Original Article

The possible ameliorating role of Aloe vera gel on the toxic Effect of Acrylamide on the jejunum of Female albino rats (Light and Scanning Electron Microscopic study)

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ABSTRACT

Introduction: Acrylamide, an industrial chemical found in many industrial applications as well as for chemical and environmental purposes. The Aloe vera plant Known for many years and was known and used for its health, beauty, medicinal and skin care properties. The name Aloe vera derives from the Arabic word "Alloeh" meaning "shining bitter substance," while "vera" in Latin means "true." 2000 years ago.

Objective: The aim of the study was to evaluate the role of the Aloe vera extract and its influence on experimental lesions in the jujenal mucosa caused by Acrylamide.

Materials and Method: Thirty adult female albino rats were used and divided equally into three groups (10 rat/eachgroup): (I)control group. (II)Acrylamide treated group (5 mg/kg/day). (III) Acrylamide&Aloe vera treatedgroup (0.4 ml/100g).The experiment extended for 3 weeks, thejejunum samples were collected, prepared and studded by light microscope and scanning electron microscope.

Results: This study revealed that acrylamide administration to rats induces pathological changes in the jejunum in the form of the fused villi;irregular mucosal folds; cellular infiltration and thickened discontinued muscularis externa, with electron microscopic study there were areas of erosion and ulceration of the villi with loss of the normal picture. Administration of Aloe vera gel restored the normal architecture of the villi and crypts with normal appearance of most layers, scanning electron microscopy showed intact epithelium withgoblet cells openings discharging mucus.

Conclusion: Acrylamide markedly affect the jujenal mucosa as seen by light and electron microscope, Aloe vera gel administration can protect the mucosa from this harmful effect.

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Key Words: Acrylamide, aloe vera, jejunum, rats.

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INTRODUCTION

Acrylamide (or acrylicamide) is a chemical material with the chemical formulaC3H5NO, it has a molecular weight of 71.08 g. Its IUPAC name is 2- propenamide. It appear as a white odorless crystalline solid, soluble in water, ethanol, ether, and chloroform[1].

Food and cigarette smoke are the major sources of acrylamide exposure[2,3].

According to the European Food Safety Agency (EFSA), processed potatoes together with coffee and cereal based food (Crisps), French Fries, Crackers, Toast, Bread Crisps, Cookies, Boxed Breakfast Cereal, Corn Chips (Crisps), Bakery Products, Coffee, Cocoa are the major sources of acrylamide in the diet[4].

Herbal drugs can be effective in improving the quality of life of patients. Aloe vera (AV) is a stemless, drought-resisting succulent of the lily family, found mainly in warm and dry areas[5].

Aloe vera gel has various properties, including anti-inflammatory[6], anti-oxidant[5], antimicrobial[7], immune system enhancer[8] and treatment of intestinal disorders[9].

Aloe vera gel was suggested and used by patients for the treatment of multiple inflammatory digestive and skin diseases, including inflammatory bowel disease[10].

This work was aimed to evaluate the effects of acrylamide on the rat jejunum and to determine the possible protective effect of Aloe vera gel as an antioxidant and anti-inflammatory.

MATERIALS AND METHOD

Chemicals

Acrylamide (99% pure) has been brought from Sigma Chemical Company (St Louis, MO, USA).Acrylamide monomer appear as a white crystalline form, soluble in water, ethanol, methanol and acetone. Its chemical formula is CH2CHCONH2.

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Aloe vera gel extraction

Fresh Aloe vera leaves were washed in clean water to remove debris,they were dried using a clean cloth. Leaves were cut longitudinally to expose gel. The gel was gently scraped into an electric blender to remove any block. The preparation was done daily without storage to prevent contamination.

Thirty adult female rats (150-200 grams) were used, obtained from the Animal House of Faculty of Medicine, Sohag University, Egypt. Rats were kept under hygienic conditions. Standard food and water ad-libitum were administrated.

Ethical approval: The experiment was performed according to the "Guide for the Care and Use of Laboratory Animals" (Institutes of laboratory Animal Research)[11] and in accordance with the guidelines of the Sohag University Animal Ethics and approved by Research Ethics Committee considering care and use of laboratory animals registration number:Soh-IACUC-12-22-5).

Experimental design (The experiment was done through February 2021): After a 7-day acclimatization period, animals were equally divided into three groups:

Group I(control): given saline only;

Group II: acrylamide monomer (AM) 5 mg/kg/ daywas dissolved in 5 ml distilled water so each 1ml water contained 1mg acrylamide[12].

Group III: acrylamide monomer (AM) 5 mg/kg/day + Aloe vera gel 0.4 ml/100g body weight[13].

Acrylamide and saline were given orally once a day using a force-feeding needle for exactly 3 weeks.

Methods

After 24 hours from the last dose, animals were decapitated; small parts of the jejunumwere immediately excised and after 10% neutral buffered formalin fixation, specimens were dehydrated, cleared and embedded in paraffin wax. Transverse sections of 5 µm were stained with hematoxylin and eosin, Periodic acid Schiff's (PAS) for demonstration of mucus in goblet cells and enterocytes and Masson's trichome stain (for demonstration of collagen fibres)[14].

