STUDY OF COLONIC CELL PROLIFERATION INDUCED BY CHRONIC TOXICITY OF ORLISTAT AND THE POSSIBLE PROTECTIVE EFFECT OF GINSENG IN EXPERIMENTAL ANIMALS

By

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ABSTRACT

Orlistat is a widely used anti-obesity agent available as a non-prescription medication in many developed countries. Its long term use may result in an increasing frequency of chronic toxicity. This work was designed to clarify the effect of Orlistat on the colonic cell proliferation and the formation of colonic aberrant crypt foci (ACF) and to investigate the possible ameliorating effect of Ginseng in experimental animals. Eighty adult male albino rats were used in this work; group I (control group) received a standard diet. Group II (Ginseng group) fed on diet containing 1% red Ginseng. Group III (high fat diet group, HFD) fed on 10% corn-oil enriched diet. Group IV (Orlistat group) received oral Orlistat (125 mg kg/day), group V (Orlistat + HFD group). Group VI (HFD + Ginseng group). Group VII (Orlistat + Ginseng group) and lastly group VIII (Orlistat + HFD + Ginseng group). After 16 weeks, animals were sacrificed and their colons were immunostained using proliferating cell nuclear antigen (PCNA) as a marker of cell proliferation which is the biomarker of increased susceptibility to gastrointestinal cancer. Results showed that Orlistat and/or HFD resulted in increased immunolabelling for PCNA causing a statistically significant increase in PCNA-labelling Index (PCNA-LI) when compared with the control groups. Ginseng when combined with Orlistat or HFD or Orlistat + HFD caused a significant decrease of both the immunolabelling and PCNA-LI. Consequently, it has been proposed to clarify the long-term impact of Orlistat treatment for physicians working in this field and for the public by the drug brochure. Also, Ginseng should be given with Orlistat as a protective agent to ameliorate its proliferative effect on the colonic mucosa.

INTRODUCTION

Obesity is an ever-expanding global health problem, which contributes significantly to individual poor health and societal burden of disease. A number of concomitant pathological processes and diseases are associated with obesity including coronary heart disease, hypertension, stroke, non-insulin dependent diabetes mellitus and certain forms of cancer. Besides changes in diet, behavior and physi-
cal activities, obesity may be treated by surgery or pharmacological therapy. Gastrointestinal symptoms have been commonly seen in 75% of the patients who had clinical treatment for obesity (Peter & Keith, 2004).

Orlistat is an anti-obesity drug used for long-term management of obesity and its long-term safety is still to be determined. It decreases absorption of dietary fat by inhibiting gastric and pancreatic lipases through covalent modification of the enzymes (PDR, 2006).

It is also a potent inhibitor of fatty acid synthase (FAS) functions through inhibition of the thioesterase activity of FAS (Dowling et al., 2009). The inactivated enzymes become unable to hydrolyze dietary fat preventing its digestion and absorption (Caner et al., 2005). Thus, the fecal fat excretion is significantly increased with Orlistat (Nishioka et al., 2003).

Many laboratory animal studies suggest that fat has a direct action on the colo­nocytes causing increase its proliferation and upward shift in the proliferation zone of the colonic crypts. This promotes the appearance of the aberrant crypt foci (ACF) - that are considered to be the earliest putative preneoplastic lesion- enhancing colorectal cancer. Many hypotheses tried to explain such mechanism. The most widely accepted hypothesis is that dietary fat leads to increase the cytotoxic fecal bile acids that induces damage to the colonic mucosal epithelial cells with subsequent compensatory hyperproliferation (Ahnen et al., 2007).

PCNA also called cyclin, was discovered through an autoantibody in the serum of a patient with systemic lupus erythematosus that specifically reacted with the nuclei of proliferating cells. It was identified as an auxiliary protein of 36 kDa of DNA polymerase-delta (Yamashita et al., 1994). PCNA has been found to be a useful marker in immunocytochemical studies of cell proliferation because its expression correlates with the proliferative state of the cell (Martínez-Lara et al., 1996). It is also a nuclear protein involved in DNA-synthesis and repair, and associated with S phase and DNA replication of the cell cycle (Motiwale et al., 2005).

Thus, the purpose of the present study was to verify the effect of chronic administration of Orlistat on the colonic cell proliferation and the formation of colonic aberrant crypt foci (ACF) determined by proliferating cell nuclear antigen (PCNA) immunohistochemistry and to investigate the role of Ginseng as a protective agent.

