

***Effect Of Aqueous Extracts of Chamomile and Arabic Gum
On Indomethacin- Induced
Toxicity In Rats***

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Abstract

Arabic gum has been used as anti-inflammatory agent and chamomile has anti-inflammatory and antioxidant properties. This study investigated the efficacy of aqueous extracts of chamomile and Arabic gum on the toxic side effects of indomethacin in male albino rats. Thirty male albino rats (185±10 g) were divided into six groups, one of them was kept as a (-ve) control group (5 rats), while the other groups 25 rats were treated orally with indomethacin (25mg / kg b.Wt.) once daily for twenty-one (21) days. One group was kept as (+ve) control group, while others were given aqueous extracts of chamomile for 2 groups and Arabic gum for the other 2 groups (500 and 1000 mg/kg) orally daily. Feed intake (FI), body weight gain % (BWG %) and feed efficiency ratio (FER) were carried out. Serum glutamic oxaloacetic transaminase (GOT) and serum glutamic pyruvic transaminase (GPT) were evaluated. Serum creatinine and urea were analyzed. Red blood cell and white blood cells count were measured. Hemoglobin concentration was estimated. Also, histopathological changes for liver were examined. The obtained results concluded that using the tested drinks improved previous mentioned parameters. The best results were obtained on using the high doses of aqueous extracts. According to the results, the author recommended the trial on human beings.

Keywords: Chamomile – Arabic gum - Aqueous Extract – Indomethacin – Side Effects.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) have been widely used in medicinal fields. In recent years, the clinical practices of these drugs are increased in spite of the increased side effects (**Fang et al., 2019**). Indomethacin (INDO) is one of non-steroidal anti-inflammatory drugs which is used as analgesics and anti-inflammatory agents. INDO causes deficiency in all blood cell types and has a dangerous effect on liver (**Abatan et al., 2006**). Indomethacin can lead to development of liver necrosis and rapidly develops acute renal failure or interstitial nephritis (**Zhelevet al., 2018**).

The central role of the liver and kidney in drug metabolism facilitate the exposure of these organs to toxic injury (**Abd El-Megid et al., 2017**). Anemia may result from excessive RBC destruction/loss or decreased RBC production and is usually a manifestation of an underlying disease process.

Arabic gum (AG) being one of the plants nominated by WHO for medicinal purposes (**khedr, 2017**). It is dried gummy exudate obtained from the stems and branches of *Acacia senegal* and *Acacia seyal* trees that is used as an emulsifier and stabilizer in pharmaceutical, cosmetic, and food industry. AG has been found to act as an antioxidant and anti-inflammatory agent as recorded by previous

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studies (*Said et al., 2019*). AG contain total phenolic compounds and flavonoids (gallic acid and catechin). AG is useful in treating patients with chronic renal failure and act as cytoprotective agent because it contains polysaccharides lie in their ability to diminish scavenging radicals (*khedr,2017*).

Chamomile is one of the oldest known herbs of traditional medicine that belongs to the *Asteraceae* family. It contains flavonoid compounds(e.g. Apigenin, Luteolin, and Quercetin), Terpenoid α -Bisabolol, and its oxides (e.g. Chamazulene)(*Narges et al., 2018*). Chamomile has anti-inflammatory, anti-oxidant properties, neuroprotective, anti-allergic, anti-bacterial properties and anti-cancer activities (*Abd El-Megid et al., 2017*).Chamomile extract may be used to protect against toxic effects of chronic alcoholism on the liver and kidney systems by antioxidant and free radical scavenging actions of chamomile (*Nwoye ,2013*).

This study was conducted to investigate the protective effects of aqueous extracts of chamomile and Arabic gum on liver and kidney toxicity and on hematological parameters induced on experimental rats caused by indomethacin.

Materials and Methods

Plant materials

Chamomile and Arabic gum were purchased from Haraz for herbs and medicinal plants Company, Cairo, Egypt.

Chemicals materials

Indomethacin (Liometacen) was purchased from The NILE Co. for Pharm. and Chemical Ind., Cairo, Egypt. Casein (85%), Vitamins mixture and salt mixture and all chemicals used for blood biochemical measurements were purchased from Modern Lab Company, Dokki, Cairo, Egypt.