For scanning electron microscopic examination, jejunum specimens were fixed in 5% gluteraldehyde, rinsed several times with cocodylate buffer and then postfixed in 1 % osmium tetroxide. speciements were dehydrated in a graded ethanol series, exposed to liquid CO2 in a drying apparatus and coated with a thin layer of gold (10-15 um) deposited over the surface in vacuum evaporator[15]. Then examined by a Jeol-JSM-5400 LV scanning electron microscope in Assiut University Center.

Morphometric study and statistical analysis

The following measurements were taken: The mucosal diameter(maximal diameter) and the goblet cells density was measured using Digimizer (image analyzer computer system) from the three groups from H&E sections (mucosal thickness), PAS section (goblet cell number), 5 sections were examined at X 20 objective from 5 animals of each group.

Measurements (mean value ± Standard deviation) were analyzed usingSSPS program version 16 using one-way ANOVA anda post-hoc test when ANOVA was statistically significant (*P value* ≤0.05)[16].

RESULTS

Histological results

Control group: H&E stained sections showed the normal structure of the jejunum which appeared formed of mucosa, submucosa, musculosa and serosa: mucosa appeared with finger like villi witha central core and covered by a simple columnar epithelium (enterocytes) with goblet cells (Figure 1).

The enterocytes showed the normal columnar picture with oval basal nuclei and apical acidophilic cytoplasmand normal brush border. Invaginations in-between villi appeared(intestinal gland or crypts of Lieberkühn)they were formed of simple tubular structures lined by simple columnar epithelium. The submucosa appeared formed of a loose connective tissue layer and blood vessels. The Muscularisexterna consisted of two layers; the inner circular and the outer longitudinal. The most outer layer forming theserosa(Figures 2,3).

In group II:The mucosa of this groupshowed that the villi fused together and appeared as a thick& irregular mucosal folds with edema and obvious mononuclear cellular infiltration in the mucosa, bundles of acidophilic structures appeared in the submucosa. Intestinal crypts of Lieberkühn showed abnormal architecture. There were thickening and discontinuations of the muscularisexternaspecially in the inner circular layer, irregular serosa also seen. (Figures 4,5,6).

Group III:The mucosa of this group showed that most villi and crypts restored their architecture with normal distribution and appearance of enterocytes and goblet cells. The muscularisexterna showed normal architecturewith normal appearance of the submucosa and serosa (Figures 7,8,9).

With PAS Staining,At control group it showed well delineated PAS positivity of the brush border of the villi, PAS positive goblet cells of both villi and crypts, apparent decrease of goblet cells in the intestinal glands of group II with week reaction specially in the brush border,Group III showed restoration of the positive reaction in the brush border and normal distribution of goblet cells (Figures 10,11,12).

Examination of Masson'strichrome stained sections from group I showed normal fine collagen fibers in the CT core of villi, in-between the crypts and in the submucosa. In group II, obvious deposition of collagen in the lamina propria of the villi, in between the crypts

and in thesubmucosa, in Group III there were restoration of the normal distribution of the collagen fibersin the CT core of villi in-between the crypts and in the submucosa (Figures 13,14,15).

Scanning electron microscopic (SEM)results

Scanning electron examination of control jejunal mucosa showed intact Leavelike villi with irregular corrugations in-between, multiple orifices of Goblet cells secreting mucus also appeared. Flat topped enterocytes appeared with a honeycomb picture on the surface of the villi with stick like microvilli on the cell surface giving a granular picture (Figures 16,17,18).

In Acrylamide treated rats: Examination of jejunal mucosa showed flatting villi with loss of cellular structure and exposure of the underlying connective tissue, with disarranged microvilli (Figures 19,20,21).

In combined acrylamide and Aloe vera treated rats: villi appeared with intact epithelial. Goblet cells orifices were seen discharging mucus,some areas showed intact microvilli, on other enterocytes were absent (Figures 22,23,24).

Morphometric results

Mucosal thickness (Table 1, Histogram 1): The mean mucosa thickness diameter (MT) in control (1076±15) which showed a very highly significant decrease in comparison to group II (1198±55) and group III (1182.9±24).

Comparison between group II and group III showed non -significant difference

Goblet cell number (Table 1, Histogram 2): The mean goblet cells density were in control (group1)224.6±36 which was very highly significant increase in comparison to group II (182.4±21) and group III (186.8±19).

Comparison between group II and group III showed non -significant difference

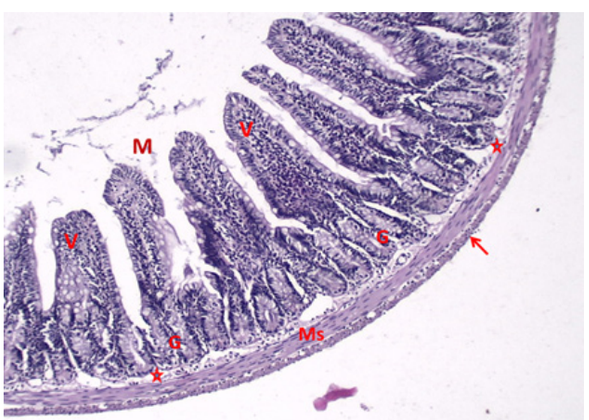


Fig. 1: A photomicrograph of a control jejunum showing normal mucosa (M) with finger like villi (V), intestinal crypts or glands (G), clear submucosa (stars), intact muscularisexterna (Ms) and intact serosa (arrow). (H&EX100)