MATERIALS & METHODS

This work was carried out in the Department of Forensic Medicine and Toxi-
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cology, Faculty of Medicine - Minia University. All aspects of animal care and treatment were carried out according to the local guide line of the Ethical Committee of Faculty of Medicine, Minia University.

Eighty adult male albino rats were used in this work with average weight of (180±20gm). They were housed in standard polypropylene cages (five rats/cage) and maintained under a controlled room temperature with 12:12 h light and dark cycle. All rats were provided with commercially available normal rat diet and water ad libitum for two weeks before experiment for acclimatization.

Rats were divided into eight experimental groups (10 rats each):

Group I (control group): Rats fed only on ordinary rat diet.

Group II (Ginseng group): Rats fed on diet enriched with 1% red Ginseng (Pharco Pharmaceuticals) according to Fukushima et al., (2001).

Group III (High fat diet group) "H.F.D": Rats fed on 10% corn oil enriched diet.

Group IV (Orlistat group): Rats received Orlistat "Eva Pharmaceutical Company" at a dose of 125 mg / kg /day according to EMEA, 2005. This dose is equivalent to 1/40 of LD50 (Hoffmann La-Roche Inc, 2008).

Group V (Orlistat + HFD group): Rats received Orlistat at a dose of 125 mg / kg /day and 10% corn oil enriched diet daily.

Group VI (HFD + Ginseng group): Rats fed on diet containing 10% corn oil and 1% red Ginseng.

Group VII (Orlistat + Ginseng group): Rats received Orlistat at a dose of 125 mg / kg / day orally and 1% red Ginseng enriched diet.

Group VIII (Orlistat + HFD + Ginseng group): Rats fed on diet containing 10% corn oil +1% red Ginseng and received Orlistat at a dose of 125 mg / kg / day orally.

Animals were observed regularly. After 16 weeks; rats were sacrificed by decapitation under light halothane anesthesia. The large bowel was rapidly removed and longitudinally opened as closely as possible to the mesenteric border throughout its full extension. The distal colon was fixed in 10% buffered formalin, segmented into small pieces and processed for different histological examinations.

The colonic segments were washed in tap water and then stained with 40 mL of 0.2% methylene blue for 3-5 minutes. Subsequently, the excess stain was washed off with tap water for five minutes. For observation by light microscopy, the mucosa
was cut into small pieces and placed under two glass slides (1 x 1 cm) and examined with mucosal surface up according to Piva-Demarzo & Garcia (2004).

Other segments were cut as a ring of the organ and were also embedded in paraffin and the paraffin blocks were cut serially maintaining the original transverse orientation so as to provide ring-shaped sections (7μm sections) used for Hematoxylin and Eosin staining and immunohistochemistry staining.

**Immunohistochemical staining and morphometry:**

Seven-micrometre sections were used for immunohistochemical staining for the Proliferating cell nuclear antigen (PCNA). In brief, sections were deparaffinized, hydrated then washed in 0.1 M phosphate buffer saline (PBS). Endogenous peroxidases were quenched by treatment with H2O2 in methanol (Peroxidase blocking solution) followed by washing in tris-buffer saline (TBS). Non-specific binding of IgG was blocked using normal goat serum, diluted in 0.1% bovine serum albumin with TBS for 30 minutes. The sections were incubated with the diluted primary antibodies; mouse monoclonal PCNA, for 30 minutes at room temperature. Sections then were washed 3 times each for 5 minutes in buffer and incubated for further 30 minutes with biotinylated goat anti-rabbit secondary antibodies diluted 1:1000, followed by washing. Following further 30 minutes incubation with Vectastain ABC kits (Avidin, Biotinylated horse radish peroxidase Complex) and washing for 10 minutes, the substrate -diaminobenzidine tetra hydrochloride (DAB) in distilled water- was added for 5-10 min. The slides were lightly counterstained by hematoxylin. This substrate gives brown color of the proliferating nucleus.

PCNA immunolabeled cells were counted in 10 adjacent non overlapping fields of the cross tissue sections of each rat. The total number of cells of the same colonic glands was also assessed by counting their all nuclei in the same fields. The ratio between numbers of PCNA-immunolabeled cells to the total number of cells was calculated in each experimental group (Kubben et al., 1994).