Preparation of extracts

The aqueous extracts of chamomile were prepared using dried chamomile powder to distilled water (1:10 w/v) and boiled for 5 min at 100°C. Then they were filtered and concentrated at 50 °C under reduced pressure using a Rota vapor. The resulting extracts were kept frozen till use (*Rezq and Elmallh, 2010*). Fine powder of Arabic gum was dissolved in distilled water to prepare 10 % extracts solution. Afterwards, the extract was left for 3 days in bottle glass in refrigerator, filtered by Whatman No. 1 filter paper (*Ayaz et al., 2017*).

Animals:

Thirty male albino rats of *Sprague Dawley* strain (185±10 g) were obtained from the laboratory animal house of Faculty of Science, Tanta University. Rats were housed individually in wire cages, maintained for one week for acclimatization under standard laboratory conditions on free supply of basal diet and water provided *ad libitum*.

Experimental Design

After the period of acclimatization, rats were divided into six groups; each group contains 5 rats as follows:

Group 1: Negative control group (-ve).

Group 2: Positive control group (+ve) (treated orally with Indomethacin 25 mg/kg only).

Group 3: Treated orally with chamomile extract (500 mg/kg Body weight /day) + Indomethacin (25 mg/kg).

Group 4: Treated orally with chamomile extract (1000 mg/kg Body weight / day) + Indomethacin (25 mg/kg).

Group 5: Treated orally with Arabic gum extract (500 mg/kg Body weight /day) + Indomethacin (25 mg/kg).

Group 6: Treated orally with Arabic gum extract (1000 mg/kg Body weight /day) + Indomethacin (25 mg/kg).

The doses of Indomethacin, chamomile extract, Arabic gum extract were selected from published literature according to **Ajeigbe et al., (2014) and Al baroudi, (2013)**.

The rats were fed on basal diet according to **(Reeves et al., 1993)** during the whole period. The extracts were given orally once daily for twenty-one days. The rats were fasted overnight before being anesthetized and sacrificed after the experimental period (21 days).

Biological evaluation

Body weight gain and the quantities of diet which were consumed after weighing the leftover diet were recorded daily during the experimental period (21 days). FI was calculated according to **(Chapman et al., 1959)**. BWG% and FER were calculated according to **Hosoya, (1980)** using the following equations:

Feed intake = Initial diet weight (g) – leftover diet weight (g)

BWG% = (Final body weight – Initial body weight) / Initial body weight × 100

FER = Weight Gain (g) / Feed intake (g)

Biochemical evaluation and hematological analysis

The rats were fasted overnight before being sacrificed after the experimental period (21 days). The blood samples were collected after slight anesthesia of rats from each rat and put immediately into glass tubes and centrifuged at 3,000 rpm for 20 min at 4 °C to obtain the serum which was kept frozen at -20°C till analysis as described by **(Schermer, 1967)**.

Different tested parameters in serum were determined using specific methods as follows: Liver enzymes (GOT and GPT) were measured in the serum according to the method described by **Reitman and Frankel (1957)**. Urea and creatinine were determined in the serum according to **Patton and Crouch (1977)** and **Faulkner and King (1976)**, respectively. Red blood cell (RBC) and white blood cells (WBC) were determined based on the method adopted by **Fischbach, (1996)**. Hemoglobin (Hb) level was measured according to **Drabkin, (1949)**.

Histopathological examination:

The liver was immediately removed from each rat, washed and cleaned from the adhesive matter by a saline solution (0.9 %, w/v), dried by filter paper and kept in formalin solution (10%) according to the method described by **Drury and Wallington (1980)** for histopathology examination.

Statistical analysis

Data were expressed as means ± standard deviation. Values were statistical analyzed by one-way analysis of variance (ANOVA test) using SPSS 10.1 software package. Differences were considered significant at P values (<0.05) **(Snedecor and Cochran, 1989)**.

Results

Biological evaluation

Administration of indomethacin showed a significant decrease ($p < 0.05$) in FI, BWG and FER when compared with negative control group. However, these parameters improved in all treated groups with extracts. The highest improvement observed in treated group with chamomile extract at dose (1000mg/kg) and was almost close to the (-ve) control group (Table 1).

