

Hypoglycemic and toxic effects of saponins from the fruit of bitter apple [*Citrullus colocynthis* (L.) Schrad] on the internal organs of Norway rat [*Rattus norvegicus* (Berkenhout)]

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ABSTRACT

Nowadays, natural products receive attention to research centers because of their special importance in safety of communities. The curative properties of these plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites. The main objectives of this study were to evaluate saponins toxic effects as reflected in the histopathological changes in the internal organs of Norway rats and to study the hypoglycemic effects induced by the same group. Powdered dried fruit parts (rinds, pulps and seeds) of bitter apple were screened for detection of saponins, glycosides, terpenes, anthraquinones, flavonoids, tannins, alkaloids, coumarins, cardiac glycosides, cyanogenic glycosides and cucurbitacins. The results of the qualitative analysis and phytochemical screening profiles indicated that, most of the natural products tested for were present in the plant material, except cyanogenic glycosides. Crude saponins were extracted from the rind and their acute toxicity was determined on Norway rats. Five dosage levels (60, 70, 80, 90 and 100 mgs saponins/rat) were administered intraperitoneally

to each batch of albino rats (4 rats/batch). The histological changes in the liver exhibited prominent nucleolus, open chromatin, congestion of central veins and liver sinusoids. The kidney showed signs of reduced renal tubules and lining of epithelial cells. The stomach and digestive system showed mucosal edema, open chromatin, prominent nucleolus and structural necrosis. Percentages reduction in glucose level in treated rats and the control after six hours were 42%, 55.4%, 60%, 62.5%, 69.3 and 0.10 %, following the same order. There was a considerable dose-dependent decrease in the mean concentration of glucose in the plasma of the treated rats.

INTRODUCTION

The search for agents to cure infectious diseases began long before people were aware of the existence of microbes. These early attempts used natural substances, usually native plants or their extracts and many of these herbal remedies proved successful. The effective substances of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents (Sofowora, 1982).

Biter apple is traditionally used as an antidiabetic medication in tropical and subtropical countries (Diwan *et al*,2000).Its fruit has been recommended for indigestion and diabetic people in traditional medicine. Cases of acute toxic colitis after ingestion of biter apple have been reported (Nmila *et al*, 2000).Toxicity studies on small ruminants suggest that the fruit causes organ damage in the liver, kidney and gastrointestinal tract. A dose of 800 mg/kg of the ethanolic extract of the leaves killed 60% of the treated rats. Pharmacological examination of the surviving animals and histopathological observations suggested hepatorenal damage (Diwan *et al*, 2000).

MATERIALS AND METHODS

Phytochemistry

Site of the experiments

The experiments were carried out in the Chemistry Laboratory of the Faculty of Agricultural Sciences, University of Gezira (U of G), Wad Medani, Sudan (14° 24'N 33° 29'E, 408 masl).

Origin of colocynth materials

Fruits (rind, pulp, and seeds) of biter apple, were used as basic materials in this study. They were collected from the natural habitat around Wad Medani town immediately after the rainy-season.

Preparation of colocynth materials

The collected samples were dried at room temperature. Pulps, rinds and seeds were manually separated from each other and then powdered using an electric blender. The powders of both rind and pulps were kept in glass bottles for further use.

Phytochemical analysis of the fruits

Qualitative analysis was carried out for the following secondary metabolites:

Saponins

The dried powder of the fruits (5g) was extracted with 20mls ethanol (50%) and filtered through filtration unit. Aliquots of the ethanol extracts (10 ml each) were evaporated to dryness under reduced pressure through filtration unit. The filtrate was manually vigorously shaken. If a voluminous froth (honey comb) was developed and persisted for almost one hr, this indicated the presence of saponins (Harborne, 1998).

Glycosides

The dried powder (30g), was boiled with an aliquot of distilled water (100 ml) and filtered. Aliquots (2ml each) of the filtrate were tested for glycosides as described by Harborne (1998). The filtrate was dissolved in 2 ml of glacial acetic acid. To this solution, two drops of ferric chloride solution were added and mixed. The mixture was transferred to a narrow test tube. Concentrated H₂SO₄ (1-2 ml) was added carefully on the side of the tube using a pipette to form a layer. In presence

of glycosides, a reddish brown layer at the interface will be formed, and the upper layer gradually acquires a bluish-green colour, which darkens on standing.