To estimate the colonic cell proliferation in the colon of all animals, PCNA immunolabeled cell nuclei were counted in 100 colonic crypts. The PCNA-labelling index (PCNA-LI) was expressed as a ratio of positively stained nuclei to a total number of counted nuclei per 100 crypts in each experimental group (Kubben et al., 1994).

**Data handling and statistics:**
The mean number (M) and standard
deviation (±SD) were determined in each group. Statistical analysis of the data was performed by using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) for windows. Student’s t-test was performed and p≤0.05 was considered statistically significant.

RESULTS

All rats survived to the final sacrifice. Diarrhea was observed in rats during Orlistat administration. Intestinal lumens of the animals fed on Orlistat were observed to be empty after they were sacrificed. No macroscopic changes were observed in the colons. The histopathological examination of methylene blue stained mucosa showed that rats of all groups lacked any ACF in the colon (Fig.1).

Immunohistochemical study:

Light microscopic examination of colons of the control and Ginseng-treated groups (I & II) revealed normal structural components of the colon which displayed normal mucosa consisting of surface lining, intestinal glands, lamina propria and muscularis mucosa. The mucosal lining showed slight immunostaining for PCNA (proliferative marker) which mainly was localized to the lower part of the glands (sites of stem cells in large intestine) (Fig. 2A). Groups III, IV, VIII showed increases in the immunolabelling for PCNA (Fig. 2B). Group V showed marked increase in the immunolabelling for PCNA (Fig. 2C). Groups VI, VII showed obvious decreases in immunolabeled cells (Fig. 2D).

Higher magnifications of sections of control groups (I & II) displayed few detectable immunolabelling for PCNA (Fig. 3A). Groups III, IV, VIII showed increases in the immunolabelling for PCNA (Fig. 3B). In group V, there was a marked increase in the immunolabelling for PCNA. It also showed areas of stratifications. The immunolabeling was mainly confined to cellular nuclei of the colonic glands but some stromal cells showed immunopositivity for PCNA (Fig. 3C). Groups VI, VII showed obvious decreases in immunolabeled cells (Fig. 3D).

Results of PCNA-labelling Index (PCNA-LI):

An Orlistat and / or high fat diet (HFD) caused an increase in the PCNA-LI positive cells when compared to the control groups. Orlistat and / or HFD with Ginseng showed a decrease in PCNA-LI but still higher in comparison with the control groups (Table 1). All groups except the Ginseng group showed significant (P=0.0001) increase in PCNA-LI in comparison with the control group (Table 2). Rats fed on Ginseng with Orlistat and / or HFD showed significant (P=0.0001) increase in PCNA-LI in comparison with Ginseng group (Table 3).
In the presence or absence of Ginseng, the association of high fat diet (HFD) and Orlistat produced a cumulative effect on the increase of the PCNA-LI when compared to Orlistat alone or HFD alone. Feeding of the Orlistat-treated rats with Ginseng decreased the PCNA-LI significantly. The PCNA-LI was significantly suppressed within the animals fed high fat diet with red Ginseng. Group VIII which were supplied with Orlistat + HFD + Ginseng showed no difference in comparison with Orlistat alone or HFD alone, but showed significant decrease in comparison with Orlistat + HFD group and significant increase when compared with Orlistat + Ginseng and HFD + Ginseng groups (Table 4).

**DISCUSSION**

A lot of studies have been done with Orlistat in humans (Joyce, 1998). Clinical and experimental studies have reported positive effects of Orlistat on lipid profile and glucose levels (Sjostrom et al., 1998), on gallbladder stones formation (FDA, 2007), on drug-induced bullous leukocytoclastic vasculitis (Lazic et al., 2011) and on liver injury (Umemura et al., 2006) and (FDA Drug Safety Communication, 2010).

Many controversies had been established about Orlistat and cancer association. While some reported positive association with cancer breast (Lee-Ping, 1997) and cancer colon (Takayama et al., 1998) and (Radtke & Clevers, 2005), others stated that breast cancer occurrence was likely to be a chance finding (EMEA, 2005). Orlistat was found by others (Dowling et al., 2009), (Kridel et al., 2004) to inhibit the proliferation of gastric tumor cells in mice and prostatic cancer cells cultured in serum free media. So this study was designed to rule out the effect of Orlistat on rat's colonic mucosa to verify any positive or negative association with hyperproliferating activity.