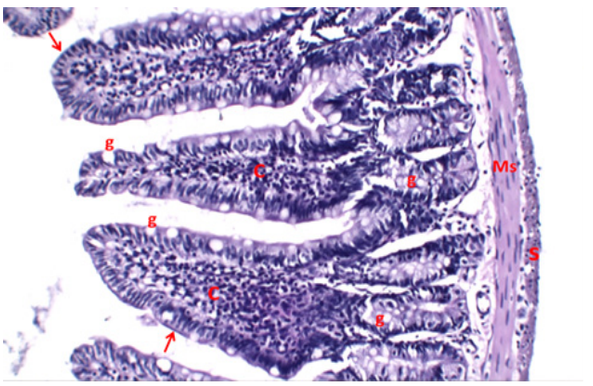


Fig. 2: A photomicrograph of a control jejunum (group1) (group1) showing; Villi with intact core (C), enterocytes (arrows) with intact brush borderand goblet cells(g) in the villi and in the intestinal glands, muscularisexterna (Ms) and serosa (S). (H&EX200)

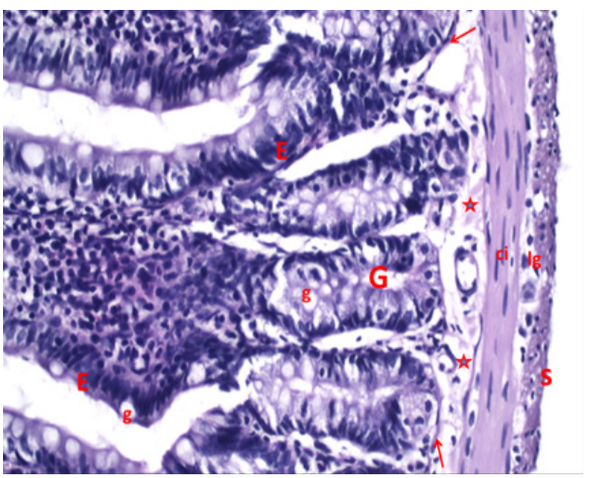


Fig. 3: Magnification of the previous sectionshowingenterocytes (E) and multiple goblet cells(g) lining the villi and intestinal glands (G) intact muscularis mucosae (arrows), submucosa (stars) with loose areolar tissue and blood vessels, inner circular muscularisexterna (ci), outer longitudinal muscularisexterna (lg) and outer serosa (S). (H&E. X400).

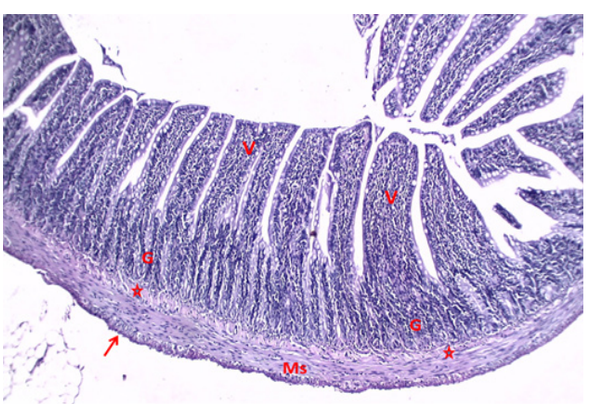


Fig. 4: A photomicrograph of a treated jejunum (group II) showing; fusion of the Villi (V),abnormal architecture of the intestinal glands(G), abnormal appearance of the submucosa (stars) with unclear appearance, thick muscularisexterna (Ms) with irregular shape of the serosa (arrow). (H&E. X100).

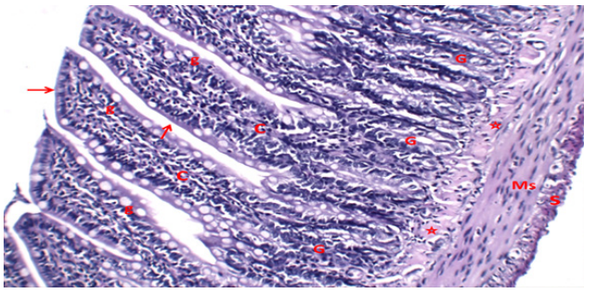


Fig. 5: A photomicrograph of a treated jejunum (group II) showing; fused flatted villi (arrow) massive inflammatory infiltration in the core of villi (C),glands(G) and submucosa (stars), multiple goblet cells(g) in the villi with little appearance in the glands(G), muscularisexterna (Ms) and Serosa (S) appear thick with disturbed cellular appearance. (H&E. X200).

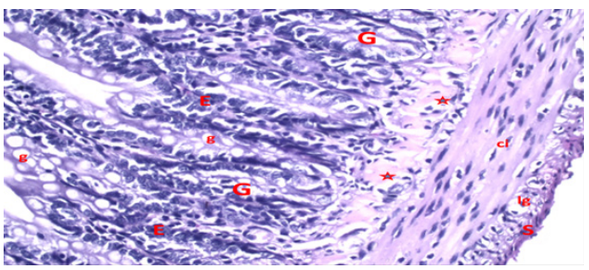


Fig. 6: Magnification of the previous sectionshowingabnormal shaped enterocytes (E) and goblet cells(g).Disturbed intestinal glands (G), abnormal disturbed submucosa (stars), inner circular muscularisexterna (ci) and outer longitudinal muscularisexterna (lg) appear with discontinuations and serosa (S) appear thick and irregular. (H&E. X400).