Flavonoids

Twenty g of the powder was macerated in 1% of HCl (50 ml) overnight, filtered and the filtrate was subjected to the following test:

Ten ml from each filtrate was rendered alkaline with NaOH (10%,w/v); the presence of yellow colour indicated the presence of flavonoids (Balbaa, 1974).

Tannins

The dried powder (5 g) was extracted with Et.OH (50%) and filtered. Ferric chloride reagent was added. The appearance of green colour, which changes to a bluish black colour or precipitate, indicates the presence of tannins (Balbaa, 1974).

Sterols triterpenes

The dried powder (1 g) was extracted with petroleum ether (10 mls each) and filtered. The filtrate was evaporated to dryness, using water bath, and the residue was dissolved in chloroform (10 ml). Aliquots of chloroform-extract (3 mls each) were mixed with concentrated acetic anhydride (3 ml), and a few drops of H₂SO₄ were added. The formation of a reddish violet ring, at the junction of the two layers, indicated the presence of unsaturated sterols and/or triterpenes (Harborne, 1998).

Alkaloids

The dried powder (5 g) was extracted with Et.OH and filtered. Aliquots from the Et.OH-extract (10 ml each) were mixed with aqueous HCl (20 ml; 10% v/v), and filtered. The filtrate was rendered alkaline with NH₄OH and extracted with successive portions of chloroform. The combined chloroform-extract was evaporated to dryness in water bath; the residue was dissolved in HCl and tested with Mayer's reagent. If a precipitate was formed (turbidity), it indicated the presence of alkaloids (Harborne, 1998).

Coumarins

The dried powder of the fruits (5 g) were subjected to sublimation and a filter paper moistened with NaOH solution was exposed to the sublimate and, then examined for any fluorescence under UV light. The appearance of blue or green colour indicates the presence of coumarins (Harborne, 1998).

Cardiac glycosides

According to Harborne (1998), since all cardiac glycosides possess a steroid nucleus, as well as an α - β unsaturated lactone ring at C17, tests used to detect the steroid nucleus and the lactone moiety were considered sufficient evidence for the presence or absence of cardiac glycosides in each plant part. In addition, cardiac glycosides possess deoxy sugars with a OH

group replaced with H. Keller's reagent describes its use to detect the principal components of cardiac glycosides. The reaction with this reagent is also known as the Keller-Kiliani reaction. Lack of the Keller-Kiliani reaction suggests the absence of deoxy sugar. The following tests were carried for the detection of cardiac glycosides:

Unsaturated steroidal nucleus (Liebermann-Burchard reaction)

The dried powder (5 g) was extracted using a Soxhlet apparatus with 95% Et.OH for 6 hr. The extract was evaporated to dryness on a waterbath (70°C), and the residue was extracted several times with portions of petroleum ether to remove parts of pigments. The defatted material was extracted with 20 mls chloroform, and the chloroformic extract was dried over anhydrous Na₂SO₄. To 5 mls of this chloroformic solution, 0.5 ml of acetic anhydride was added, followed by two drops of H₂SO₄.

A change in colour to green or blue indicated the presence of sterols.

The lactone moiety

Baljet's colour reaction: The chloroformic-extract and the alcoholic extract were spotted on a piece of filter paper. The spots were completely dried under a current of warm air and, then sprayed with Baljet reagent and dried. A colour change to orange or red ring around the spots is considered as a positive test (Hwida, 2010).

The deoxy sugar

The dried powder of the fruits (5g) was extracted with Et.OH and filtered. Five ml of the extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. The mixture was under lied with 1 ml of conc. H₂SO₄. A brown ring of the interface indicated a deoxy sugar.

Cyanogenic glycosides

Five grams of the dried powder of the fruits of bitter apple plant were placed in a 125 Erlenmeyer flask and sufficient distilled water was added to moisten the sample, followed by 1ml of chloroform. A piece of freshly prepared sodium picrate dried paper was carefully inserted between the splitted cork used to stopper the flask. A change in the colour of the sodium picrate paper from yellow to different shades of red is taken as an indication of the presence of cyanogenic glycosides (Harborne, 1998).

Anthraquinones

To 5 g of the dried powder of the fruit, 20 mls of 20% aqueous sulphuric acid and, two ml of 2% aqueous ferric chloride solution were added and refluxed for 30 min. The mixture was allowed to cool and filtered.