According to the results of the present study; Diarrhea was observed in rats during the 6 weeks of Orlistat administration which is compatible with results of Caner et al., (2005) and Li & Cheung (2009). EMEA, (2005) attributed all GIT symptoms due to the changes in vitamins D, E, and β-carotene levels.

Results showed that HFD or Orlistat or even both together lacked aberrant crypt foci in rat's colons, these findings met with results of Garcia et al. (2005). But, The FDA pharmacology review of Roche's high fat / low calcium study noted that there was a treatment-related increase in the number of colonic aberrant crypt foci in rats with Orlistat (David, 1997).

Increased cell proliferation has been proposed to be a biomarker of increased susceptibility to gastrointestinal cancer.
PCNA can be used as a proliferating index of a broader spectrum of cells than other parameters (Yamashita et al., 1994).

PCNA-LI positive cells were observed in high number in rats fed on Orlistat. An important explanation for these findings is what observed by Melia et al. (1996) who reported that Orlistat significantly reduces the absorption of vitamin E. As vitamin E supplementation (90 mg/kg diet) caused a significant decrease in the number of aberrant crypt foci (Narayan et al., 1995) and (Victor & Ronald, 2006) and vitamin E enriched-diets was found to have a protective effect on oxidative DNA damage (Morin et al., 2008). So, Vitamin E deficiency combined with free radicals generation was highly involved in the proliferation of the aberrant crypt foci. Furthermore, Orlistat may inhibit the absorption of vitamin D, which plays an important role in the colonic cancer prevention (Murillo et al., 2010).

A recent explanation of these results that showed increasing in PCNA-LI positive cells in rats fed on HFD that was designed by Bernstein and his coworkers (2011) that a high fat diet is associated with increased risk of cancer colon. HFD may have its carcinogenic potential mediated through the action of bile acids which act as tumor promoters, and that some dietary anti-oxidants may ameliorate this carcinogenicity.

The higher number of the preneoplastic PCNA-LI positive cells observed in rats fed on Orlistat and HFD was due to the linear-accumulative effect on tumor cells (Dowling et al., 2009). The exact mechanism is not fully known; while David (1997) noted fatty changes and fatty infiltrations of rat tissues “probably attributed to inhibition of cellular lipases” due to absorption of the drug. Thornton & MacDonald (Thorton & MacDonald, 1997) proposed that the dietary fat induces changes in the cell membrane lipid composition and proliferation in the colon and these changes may be related to the development of tumors. The free radicals formation may be involved in these changes, since a diet rich in fat increases the in vitro formation of a reactive oxygen species (ROS) in feces (Erhardt et al., 1997). ROS was found by Jain et al. (2007) to cause colonic mucosal damage.

The authors thought that the possibility of colon cancer was raised in the view of increased lipid content in the colonic fecal matter and current epidemiological data linking human colon cancer and increased fat intake. Contrary to the present results; EMEA, (2005) stated that the available epidemiological evidence showed that the relationship between fat intake and colonic cancer is doubtful. Increased energy intake was incriminated as the main risk factor whereas fat intake could be a confounding factor.
To best of the knowledge; this is the first time to try Ginseng to prevent the possible premalignant pathological changes induced by Orlistat and or high fat diet. Ginseng is believed to have been used in medicinal preparations for about 2000 yr in Oriental countries. Several pharmacological activities have been reported for Ginseng (Fukushima et al., 2001). Preventive effects of Ginseng against cancer development have been observed by many authors (Saw et al., 2010), (Choi et al., 2011), (Hao et al., 2011) and (Toh et al., 2011). The present results proved that Ginseng significantly decreased the PCNA-LI positive cells in both Orlistat and or high fat diet-treated rats. Saponins were considered to be the major active components of red Ginseng. Saponins have been reported to have antimutagenic activity, inhibiting tumour angiogenesis and metastasis as well as reducing growth of several tumour cell lines (Li et al., 2000).

CONCLUSION & RECOMMENDATION

1- Orlistat and or HFD increased the proliferating marker PCNA in the colon. So, people with vulnerability to cancer colon must avoid Orlistat intake as well as HFD. This is an important alarm to change bad habits in feeding and avoid unnecessary medications.

2- Broadcasting this effect must be done for both physicians and people.