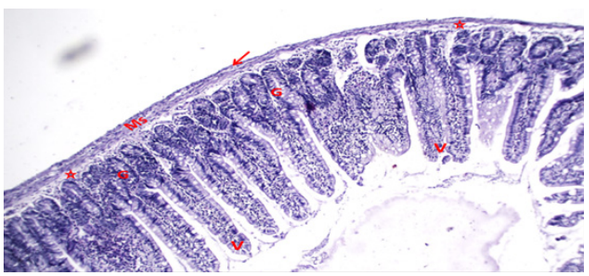


Fig. 7: A photomicrograph of a treatedjejunum(group III)showing; normal appearance of the villi (V), normal appearance of the intestinal glands (G), submucosa (stars) appear clear,muscularisexterna (Ms) and serosa (arrow) show normal picture. (H&EX100)

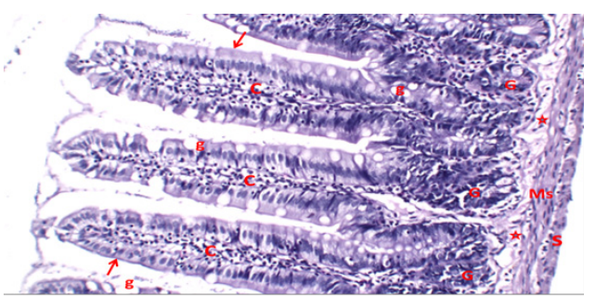


Fig. 8: A photomicrograph of a treatedjejunum(group III) showing; villi with intact core (C), striated border of enterocytes (arrows)and goblet cells(g) with normal distribution in the villi and glands. Submucosa (stars),intact muscularisexterna (Ms) and serosa (S) with regular normal picture. (H&EX200)

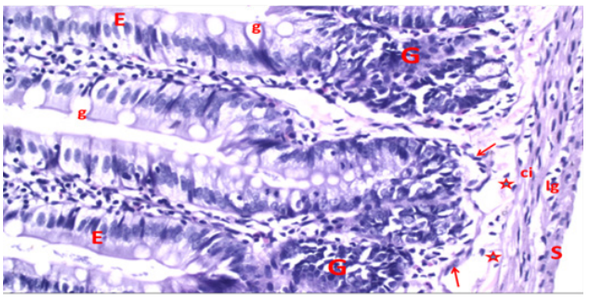


Fig. 9: Magnification of the previous sectionshowingenterocytes (E) and multiple goblet cells(g) with normal picture in the villi and intestinal glands (G) muscularis mucosae (arrows), submucosa (stars) with areolar normal picture. Inner circular muscularisexterna (ci), outer longitudinal muscularisexterna (lg), serosa (S) with normal appearance.(H&E. X400).

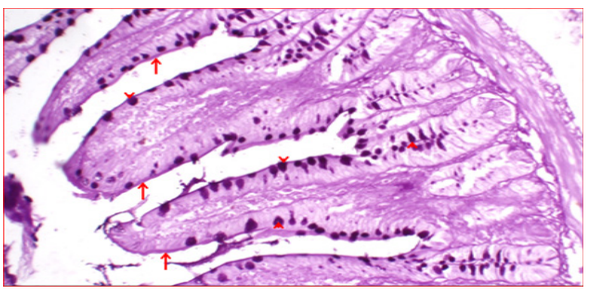


Fig. 10: A photomicrograph of a control jejunum showing strong intensity of PAS reaction in the goblet cells (arrowhead) and in the brush border of enterocytes (arrows)(x200)

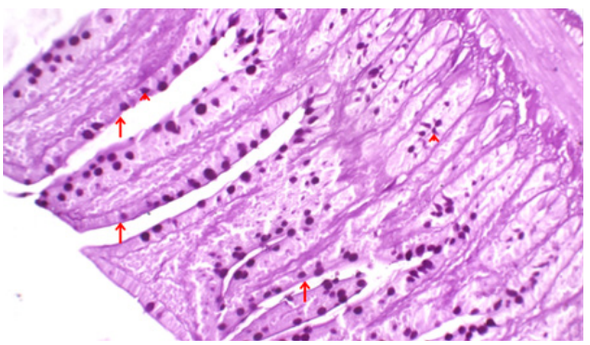


Fig. 11: A photomicrograph of a treated jejunum (group II)showing apparently few goblet cells in the intestinal glands (arrow head) with apparent weak intensity of PAS stain. Week reaction brush border of most enterocytes (arrows) (x200)

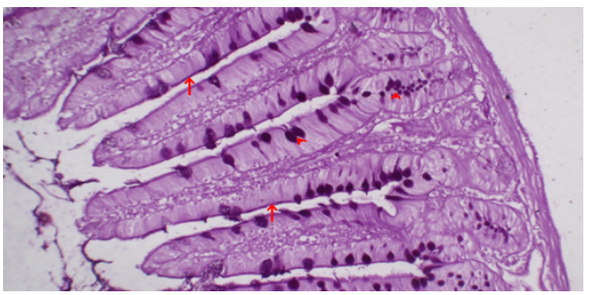


Fig. 12: A photomicrograph of a treated jejunum (group III) showingstrong intensity of PAS reaction in the goblet cells (arrowhead) and in the brush border of enterocytes (arrows)(x200).