The filtrate was extracted with 20 ml chloroform. Ten ml of the chloroform solution was shaken with 5 ml of 10% ammonium hydroxide solution, and the two layers were allowed to separate. A change in the colour of the alkaline layer from a pink to red colour was taken as indication of the presence of anthraquinones (Harborne, 1998).

Cucurbitacins

Five g of the dried powder of each part of the bitter apple plant were transferred into a test tube; absolute Et.OH (15 ml) was added to each test tube, then maintained in a water bath at 60°C for two hr and filtered. The filtrate was completed to 50 ml with absolute Et.OH, and one ml of phosphomolybdic acid (2%) in absolute Et.OH was added. The formation of a green colour, which changes to a bluish black colour or precipitate, indicates the presence of cucurbitacin compounds (Attard and Scicluna-Spiteri, 2001).

Isolation of crude saponins

The saponins were isolated according to a method described by Feroz *et al.* (1993), and Otsuka *et al.* (1997). In brief, 30g of the rind (epicarp and outermost layer of mesocarp) of the fruit was sun-dried, powdered and defatted in a Soxhlet with petroleum ether at 40-60°C for 16 hr .The residue was added to absolute methanol and left overnight under reflux at 70°C. It was then filtered, and the filtrate was evaporated to dryness. The yield was dissolved in distilled water extracted in a separatory funnel with 1-butanol (three times), and dried by evaporation. Finally, the extract was dissolved in absolute Me. OH and saponins were precipitated by adding diethyl ether. A yellowish-brown dry powder of pure saponins was collected (5g in four intervals) and identified by their frothing and formation of the honey comb.

Test animals

Adult Sprague Dawely rats (3 months old), of both sexes and weighing 130-140g were used in the study. Rats were supplied by the Animal House, National Institute for Medicinal and Aromatic Plants, The National Center for Research (NCR), Khartoum, Sudan. The rats were housed in plastic cages

with bar lids used to hold water bottles in accordance with the protocol set by Institutional Animal Care and Use Committee (IACUC)

(Rand,2001).These rats were kept under controlled conditions of temperature (20-30°C), R.H. (50%) and L:D (Ligt-Dark) periods (12:12-hrs).

Rats were fed with meals composed of minced meat, flour and drinking water. All rats were apparently healthy. An acclimatization period of 7 days was allowed before experimentation.

Intraperitoneal (I.P.) administration of saponins

Twenty four rats were used in this experiment, divided into 6 groups of 4 animals each, 2 males and 2 females/ group. Groups 1-5 received 1ml of saponins solution at a dosage level of 60, 70, 80, 90, and 100 mg/rat, respectively. Group 6 was used as a control and received 1 ml of saline solution /rat. The animals were kept under observation for 96 hr.

Histochemistry

Site of the experiments

Histopathological studies were conducted in the Medical Laboratory Faculty of Medicine, U of G, Wad Medani, Sudan.

Tissue processing

The experimental animals were dissected immediately after death and their stomachs, small and large intestines, liver, heart and kidneys were excised. The organs were cut in appropriate sizes and fixed in 10% buffered formalin for two days. The samples were transferred to a cassette, a container designed to allow reagents to freely act on the tissue inside. This cassette was immersed in a processing machine with multiple baths of progressively more concentrated ethanol to dehydrate the tissue, followed by xylene, and finally extremely hot liquid paraffin. During this 16 hr process, paraffin replaced the water in the tissue, turning soft, moist tissues into a sample miscible with paraffin (Kumar *et al.*, 2009).

Embedding

The processed tissues were then taken out of the cassettes and set in a dispenser Through this process of embedding, additional paraffin was added to create a paraffin block, which was attached to the outside of the cassette. The process of embedding then allowed the sectioning of tissues into very thin (2-7 μm) sections using a microtome for microscopic examination. The slices were thinner than the average cell, and are layered on a glass slide for staining.

Staining

The organ tissues were stained with H&E .The sections were examined by a phase contrast microscope (Leica), and were photographed by a digital camera (Cassio Exailim 12.1 mega pixel).

Examination of slides

The histological slides were examined under a microscope by a pathologist from the Medical laboratory, Faculty of Medicine, U of G.