Table (1):
Effects of aqueous extracts of chamomile and Arabic gum on FI, BWG and FER in the different experimental groups

Groups (n= 5 each)	FI(g)	BWG(%)	FER
(- ve) Control (G1)	24.92±1.15 ^a	24.25± 2.07 ^a	0.045 ±0.005 ^a
(+ ve) Control (G2)	11.17±0.97 ^f	4.33 ±0.38 ^d	0.023 ±0.004 ^d
500mg/kg Chamomile extract (G3)	19.90±1.09 ^c	17.84±3.63 ^b	0.040±0.001 ^{bc}
1000mg/kg Chamomile extract(G4)	22.47 ± 0.64 ^b	23.29 ±2.66 ^a	0.042± 0.002 ^{ab}
500 mg/kg Arabic gum extract(G5)	15.36±1.67 ^e	14.10±1.00 ^c	0.036 ±0.003 ^c
1000mg/kg Arabic gum extract(G6)	18.35±1.12 ^d	16.91±3.42 ^b	0.038 ±0.001 ^{bc}

Values were expressed as Means ± SE.

Values at the same column with different letters are significantly different at $P < 0.05$.

Liver function

It is clear from table (2) that there were significant increase for GPT and GOT levels in the serum of (+ve)control group as compared to negative control group .Results denote that all treated groups had significant decrease in serum levels of SGPT and SGOT activities when compared with indomethacin treated (+ve control group). Treated group with high dose of Arabic gum and chamomile extracts (1000 mg/kg) showed the highest decrease of SGPT and SGOT enzyme levels in serum, values were nearest to negative control group as shown in table (2).

Table (2):
Effects of aqueous extracts of chamomile and Arabic gum on liver enzymes (GPT and GOT) in the different experimental groups

Groups (n= 5 each)	GPT (IU/L)	GOT (IU/L)
(- ve) Control (G1)	21.00 ^f ± 3.46	87.40 ^f ± 11.41
(+ ve) Control (G2)	58.27 ^a ± 5.41	288.27 ^a ± 29.77
500mg/kg Chamomile extract (G3)	51.05 ^b ± 5.80	232.50 ^b ± 16.79
1000mg/kg Chamomile extract(G4)	26.30 ^d ± 2.55	137.87 ^d ± 19.45
500 mg/kg Arabic gum extract(G5)	39.37 ^c ± 3.37	192.33 ^c ± 17.67
1000mg/kg Arabic gum extract(G6)	23.03 ^e ± 3.37	112.10 ^e ± 16.93

Values were expressed as Means ± SE.

Values at the same column with different letters are significantly different at $P < 0.05$.

Kidney function

Indomethacin caused a significant increase of serum urea and creatinine compared with (-ve) control, while oral administrations of chamomile and Arabic gum extracts recorded a significant decrease in both urea and creatinine when compared with the positive control group. The best results

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were obtained on giving high dose (1000 mg/kg) of Arabic gum extract followed by high dose of chamomile extract.

Table (3):

Effects of aqueous extracts of chamomile and Arabic gum on urea and creatinine parameters in the different experimental groups

Groups(n= 5 each)	Urea (mg/dL)	Creat. (mg/dL)
(- ve) Control (G1)	22.93 ^f ± 3.30	0.54 ^e ± 0.060
(+ ve) Control (G2)	76.23 ^a ± 9.38	1.12 ^a ± 0.129
500mg/kg Chamomile extract (G3)	42.27 ^c ± 3.42	0.75 ^c ± 0.095
1000mg/kg Chamomile extract(G4)	32.53 ^d ± 5.26	0.64 ^d ± 0.087
500 mg/kg Arabic gum extract(G5)	48.30 ^b ± 8.63	0.86 ^b ± 0.042
1000mg/kg Arabic gum extract(G6)	24.40 ^e ± 3.57	0.54 ^e ± 0.050

Values were expressed as Means ± SE.

Values at the same column with different letters are significantly different at P<0.05.

Hematological parameters (RBC , Hb and WBC)

Administration of indomethacin showed a significant decline (p<0.05) in red blood cell (RBC) and hemoglobin (Hb) While, white blood cells (WBC) were increased in indomethacin (+ve control) when compared with the (-ve) control group. However, these parameters improved in all treated groups with aqueous extracts of chamomile and Arabic gum. The best observed result was in the group treated with chamomile at dose (1000 mg/kg) and close to the negative control group RBC and Hb, while WBC was nearest to negative control group in (Table 4).