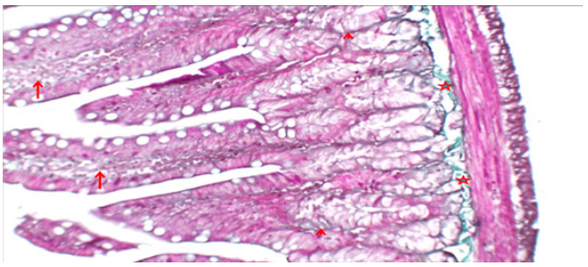


Fig. 13: A Photomicrograph of a controljejunumof a control rat stained with Masson's trichromeshowing fine green stained collagen fibers in thevillus core (arrow) and in between the crypts (arrowheads). Denser collagen fibers are seen in submucosa (stars)X200.

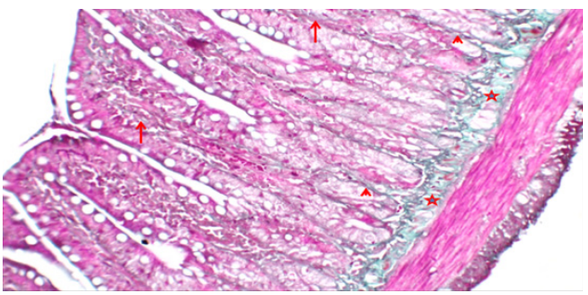


Fig. 14: A Photomicrograph of a treatedjejunum(group II)stained with Masson's trichromeshowing widespread green-stained dense thick collagen fibers in the villus core (arrow) and in between the crypts (arrowheads). Obvious deposition of collagen is seen in thesubmucosa (stars)(x200).

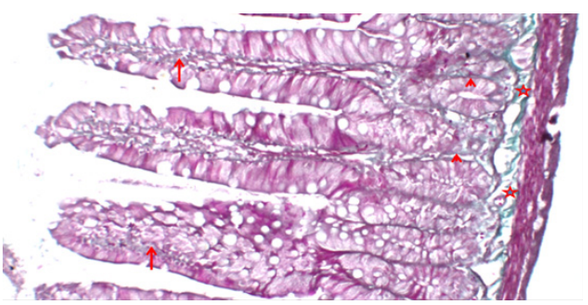


Fig. 15: A Photomicrograph of a treatedjejunum(group III)stained with Masson's trichromeshowing scattered green-stained collagen fibers in the lamina propriaof the villous core (arrows), in between the crypts (arrowheads) and in thesubmucosa (stars)(x200).

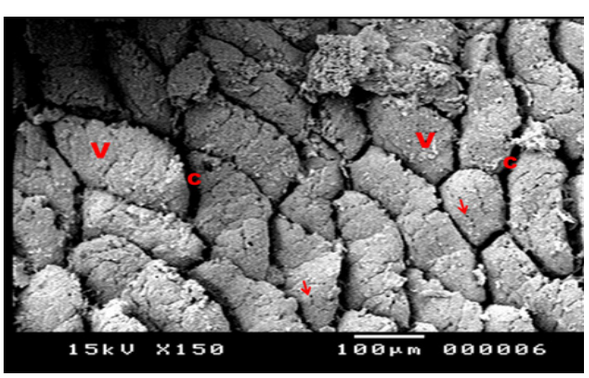


Fig. 16: A scanning electron micrograph of a control jejunum showing normal appearance of the villi (V) with crypts (C) in-between, multiple orifices of goblet cells (arrows) also appear. (Org. Mic. Mag. X 150)

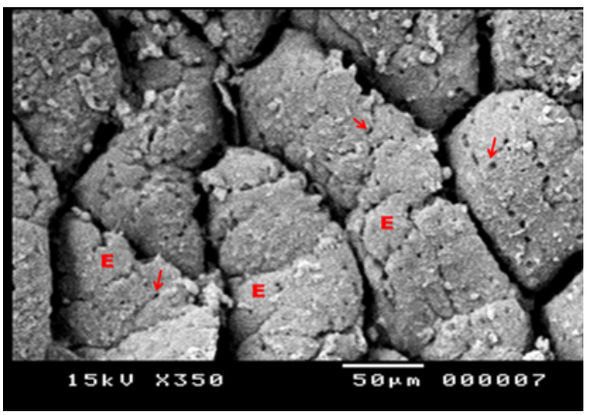


Fig. 17: A magnification of the previous picture showing normal corrugations of the villi in which enterocytes (E) appear with honeycomb picture, goblet cell orifices also appear prominent (arrows). (Org. Mic. Mag. X 350)