Hypoglycemic effect

The ability of saponins in lowering rat blood glucose was studied. The albino rats were subjected to fasting for 6 hr prior to the collection of blood from the tail veins in three intervals (2, 3 and 6 hr) for glucose estimation. Ten minutes after collection of the fasting blood samples, the saponins-extract was administered I.P. at 60, 70, 80,90 and 100 mg / rat. The amount of BG in the blood was measured in each treated rat by using a glucometer.

The percentage of induced glycemia was calculated as a time function by applying the following equation, according to Dinesh (2001):

$$\frac{(G_0 - G_i)}{G_0} \times 100$$

where:

G_0 = initial glycemia G_i = glycemia at the hours (1,2...6 hr)

RESULTS AND DISCUSSION

Phytochemistry

The results of the qualitative phytochemical analysis (Tables 1 and 2) indicated that most of the compounds tested for, were present in the fruit rind, pulp and seeds, except cyanogenic glycosides. These are in line with the findings of Karthikeyan *et al.* (2009) and Lozoya and Lozoya (1989). The latter stated that the toxic properties of some plants were mainly due to the presence of various blends of different composition, which occur as secondary metabolites. They are grouped as saponins, glycosides, flavonoids, tannins, alkaloids, coumarins, cardiac glycosides, cyanogenic glycosides and cucurbitacins. Moreover, secondary metabolites are involved in the process of co-evolution between plants and other organisms (Berenbaum, 1995), or antagonistic fungi (Vander *et al.*, 2000), dissuasive substances to resist insects (Wierenga and Hollingworth, 1992), pathogenic microorganisms (Berenbaum, 1995) and competitive plants. Among substances involved in plant defense, saponins, which are heterosides synthesized by several plants, were reported to have a defensive role, which was highlighted for the first time by (Appelbaum *et al.*, 1969)

Table 1. Secondary metabolites of the fruits of bitter apple

Secondary metabolites	Rind	Pulps and seeds
Saponins	+	+
Flavonoids	+	+
Tannins	+	+
Sterols and triterpenes	+	+
Alkaloids	+	+
Coumarins	+	+
Cardiac glycosides	+	+
Cyanogenic glycosides	-	-
Anthraquinonesglycosides	+	+
Cucurbitacins	+	+

+: detected, - : not detected.

Table 2. Qualitative analysis of the fruits of bitter appl

Secondary metabolites	Reagents	Observation/ result
Saponins	Ethanol (50%)	Voluminous froth (honey comb) was developed.
Glycosides	Glacial acetic acid, H ₂ SO ₄ , Fe Cl ₃	Reddish brown layer at the interface was formed and the upper layer gradually acquired a bluish-green colour, which darkened on standing.
Flavonoids Tannins	HCl , NaOH Et.OH Fe Cl ₃ (5%, w/v in methanol)	A yellow color was formed appearance of green colour, which changes to a bluish black colour or precipitate.
Sterols and triterpenes	Petroleum ether, chloroform, acetic anhydride +H ₂ SO ₄	Formation of a reddish violet ring at the junction of the two layers.
Alkaloids	Mayer's	A precipitate was formed (turbidity).
Coumarins	Sodium hydroxide solution	appearance of blue or green colour under UV.
Cardiac glycosides	Liebermann-Burchard , Baljet Keller-Killiani's	A change in colour to green or blue. A colour change to red or orange ring around the spots. A brown ring is formed in between the two layers.
Cyanogenic glycosides	Sodium picrate	No change was observed
Anthraquinones Glycosides	FeCl ₃ , H ₂ SO ₄ , chloroform and Ammonium hydroxide	A change in the colour of the alkaline layer from a pink to red colour
Cucurbitacins	Ethanol, phosphomolybdic acid	Formation of a green colour which changes to a bluish black colour or precipitate

Histochemistry

The histopathological changes were confined to the small intestine, liver and kidneys, while the liver showed opened chromatin (karyorrhexis) (Table 3) that is, the destructive fragmentation of the nucleus of a dying cell whereby its chromatin is distributed irregularly throughout the cytoplasm (Zamzami and Kroemer, 1999) also a prominent nucleolus, an important diagnostic feature of malignant cells, in addition to congestion of central veins and liver sinusoids, which indicated right heart failure results in

delayed emptying of the great veins and retention of blood primarily in the central veins of the liver (Brenner and Richard, 2003).Kidneys exhibited reduced number of lining epithelium cells, which indicated symptoms of renal failure(Table 3)

Regarding the stomach (Table 3), the effects were clearly represented in mucosal edema, which is an indication of abnormally large fluid volume in the in tissues between the body cells (interstitial spaces) and acute inflammation of the intestine prominent nucleolus, open chromatin and structural necrosis (ischemic necrosis) as a result of hypoxia due to local deprivation of blood supply (WHO, 2004) (Table 3).