Table (4):

Effects of aqueous extracts of chamomile and Arabic gum on hematological parameters (RBC, Hb and WBC) in the different experimental groups

Groups (n= 5 each)	RBC(10 ⁶ cell/μL)	Hb(gm %)	WBC(x10 ³)
(- ve) Control (G1)	9.36 ± 0.17 ^a	15.67 ± 0.21 ^a	8.47 ± 0.45 ^f
(+ ve) Control (G2)	5.51 ± 0.46 ^d	11.63 ± 0.15 ^e	21.00 ± 1.00 ^a
500mg/kg Chamomile extract (G3)	8.51 ± 0.36 ^b	13.73 ± 0.67 ^c	13.40 ± 0.36 ^c
1000mg/kg Chamomile extract(G4)	9.10 ± 0.20 ^a	15.27 ± 0.15 ^a	10.70 ± 0.20 ^e
500 mg/kg Arabic gum extract(G5)	7.67 ± 0.15 ^c	12.23 ± 0.06 ^d	18.10 ± 0.10 ^b
1000mg/kg Arabic gum extract(G6)	8.20 ± 0.20 ^b	14.50 ± 0.46 ^b	12.50 ± 0.20 ^d

Values were expressed as Means ± SE.

Values at the same column with different letters are significantly different at P<0.05.

Histological results

Table (5) shows the scores of various abnormalities in the liver cells of the experimental animals are Portal congestion, Sinusoidal dilation, Portal fibrosis, Biliary hyperplasia, Portal inflammation, Hepatocytes coagulative necrosis and Microvesicular steatosis or ballooning degeneration in hepatocytes. The changes in the liver cells are illustrated in figure(1) where the microscopic examination of the stained section with H&E revealed abnormal liver cells in the (+ve) control group (B) compared with the histological picture in the (-ve) control group (A). The histopathological picture of the treated groups (C, D, E, F) were in better shape than the (+ve) control group.

**Table (5):
Scoring of histological lesions in liver in all the experimental groups**

Liver	-ve	+v	500mg/kg Chamomile extract	500mg/kg Chamomile extract	500mg/kg Arabic gum extract	1000mg/kg Arabic gum extract
Portal congestion	-	+++	++	+	++	+
Sinusoidal dilation	-	+++	++	+	++	+
Portal fibrosis	-	++	±	±	++	+
Biliary hyperplasia	-	++	+	±	++	-
Portal inflammation	-	++	+	-	+	-
Hepatocytes coagulative necrosis	-	+++	++	+	++	+
Microvesicular steatosis or ballooning degeneration in hepatocytes	-	+++	+	-	++	++

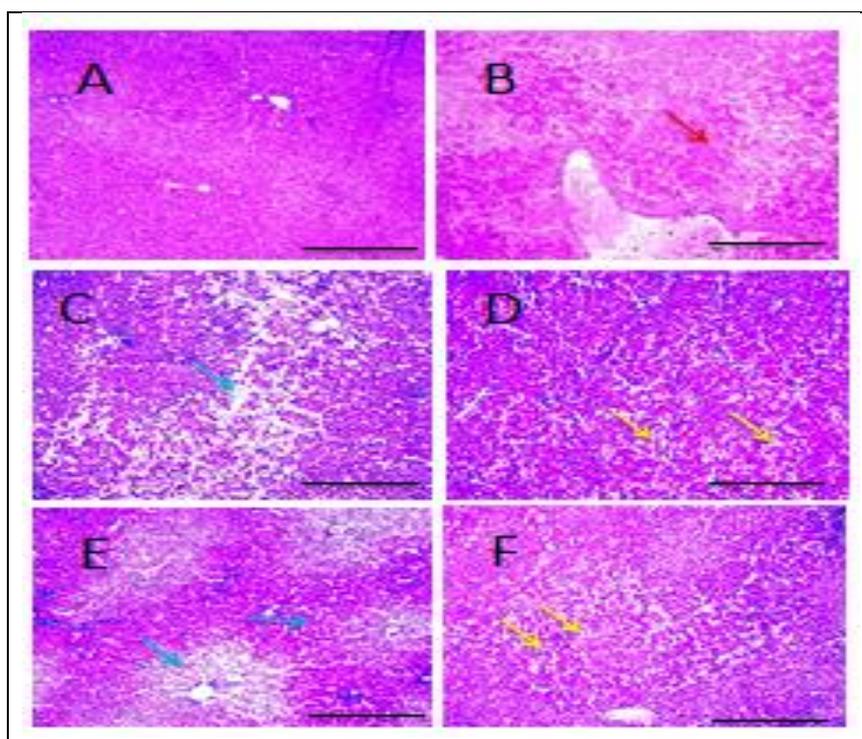


Figure (1): Microscopic images hematoxylin and eosin (H & E)-. **(A)** Negative control group. **(B)**Indomethacin (+ control) group. **(C)** Chamomile aqueous extract group (low dose) **(D)** Chamomile aqueous extract group (high dose).**(E)**Arabic gum group (low dose). **(F)**Arabic gum group (high dose).