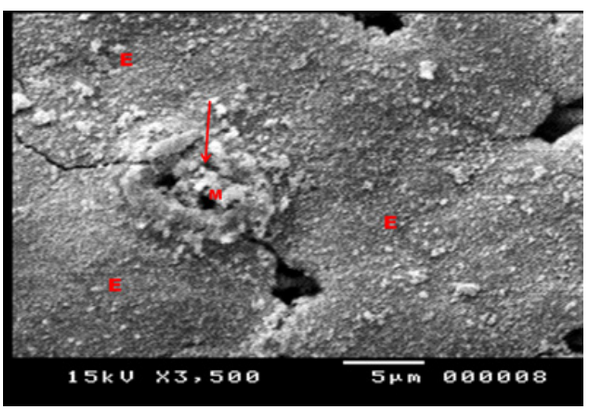


Fig. 18: magnification of the previous enterocytes (E) with closely backed microvilli and goblet cell orifice (arrow) discharging mucus (M). (Org. Mic. Mag. X 3500)

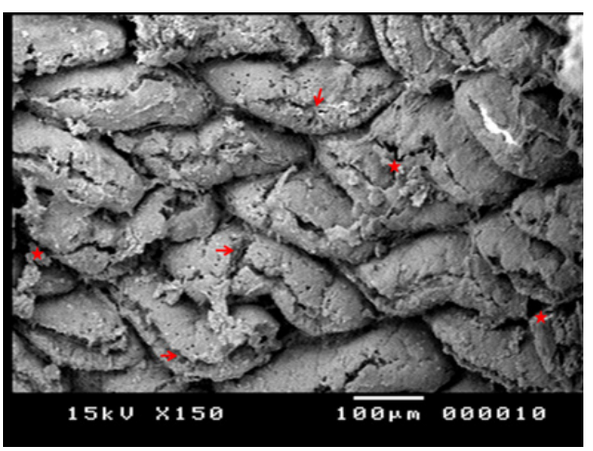


Fig. 19: A scanning electron micrograph of the jejunum of group II showing flatting of villi with loss of their cellular structure (arrow), with abnormal crypts inbetween (stars). (Org. Mic. Mag. X 150)

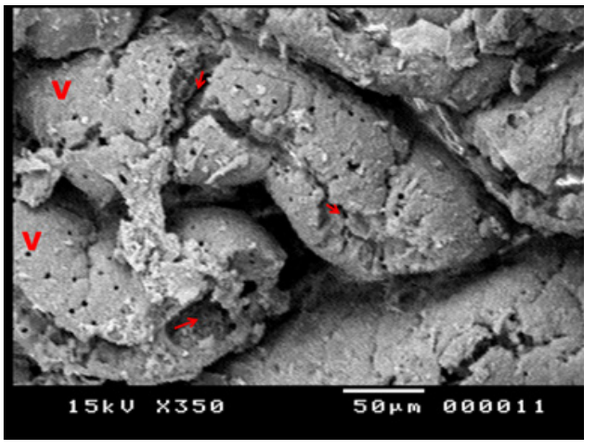


Fig. 20: A magnification of the previous picture showing destruction of the tops of the villi (V) with exposure of their underlying connective tissue core (arrows). (Org. Mic. Mag. X 350)

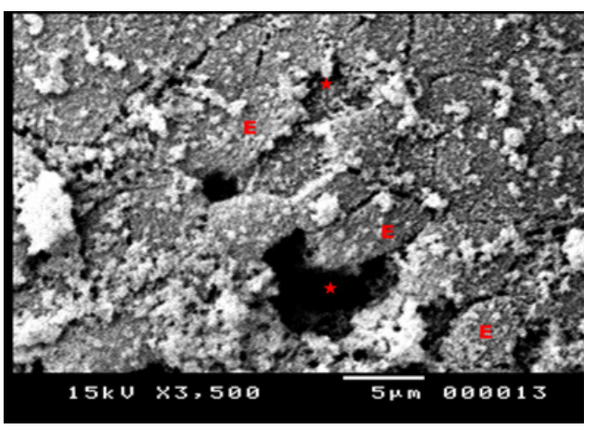


Fig. 21: A magnification of the previous enterocytes (E) which appear destructed with exposure of the underlying connective tissue (stars). (Org. Mic. Mag. X 3500)

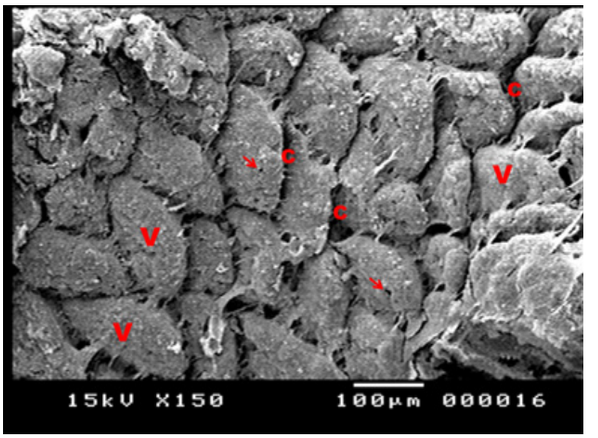


Fig. 22: A scanning micrograph of treated jejunum (group III) showing intact villi (V) and crypts (C) and goblet cell openings (arrows). (Org. Mic. Mag. X 150)