3- Ginseng caused a marked decrease of the high PCNA-LI induced by chronic Orlistat administration and or HFD. Thus, Ginseng must be added to Orlistat as a protective agent to ameliorate its hyperproliferating effect.
Table (1): Mean values for PCNA-LI positive cells among studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>$M \pm SD$</th>
<th>$M \pm SD$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7.60 ±2.37</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>8.30 ±2.21</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>23.60 ±2.22</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>22.700 ±2.31</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>32.800 ±2.29</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>15.2 ±1.93</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>15.1 ±2.13</td>
<td></td>
</tr>
<tr>
<td>VIII</td>
<td>23.3 ±1.77</td>
<td></td>
</tr>
</tbody>
</table>

Group I: control group
Group III: High Fat Diet group (HFD)
Group V: Orlistat + HFD group
Group VII: Orlistat + Ginseng group

Table (2): Statistical comparison for PCNA-LI positive cells between control and other studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>I vs II</td>
<td>0.700</td>
<td>0.657</td>
<td>0.527</td>
</tr>
<tr>
<td>I vs III</td>
<td>16.00</td>
<td>17.411</td>
<td>0.0001**</td>
</tr>
<tr>
<td>I vs IV</td>
<td>15.10</td>
<td>15.733</td>
<td>0.0001**</td>
</tr>
<tr>
<td>I vs V</td>
<td>25.200</td>
<td>27.139</td>
<td>0.0001**</td>
</tr>
<tr>
<td>I vs VI</td>
<td>7.60</td>
<td>8.143</td>
<td>0.0001**</td>
</tr>
<tr>
<td>I vs VII</td>
<td>7.500</td>
<td>6.277</td>
<td>0.0001**</td>
</tr>
<tr>
<td>I vs VIII</td>
<td>14.700</td>
<td>17.99</td>
<td>0.0001**</td>
</tr>
</tbody>
</table>

Group I: control group
Group II: Ginseng group
Group III: High Fat Diet group (HFD)
Group IV: Orlistat group
Group V: Orlistat + HFD group
Group VI: HFD + Ginseng group
Group VII: Orlistat + Ginseng group
Group VIII: Orlistat + HFD + Ginseng group

P ≤ 0.05: significant
P < 0.001: highly significant

vs: versus
Table (3): Effect of Ginseng treatment in PCNA-LI positive cells in the studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>II vs VI</td>
<td>6.90</td>
<td>6.866</td>
<td>0.0001**</td>
</tr>
<tr>
<td>II vs VII</td>
<td>6.80</td>
<td>8.500</td>
<td>0.0001**</td>
</tr>
<tr>
<td>II vs VIII</td>
<td>14.00</td>
<td>19.170</td>
<td>0.0001**</td>
</tr>
</tbody>
</table>

Group II: Ginseng group  
Group VII: Orlistat + Ginseng group  
P ≤ 0.05: significant
vs: versus

Table (4): Comparison of PCNA-LI positive cells among different treated groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>III vs V</td>
<td>9.20</td>
<td>9.783</td>
<td>0.0001**</td>
</tr>
<tr>
<td>III vs VI</td>
<td>8.4</td>
<td>18.578</td>
<td>0.0001**</td>
</tr>
<tr>
<td>III vs VIII</td>
<td>1.300</td>
<td>1.414</td>
<td>0.191</td>
</tr>
<tr>
<td>IV vs V</td>
<td>10.10</td>
<td>8.787</td>
<td>0.001**</td>
</tr>
<tr>
<td>IV vs VII</td>
<td>7.60</td>
<td>9.601</td>
<td>0.0001**</td>
</tr>
<tr>
<td>IV vs VIII</td>
<td>0.40</td>
<td>0.524</td>
<td>0.613</td>
</tr>
<tr>
<td>V vs VIII</td>
<td>10.50</td>
<td>14.932</td>
<td>0.0001**</td>
</tr>
<tr>
<td>VI vs VIII</td>
<td>7.10</td>
<td>7.398</td>
<td>0.0001**</td>
</tr>
<tr>
<td>VII vs VIII</td>
<td>7.20</td>
<td>7.137</td>
<td>0.0001**</td>
</tr>
</tbody>
</table>

Group III: High Fat Diet group (HFD)  
Group V: Orlistat + HFD group  
Group VII: Orlistat + Ginseng group  
P ≤ 0.05: significant
vs: versus