Discussion

In the present study, indomethacin decreased FI BWG%, and FER because it interfered with the metabolic pattern of experimental rats and caused poor gastro intestinal functions with less absorption of nutrients according to **Bagoji et al.,(2015)**. These results agree with **Fjære et al., (2014)** who reported that indomethacin reduces Feed efficiency and obesity in mice treated with high fat/high sucrose. On the other hand, chamomile aqueous extract at 1000 mg/kg improved FI BWG%, and FER and achieved the best result which is close to normal. The positive improvement in these parameters may be related to the active compounds which influence the gastrointestinal eco system, increasing production of digestive enzymes and improving digestion system according to **Al-Mashhadani et al., (2013)**. In this respect, **Al baroudi (2013)** reported that 2, 4-Dichlorophenoxyacetic acid reduces the body weight in rats, while chamomile aqueous extract increased the body weight of rats given chamomile. It contains high levels of bioactive compounds including flavonoids and phenolic compounds. It enhances food consumption by rats given chamomile. Also, in this study the decrease in body weight gain of aqueous extract of Arabic gum may be due to the high dietary fiber content of AG which has an effect on fat metabolism or lowering caloric density of food according to **Ahmed et al., (2015)**.

Non-steroidal anti-inflammatory drugs are widely used worldwide (**Scheiman et al.,2006**) because they exert excellent efficacy in the management of pain, fever and inflammation (**Simone, 2006**).The use of NSAIDs were accompanied with serious adverse effects not only in stomic but in the small intestine, cardiovascular system and liver (**Filaretova et al., 2011**). The liver has central role in

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detoxification of drugs and toxic substances, since it is the target organ for all toxic effects (**Mahmoed and Rezaq, 2013**). In this work, INDO-induced toxic effect on liver is evidenced by significant increase in the levels of serum GOT and GPT. On another hand, treatment with extracts of chamomile and Arabic gum abated the hepatic lesions produced by indomethacin as evidenced by the reduction of the elevated serum levels of liver enzymes. In the present study, the group treated with high dose of Arabic gum and Chamomile extracts showed the highest decrease in the serum level of liver enzymes. This is in line with previous findings by **Najla et al., (2017)** and **Elshama (2018)** who reported that Arabic gum has significant antioxidant properties which has an effective protective in hepatotoxicity. Also, previous studies confirmed the antioxidant effect of Arabic gum by the significant reduction in MDA and increase in glutathione, total antioxidant capacity, and antioxidant enzyme activities in kidney tissue of the adenine + AG group (**Said et al., 2019**). These results are compatible with those of **Gupta and Misra (2006)** who concluded that chamomile extract functions as a hepatoprotective agent cause a reduction of the elevated serum levels of GOT and GPT. These results agreed with the hypothesis that chamomile (*Matricaria recutita* L.) exerts therapeutic effects by antioxidant and free radical scavenging actions (**Abd El-Megid et al., 2017**). Also, **Gupta and Misra (2006)**, reported that the administration of chamomile extract to rats caused significant decrease in elevated SGPT and SGOT in the paracetamol induced hepatotoxicity. This activity may be due to the protective effect of chamomile extract and the maintenance of the functional integrity of hepatic cells. The mechanism by which chamomile extract offered protective effects against drug-induced hepatotoxicity may be related to antioxidant properties, which responsible for protecting the liver against the oxidative stress, possibly by elevating the endogenous defensive capacity of the liver to combat oxidative stress.