Table 3. Histopathological changes in the internal organs of Norway rats treated with different dosages of saponins.

Organ	Lesions	Significance
Liver	Open chromatin (karyorrehexis). Prominent nucleolus.	Destructive fragmentation of the nucleus of a dying cell.
	Congestion of central veins and liver sinusoids.	Important diagnostic feature of malignant cells. Right heart failure
Kidney	Reduced number of lining epithelium	Renal failure.
Stomach	Mucosal edema	Acute inflammation of the intestine.
	Open chromatin (karyorrehexis)	As above
	Prominent nucleolus	As above
	Structural necrosis	Ischemic necrosis as a result to hypoxia

These findings also agreed with those of Diwan *et al.* (2000) who stated that forced administration of saponins to rats caused some histopathological changes; liver sections showed small haemorrhage in many lobules and congestion of central veins and liver sinusoids. Moreover, destruction of the liver architecture, due to the necrosis of liver cells, was also seen. In addition to exudation and haemorrhage in the glomeruli, the kidney section showed focal destruction of the renal tubules. Traces of saponins were found in only a few places of the submucosa of the stomach and large intestine and in heart cavities. In contrast, larger amounts of saponins appeared in the lumen and submucosa of the small intestine. Sections of the small intestine showed acute erosion of the superficial or middle parts of

some intestinal villi. Hemorrhage was evident in these parts and inside the lamina propria. The results of the present study were also in line with the finding of Dehagani and Panjehshahin (2006) who found that I.P. administration of *C. colocynthis* saponins to rats in the experimental groups caused some histopathological changes in their livers. Sections of the liver showed small haemorrhages in several lobules and congestion of central veins and sinusoids accompanied with mild nonspecific inflammation with hepatocellular necrosis. Mixed neutrophil and lymphocyte infiltrate involving the parenchyma was observed, but no bile duct injury. A morphological change in hepatocyte, including karyorrehexis, chromatolysis and granulation of the cytoplasm was seen using H&E staining, especially with doses of 200, 400 mg/rat.

There is growing concern about the hepatotoxicity of herbal remedies. Herbal hepatotoxicity has been recognized for many years, but new agents are constantly being identified (Chitturi and Farrell, 2000). Bitter apple extracts were found to be free of hepatotoxic effects at concentrations up to 100 µg/ml (Barth *et al.*, 2002). However, higher concentrations seem to have some degree of hepatotoxicity. Male Wistar rats that were fed diets containing 10% bitter apple ripe fruits showed body weight loss, inefficiency of feed utilization, diarrhoea, ruffled hair and enterohepat on ephrotoxicity (Adam *et al.*, 2001). Itzhaki *et al.* (2003) reported that the effect of different concentrations of saponins on the liver was investigated. The results showed some histological changes in the nucleus and cytoplasm of hepatocytes. The changes observed in the nuclei included chromatolysis (open chromatin) and prominent nucleolus. The mechanism for these changes is not clear, but other reports have shown that bitter apple has a damaging effect on different cells.

Hypoglycemic effect

Percentage reduction in glucose level of albino laboratory rats treated with different dosages of saponins were 42, 55.4, 60, 62.5, 69.3 and 0.10% corresponding to the dosage levels 60%, 70%, 80%, 90%, 100% mg and the control treatment, respectively (Table 4). The maximum hypoglycemic effect was found with the dosage of 100 mg/rat (Table 4). It is obvious that there was a clear decrease in the average of the amount of glucose in the plasma of the treated rats.

Table 4. Percentage reduction in glycemia after 6 hr of treatment with saponins.