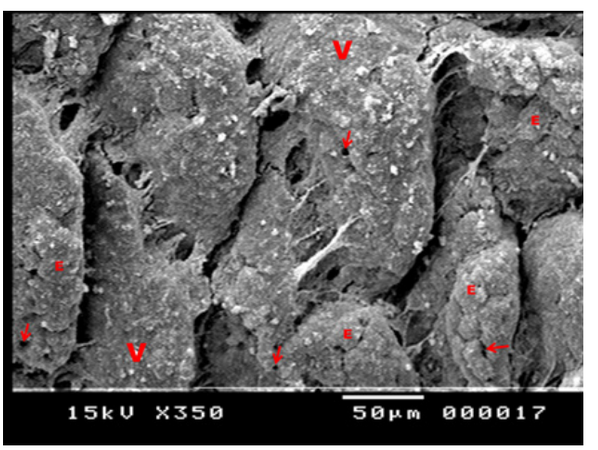


Fig. 23: A magnification of the previous picture showing intact villi (V), with normal enterocytes (E), most areasshowed prominentorifices of the goblet cell (arrows). (Org. Mic. Mag. X 350)

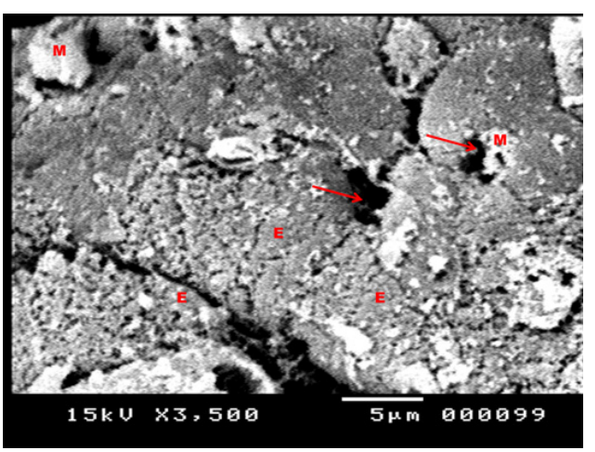
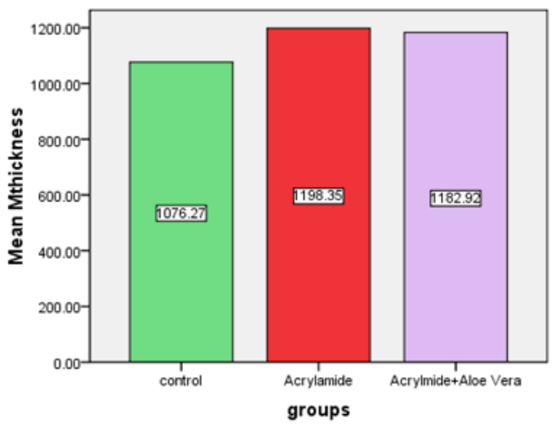


Fig. 24: A magnification of the previous disturbed enterocytes (E) with goblet cell orifice (arrow) discharging mucus (M). (Org. Mic. Mag. X 3500)

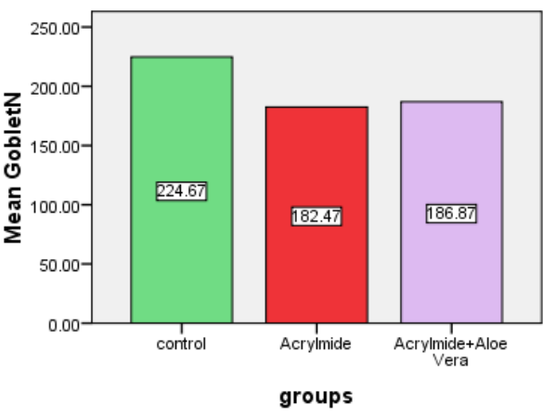
Table 1: Mucosal thickness and Goblet cell numbers in control and treated groups.(Mean± slandered deviation)

Groups	Group 1	Group 2	Group 3	ANOVA	P1	P2	P3
Mucosal thickness	1076.2±15	1198.3±55	1182.9±24	.000	.000***	000***	.48NS
Goblet cell number	224.6±36	182.4±21	186.8±19	.000	.000***	.001***	.89NS

P1=Difference between group 1 and 2
P2= Difference between group 1 and 3
P3= Difference between group 2 and 3
***: Very highly significant
NS: Non-significant



Histogram 1: Mean thickness of jejunal mucosa in control and treated groups



Histogram 2: Mean goblet cell number in control and treated groups

DISCUSSION

The importance of acrylamide as a food contamination was shown when Tareke *et al*. proved that rats fed with fried food showed an increase in the hemoglobin adduct level[17].

In this study, light microscopic examination of acrylamide treated jejunum showed fused, thick& irregular villi with edema and cellular infiltration in the mucosa and submucosa, intestinal crypts showed abnormal architecture, with thickened and discontinuations of the muscularisexterna and serosa

These results are in acceptance with previous results[18] which showed that acrylamide induced histological alteration in the jejunum of adult male albino rats in the form of broadening and shortening of villi and inflammatory cell infiltration in the lamina proria. Also acrylamide was suggested to causes histopathological changes of ileum, DNA changes which lead to pyknosis of the nuclei and necrosis of the cells[19].

