sayyad et al., (2011c) and Gad-Allah et al., (2013). This neurotoxicity may be related to the damage of neural elements that is crucial for the normal development of the circumvallate papillae (Mistretta et al., 1999; Sbarbati et al., 2000).

The damage in the cellular level observed in the present work in lingual papillae as well as muscle fibers might be attributed to the pro-apoptotic activity caused by acrylamide. These findings coincide with several researches reported the oxidative stress and apoptosis play an important role in potato chips and acrylamide induced inflammation and/or toxicity (Naruszewicz et al., 2009; Sumizawa, 2009; Rodriguez et al., 2011; Lakshmi et al., 2012; Ali et al., 2014; Al-Serwi and Ghoneim, 2015).

Our results revealed destructive changes in the muscle fibers with fatty degeneration in many areas. This agreed with previous work conducted by Al-Serwi and Ghoneim, (2015) who reported fatty infiltration between the muscle fibers in animals subjected to oral acrylamide intake. Fatty degeneration induced by acrylamide was also, observed by Almoeiz et al., (2013) in their study on liver. We observed spotty collagenous deposition infiltrating the muscle layers in many samples which was confirmed histochemically via Masson trichrome staining. This coincides with Maronpota et al.,(2015) who reported association between acrylamide intake and muscle fiber atrophy with interstitial fibrosis. There are more wide researches clearly detected the adverse effects of acrylamide on the skeletal muscle due to the monomeric neurotoxic activity which predisposes skeletal muscle weakness (LoPachin et al., 2002 and 2004) as well as the oxidative stress effect of acrylamide (Hori et al., 2013).

Generally, the teratogenic effects that reported in this study could be explained by the ability of acrylamide to cross the placenta reaching to fetus (el-Sayyad et al., 2017). Previous work reported that a potato chip as well as administration of acrylamide to pregnant rats has been shown to produce developmental and post-natal effects in rodent offspring (Annola et al., 2008; Elsayyad et al., 2011a).

More work is needed for evaluation of the reversibility of the teratogenic effects of the fried potato chips, the dose dependence of these defects, the emergence of protective materials and nutritive substances which might counteract and/or cure the deleterious effects of fried potato chips and acrylamide.

So, within the limitations of this study the following conclusions could be drawn:

a- Fried potato chips adversely affect the tongue development.
b- The most affected tissue is the muscle layer with fibrous and fatty degenerative changes.
c- Potato chips prevent the development of circumvallate papillae and caused filiform and fungiform distortion while foliate papillae are the least affected one.
d- Salivary glands are the most resistant tissue to the teratogenic effects of fried potato chips.

Table 1: Mean and standard deviation of the tongue gross anatomy measurements.

|                | Weight | Circumference | Area   |
|----------------|--------|---------------|--------|
|                | Control | Chips | Control | Chips | Control | Chips |
| Mean           | 14.40   | 7.50   | 17.72   | 15.12 | 14.85   | 10.49 |
| S.D.           | 3       | 4     | 0.95    | 0.93  | 1.13    | 1.52  |
Table 2: Mean and standard deviation of the epithelial and connective tissue thickness, muscle bundle width (H&E) and collagen percentage (Masson trichrome).

|                      | Epithelial thickness | Connective tissue thickness | Muscle bundle width | Percentage of collagen |
|----------------------|----------------------|-----------------------------|---------------------|------------------------|
|                      | Control | Chips | Control | Chips | Control | Chips | Control | Chips |
| Mean                 | 32.72   | 25.07 | 42.20   | 33.14 | 11.03   | 10.92 | 1.65    | 4.04  |
| S.D.                 | 9.95    | 2.77  | 15.38   | 5.52  | 1.13    | 0.26  | 0.71    | 1.09  |

Table 3: Summary of the main effects of fried potato chips on the tongue development.