Dosage (mg/rat)	Albino rats (No and sex)	Average wt/ rat (g)	Initial glycaemia (mg/dl) at 1hr	Value mg/dl at 6 hr	Reduction in glycaemia (%)
Control	2M, 2 F	148	92.6	92.9	0.10
60	2M,2 F	133	89.2	77.5	42.00
70	2M,2 F	140	81.2	67.4	55.40
80	2M,2 F	141	86.1	66.5	60.50
90	2M,2 F	131	79.2	61.6	62.00
100	2M,2 F	140	80.6	59.9	69.30

Nmila *et al.* (2000) stated that the chromatographic fraction of bitter apple given to rats at a concentration of 0.1 mg/ml showed insulin-release stimulation in the pancreas, using glucose induced insulin secretion. When 10% of seed was mixed in chicks ration, the following changes were noticed in the serum; alkaline phosphatase stimulation, creatin kinase stimulation and lipid synthesis stimulation. Also, hepatotoxic activity was found in the liver of the chicks (Bakhiet and Adam, 1995). Abdel-Hassan *et al.* (2000) investigated the effects of the aqueous, glycosidic, alkaloidal and saponins-extracts of the rind of bitter apple on the plasma glucose levels were in rabbits. Oral administration of aqueous-extract (300 mg/rat) produced significant reduction in plasma glucose after 1 hr, and a highly significant effects after 2, 3 and 6 hr.

CONCLUSION

It can be concluded that bitter apple saponins caused damage to the liver, kidney and stomach. Furthermore, it reduced blood glucose levels and consequently caused the death of rats.

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على الأعضاء الداخلية *Citrullus colocynthis* (L.)Schrad الأثر الخافض للسكر والآثار السامة لصابونينات نبات الحنظل *Rattus norvegicu* (Berkenhout) للجرذ النرويجي

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⁴المعمل الطبي، كلية الطب، جامعة الجزيرة، واد مدني، السودان.

الخلاصة

أصبحت المنتجات النباتية الطبيعية في الآونة الأخيرة محوراً مهماً للبحث في الكثير من المراكز البحثية، وذلك لأهميتها الخاصة في سلامة المجتمعات. تعود الخواص العلاجية لهذه النباتات لاحتوائها على العديد من المركبات الكيميائية المعقدة ذات التراكم المتباينة والتي توجد فيها على صورة نواتج ثانوية لعملية الأيض. هدفت هذه الدراسة إلى تقويم الآثار الخافضة للسكر والآثار السامة لصابونينات الحنظل والتغيرات النسيجية التي تحدثها على الأعضاء الداخلية للجرذ النرويجي. تم كل ذلك عبر عدة طرق وشملت هذه *C. colocynthis* (L.)Schrad هدفت للكشف عن وتسمية مجاميع المواد الفاعلة في مسحوق ثمار نبات الحنظل المواد الصابونينات، الجلايكوسيدات والفلافونويدات والقلويدات والأنثروكينونات، والتربينات والتانينات والكومارينات، الجلايكوسيدات القلبية والجلايكوسيدات والسيانوجينية والكيوكيربتسينات. كانت النتائج كلها إيجابية مع غياب الجلايكوسيدات السيانوجينية. تم اختبار السمية الحادة للصابونينات المستخلصة من قشور ثمار نبات الحنظل علي طريق الحقن عبر الغشاء (. بناءً على تجارب أولية تم اختبار خمس جرعات Berkenhout (*Rattus norvegicus*) البروتوني للجرذان النرويجية البهقاء وهي 60، 70، 80، 90 و100 مجم صابونين خام/جرذ. فيما يختص بالتغيرات النسيجية على الأعضاء والأنسجة الداخلية للجرذان المعاملة بالصابونين فقد لوحظ أنها شملت تشوه الأنوية وانفتاح الكروماتين واحتقان الأوردة في خلايا الكبد مع انخفاض الخلايا الطلائية المبطنة للأنيبيبات البولية في الكلى. أما بالنسبة للتغيرات في المعدة والجهاز الهضمي فشملت وزمة الغشاء المخاطي، وانفتاح الكروماتين مع تشوه الأنوية وموت الأنسجة. تمت أيضاً دراسة أثر الصابونينات علي خفض معدل السكر في دم الجرذان المعاملة بالجرعات السابقة وكذا الشاهد حيث تم أخذ متوسط النسب المئوية لانخفاض السكر في الدم خلال ست ساعات وكانت المتوسطات 42%، 55.4%، 60%، 62%، 69.3%، 0.10% وللشاهد على الترتيب. وقد لوحظ أن هنالك تدني ملحوظ في نسبة الجلوكوز في دم الجرذان المعاملة يتناسب عكسياً مع الجرعة المستخدمة.