Previous studies demonstrated that acrylamide induced oxidative stress. acrylamide affect the cellular redox chain and generate reactive oxygen species (ROS), which lead to activate the mitogenactivated protein kinase (MAPK)-JNKs that play a big role in regulation of apoptosis(the most important cell death type)[20,21].

Paulsson *et al*. (2002) reported neurotoxic and carcinogenic effect of acrylamide in animals which appear in mice more than rats[22].

Previous studies also suggested that acrylamide induce apoptosis in testes, bovine lens, neuroblastoma cells, human promyelocytic leukemia cells, and astrocytoma cells[23-27].

In this study rats with Aloe vera gel (AVG) administration showing restoration of the normal shape of the villi and crypts, disappearance of the inflammatory cells infiltration with normal appearance of muscularisexterna and serosa.

Aloe vera gel was used widespread by ancient and modern cultures for its anti- inflammatory and wound healing propertie[28].

The dietary use of AVG decrease the Intestinal polyps number resulted from high fat diet in mice[29].

Scanning electron microscopic of the jejunal mucosa in Acrylamide treated ratsin the present study revealed ulcerationsof the villiand disarranged microvilli.

These results are in acceptance with previous results[19] who found that the SEM study revealed that the ileal villi after acrylamide administration showed loss of the normal structure of the villi accompanied with prominent damage and bleeding.

This is in acceptance with previous studies which showed that acrylamide is known as an animal carcinogen and maybe human carcinogen present mainly in heated carbohydrates. acrylamide suggested also to be a germ cell mutagen, inducing predominant lethal mutations and chromosomal translocations in sperms of treated mice[30].

Studies also showed that acrylamide, by binding to glutathione (GSH) stores, led to a change in the redox status of cells and, so, induced apoptosis[31]. Acrylamide-also induced oxidative stress which may mediate the activation of glial cells and the release of proinflammatory cytokines and then neuronal damage[32].

Acrylamide induced Oxidative stress and the deficiency of antioxidants may play an important role in gastrointestinal tract damage[33].

Scanning electron microscopy of Aloe vera gel group showed intact epithelial with goblet cells openings discharging mucus.

Aloe vera gel consist of polysaccharides (55%), sugars (17%), proteins (7%), lipids (4%), minerals (16%), phenolic compounds (1%), and various vitamins such as vitamin A, C, E, B1,B 2,B12[34], it was Know that polysaccharides responsible for the most biological effects of Aloe vera gel[35].

Previous researches reported the positive regulation of natural polysaccharides on intestinal mucin expression as polysaccharides can be a key player in intestinal mucin expression and mucosal protection[36,37].

CONCLUSION

Acrylamide is a very toxic material and can damage the mucosa of the jejunum;the addition of Aloe vera gel has an ameliorating effect on this damage.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

الدور التحسني المحتمل لجل الصبار في التأثير السام لمادة الأكريلاميد على صائم اناث الجرذان البيضاء
(دراسة بالميكرسكوب الضوئى والالكترونى الماسح)

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المقدمة: مادة الأكريلاميد مادة كيميائية صناعية تستخدم على نطاق واسع في العديد من التطبيقات الصناعية وكذلك للأغراض الكيميائية والبيئية. نبات الصبار معروف ويستخدم منذ قرون لخصائصه الصحية والجمالية والطبية والعناية بالبشرة. يشتق اسم جل الصبار من الكلمة العربية "Alloeh" التي تعني "مادة مرارة مشرقة" ، بينما تعني كلمة "vera" في اللاتينية "true". قبل ٢٠٠٠ سنة.

الهدف: أجريت هذه الدراسة لتقييم دور مستخلص الصبار وتأثيره على التغيرات المرضية في الغشاء المخاطي للصائم التي تسببها مادة الأكريلاميد.

المواد والطريقة: تم تقسيم ثلاثين أنثى بالغة من الجرذان البيضاء إلى ثلاث مجموعات (١٠ جرذ لكل مجموعة): (I) مجموعة ضابطة. (II) المجموعة المعالجة بالأكريلاميد. (III) المجموعة المعالجة بالأكريلاميد والصبار. امتدت التجربة لمدة ٣ أسابيع ، وتم جمع عينات الصائم لدراستها بالمجهر الضوئي و الميكروسكوب الإلكتروني الماسح

النتائج: أظهرت هذه الدراسة أن مادة الأكريلاميد تسبب تغيرات مرضية في الصائم على شكل الزغابات المندمجة. طيات مخاطية غير منتظمة ,انتشار للخلايا الالتهابية وتسميك عضلي خارجي ، مع المجهر الإلكتروني كانت هناك مناطق تآكل وتقرح في الزغابات. أعاد استخدام جل الصبار البنية الطبيعية للزغابات والخبايا مع استعادة المظهر الطبيعي لمعظم الطبقات ، وأظهر الفحص المجهري الإلكتروني غشاء مخاطى سليم فى معظم المناطق.

الخلاصة: تؤثر مادة الأكريلاميد بشكل ملحوظ على الغشاء المخاطي للصائم كما اتضح بالمجهرالضوء والإلكتروني الماسح ، ويمكن لجل الصبار حماية الغشاء المخاطي من هذا التأثير الضار.