The kidney functions were affected significantly on indomethacin administration as evidenced by the significant increase in renal function marker levels such as serum urea and creatinine compared to the (-ve) control. While administration of extracts caused significant reductions in serum concentrations of urea and creatinine compared to indomethacin group. These results were in agreement with the findings of **Alnahdi, (2016)** who reported that Arabic gum is used as a prophylactic agent against the renal toxicity. Also, (**Osman et al., 2011**) concluded that Arabic gum caused decline in serum concentrations of urea, creatinine, BUN, and uric acid to near normal. These results may be due to the ability of Arabic gum to decrease the uric acid level and other purine metabolites, and to inhibit the colonic bacterial ammonia generation (**Nasir, 2013**).

Changes in hematological parameters showed the decrease in hemoglobin concentration, RBCs count while increase in WBC count, that resulted from indomethacin (INDO). The present results agree with **Bagoji et al., (2015)** who reported that INDO caused decline RBC and Hb parameters in positive control group when compared to normal group. Also **Lateef and Taiwo (2006)** showed that INDO caused significant increase in WBC count. On the other hand, chamomile aqueous extract at 1000 mg/kg increased RBC count and Hb concentration, while decreased the WBC count that might be due to its antioxidant and inflammatory properties according to (**Jabri et al., 2016**). In this respect, **Nwoye(2013)** reported that oral administration of chamomile aqueous extract has safe use on hematological parameters. Also, arabic gum aqueous extract increased RBC count and Hb concentration, while decreased the WBC count according to **Elderbi et al.,(2009)**.

Moreover, these results are confirmed by histopathological examination as liver sections in indomethacin (+ve) control group showed severe diffuse necrotic lesions. These results agree with **Bagoji et al., (2015)** who demonstrated that INDO damage the liver and caused necrosis of hepatocyte and relies the number of Kupffer cells. The aqueous extracts have protective effect against oxidative stress caused by indomethacin. These results are supported by **Al baroudi (2013)** who

reported that Chamomile extract protect the hepatocyte from oxidative damage and stop the harmful effect on liver cells. Also, **Saad et al., (2018)** reported that administration of Arabic gum led to little improvements in hepatic tissues against adenine had been observed.

Conclusion

Indomethacin has toxic side effects on experimental animals proved by biochemical and histological results. The results concluded that using high doses of aqueous extracts improved liver and kidney function and hematological parameters. The Author recommended that the aqueous extracts of chamomile and Arabic gum could be tried on patients using indomethacin.

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تأثير بعض المستخلصات المائية (البابونج والصبغ العربي) على الآثار الضارة
للإندوميثاسين في الجرذان

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الملخص العربي

أجريت الدراسة لمعرفة تأثير المستخلصات المائية (البابونج والصبغ العربي) على الآثار الجانبية السامة للإندوميثاسين باستخدام ثلاثين من ذكور الجرذان البيضاء والتي تبلغ أوزانهم (185 ± 10 جم) وتم تقسيمهم الى ست مجموعات احدهم هي المجموعة الضابطة السليمة (5 جرذان)، بينما المجموعات الأخرى (25 جرذ) تم معاملتهم بجرعة يومية من الإندوميثاسين (25 ملجم / كجم من وزن الفأر) لمدة 21 يوم ، بقيت احدي المجموعات كمجموعة ضابطة موجبة بينما المجموعات الأخرى تم اعطائهم عن طريق الفم المستخلص المائي للبابونج والصبغ العربي بجرعتين هما 500 و1000مجم / كجم من وزن الجسم . استمرت التجربة لمدة ثلاث أسابيع . تم اجراء التقييم البيولوجي ويشمل النسبة المئوية لوزن الجسم المكتسب , المأخوذ الغذائي و معدل الاستفادة من كفاءة الغذاء . تم تقدير انزيم الأمينوترانز أمينيز و أمينو ترانسفيريز في السيرم , تم تقدير مستوى الكرياتينين واليوريا في السيرم ، وتم تقدير عدد كرات الدم الحمراء والبيضاء وتركيز الهيموجلوبين في الدم. وكذلك التغيرات الهستوباثولوجية في الكبد تم فحصها , ووجدت افضل النتائج في المجموعات المعالجة بالجرعات العالية من المستخلصات المائية . وفقا لهذه النتائج يمكن تجربة استخدام المستخلصات المذكورة على الإنسان للوقاية من الآثار الجانبية للإندوميثاسين .

الكلمات المفتاحية :

الكاموميل - الصبغ العربي - المستخلصات المائية - الإندوميثاسين - آثار جانبية.