Group IV: Orlistat group  
Group VI: HFD + Ginseng group  
Group VIII: Orlistat + HFD + Ginseng group  
P<0.001: highly significant**
Figure (1): Photomicrograph of control rat colon showing normal appearance of colonic mucosa lacking criteria of aberrant crypt foci (ACF). The surface openings of the glands appeared of equal sizes, not elevated, and patent with no slit-like openings (Methylene blue, X100).
Figure (2): Photomicrographs of rat colons labeled for PCNA, showing: A) control groups with normal mucosa which traversed by intestinal crypts (glands), lamina propria (lp), and muscularis mucosa (mm). The mucosal lining showing slight immunolabeling which mainly localized to the lower part of the glands (arrows). B) Groups III, IV, VIII showed increased immunolabeling of the glands. C) Group V showed extensive immunolabeling. D) Groups VI & VII showed decreased immunolabeling (Immunohistochemistry, X100).
Figure (3): Photomicrographs of cross sections of rat colons labeled for PCNA, showing: A) control groups with few immunolabeled cells (arrows). B) Groups III, IV, VIII showed increased immunolabeling of cells "arrows". C) Group V showed extensive immunolabeling of cells "arrows". Notice areas of stratifications "circles". D) Groups VI, VII showed decreased immunolabeled cells "arrows" (Immunohistochemistry, X 1000).
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دراسة التكاثر الخلوى القولوني الناتج عن التسمم المزمن بعقار الأورليستات والتآثر الوقائي المحتمل للجنسن في حيوانات التجارب

المشتركون في البحث

إيبان إسماعيل خسن
جعنة عطية اسحق
*نشوة فتحي الطحاوي

من قسم الطب الشرعي والمسموم والبهوثي - جامعة المنها

يستخدم عقار الأورليستات كمضاد للسمة وهو مناج كعقار يمكن استخدامه لفترات طويلة بدون أن يسبب أي من سوء التقدم ما يؤدي إلى حدوث تسمم مزمن. تمت هذه الدراسة لتوسيع تأثير عقار الأورليستات على التكاثر الخلوي القولوني في دورة في تكوين الور الجريبية القولونية للمعدة، ودراسة التأثير المحتمل لجنسن على حيوانات التجارب. أجريت هذه الدراسة على ثمانية ذكور أليساير بالعظام التي قسمت إلى 8 مجموعات: المجموعة الأولي (الضابطة) غذيا على غذائها الطبيعى، المجموعة الثانية (المستضيفة) أضيف 1% جنسن أحمى إلى غذائها، المجموعة الثالثة (الغذائية عالية الدهون) أضيف إلى غذائها 10% زيت لديك، المجموعة الرابعة (الأورليستات) أعطيت العقار بجرعة 125 مجم / كجم / اليوم من طرق الفم، المجموعة الخامسة أعطيت الأورليستات + التغذية عالية الدهون، المجموعة السادسة أعطيت الورجنسن + الجنسن + الفحص، المجموعة السابعة أعطيت الأورليستات + التغذية عالية الدهون + الجنسن، المجموعة الثامنة أعطيت النسب الأولى الجنسن + الفحص. وبعد 16 أسبوعا، تم ذبح الفئات وصيغ القولون لكل منهما عمق 100 مل ذئب اللد بالإضافة للتكاثر الخلوي النوري كкалاغي للتكاثر الجلدي وكمؤشر لزيادة التقابلية للبكتيريا الجهاز الهضمي. وقد أظهرت النتائج أن كل من الأورليستات أو النسبة عالية الدهون أو كليهما سبب في زيادة المحتوى النوري لردة الولد والتكاثر النوري ممنهمة باستخدام عقار الأورليستات + التغذية عالية الدهون. molest لكي النتيجة مقارنة بالمجموعة الضابطة. وقد سبب الجنسن مضافا إلى الأورليستات أو التغذية عالية الدهون أو كليهما معا نقص ذو دلالة إحصائية في كلا من المحتوى النوري وتوزيع الولدة المختلفة. ولهذا، نوصي الدراسة بتوسيع تأثير التسمم المزمن بعقار الأورليستات للأفياء المماثلة في هذا المجال أيضا لعدة من خلال نشر النتائج. ونوصي بإضافة الجنسن مع الأورليستات كعامل وقائي للحد من تأثيره التكاثري على الغشاء الخاطئ للقولون.