|                      | H&E stain | Masson trichrome | Histomorphometry | SEM | Other tests |
|----------------------|-----------|------------------|------------------|-----|-------------|
| Filiform papillae    | 1- Distorted 2- Atrophied 3- Torn keratin | No observed change | Not applicable | Distorted |
| Fungiform papillae   | Poorly developed | No observed change | Not applicable | 1- Depressed 2- Degenerated surface |
| Circumvallate papilla | Lost | Lost | Not applicable | Lost |
| Foliate papilla      | Minute keratin tearing | No observed change | Not applicable | No observed change |
| Ventral surface of the tongue (epithelium) | 1- Thin 2- Detached keratin | No observed change | Decreased thickness | Not applicable |
| Ventral surface of the tongue (Lamina propria) | 1- Thin 2- Minute degeneration | No observed change | Decreased thickness | Not applicable |
| Muscle               | 1- Fatty degeneration 2- Fibrosis | Increased collagen fiber | Increased collagen percentage | Not applicable |
| Salivary glands      | Minimal epithelial discontinuity | No observed change | Not applicable | Not applicable |
| Tongue weight        | Not applicable | Not applicable | Not applicable | Not applicable | Decreased |
| Tongue circumference | Not applicable | Not applicable | Not applicable | Not applicable | Decreased |
| Tongue area          | Not applicable | Not applicable | Not applicable | Not applicable | Decreased |

Figure 1: Demonstration of the method of measuring the samples areas and circumference using Image J software.
Figure 2: Bar chart showing the difference between the studied groups in sample weight (A), circumference (B) and area (C).

Figure 3: Photomicrograph of control group showing: (A): conical and (B): slender filiform papillae with regular outline and intact keratin. (C): Fungiform papilla “arrow” and (D): developing foliate papilla “arrow” (H&E x 400).
Figure 4:- Photomicrograph of control group showing (A): Ventral surface of the tongue with thin keratinized epithelium “1” and dense fibrous lamina propria “2” overlying the lingual muscles “3” (B): Mixed lingual minor salivary gland with intact serous “green arrows” and mucous acini “black arrows”. (C): Densely packed long muscle bundles with observable striations “green arrows” and peripheral elongated nuclei “black arrows” (H&E A and C: x400 / B: x200).
Figure 5: Photomicrograph of chips group showing (A): distorted filiform papillae “black arrow” with torn keratin “blue arrow” (B): atrophied filiform papilla “arrow”. (C): Areas of lost filiform papillae “arrow”. (D): Poorly developed fungiform papilla “arrow” and (E): Foliate papillae with minute tearing in the keratin (fig. 5e). (F): Ventral surface of the tongue with thin lamina propria “black arrow”, minute keratin detachments “red arrow” and degenerative changes in the lamina propria “green arrows” (H &E x 400).
Figure 6: Photomicrograph of chips group showing (A): minor salivary glands with occasional epithelia discontinuities “arrows”. (B): Muscle bundles with fragmentation “black arrow” and fatty degeneration “blue arrow”. (C and D): Patchy areas of fibrous tissue infiltrating the muscle layer in many regions with variable sizes “arrows” (H&E A, B and D: x400 / C: x200).
Figure 7: Photomicrograph showing (A): Red stained epithelial lining “1” and blue stained collagenous lamina propria “2” in control group (A) and chips group (C). The muscle layer of the control group revealed minimal percentage of collagen fibers between the muscle bundles (B) while the chips group showed more collagen fibers “arrows” (D) (Masson trichrome A and B: x200 / C and D: x400).

Figure 8: Bar chart showing the difference between the studied groups in epithelial and connective tissue thickness and the muscle bundle width.
Figure 9: - Bar chart showing the difference between the studied groups in collagen fibers percentage in the muscle layer.
Figure 10: SEM micrograph of control group showing: (A): velvet shaped regular surface with scattered fungiform papillae (arrows). (B): Conical filiform papillae with concave sides “white arrow” and convex side “yellow arrow”. (C): slender filiform papillae, (D): Fungiform papilla, (E): Circumvallate papilla with central part “white arrow” surrounded by elevated flanking papillary structure “blue arrow” and the furrow “red arrow”. (F): The foliate papillae on the lateral borders posteriorly with parallel grooves “red arrows” separating the folia “white arrows”.

Figure 11: SEM micrograph of chips group showing: (A): distorted body with many irregularities (arrow). (B): Irregular filiform papillae with degenerated keratin. (C): Fungiform papillae showed a depressed dorsal surface and (D): degenerated surface layers (E): The foliate papillae were almost like those of the control group with their grooves “red arrows” separating the folia “white arrows”.
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