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Study The Effect of Aqueous Extract of *Piper nigrum* on Some physiological parameters and Histopathological in Female Rabbit Induced Gastric Ulceration by Aspirin

دراسة تأثير المستخلص المائي لثمرة نبات الفلفل الأسود على المعايير الفسلجية والامراضية النسيجية في إناث الأرانب المستحدثة فيها القرحة المعدية بواسطة الأسبرين

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Summary

This study was carried out at the animal house of the College of Veterinary Medicine-Basrah University. An attempt was done to induce gastric ulceration by using oral administration of 400 mg/kg B.W. of aspirin. The aim of this study was to evaluate the effect of aqueous extract of *Piper nigrum* (500 mg/kg B.W.) on hematological and biochemical parameters and histopathological examination of liver, stomach in female rabbits induced gastric ulceration. Twenty four female rabbits weighted (1250-1500g) and ages (7m) were divided into 4 equal groups (6 rabbits/group). The aspirin and aqueous extract of *Piper nigrum* were administered daily for 30 days and blood samples were collected for analyses.

Treatment of rabbits for 30 days with 400 mg/kg B.W. of aspirin caused significant ($p<0.05$) reductions in RBC,WBC, PCV, Hb, DWBC relative to their respective controls; but caused significant ($p<0.05$) increments in the activities of AST and ALT relative, creatinin and bilirubin to their respective controls. On the other side aspirin+*Piper nigrum* extract, *Piper nigrum* extract groups showed a significant increase in RBC,WBC, PCV, Hb but showed a significant decrease in the glucose, cholesterol and activities of AST and ALT relative. These observations showed that aqueous extract of *Piper nigrum* possess antiulcer potential.

Aspirin was found to cause stomach erosion, mucosal injury, infiltration of inflammatory cells and an increase in gastric pits depth. In addition to degenerated parietal and chief cells which clearly seen near the muscularis mucosa. While the stomach sections related to animals treated with extract and aspirin (400mg/kg.dose) showed mild mucosal injury, little number of congested blood vessels, mild infiltration of leucocytes in submucosa and no haemorrhage. Also an increase in mucus layer thickness which covers the surface mucosal layer, compared with animals treated with aspirin only.

These findings probably indicates that aspirin have deleterious effect on the blood chemistry of female rabbits. The results of the effect of aqueous extract of *Piper nigrum* (alone) revealed lack of any effect of treatment with aqueous extract of *Piper nigrum*(500 mg/kg) was found to inhibit the ulcers induced by aspirin. It prevented the increase of gastric acid secretions, depletion of stomach wall mucus and prevented the histological changes caused by aspirin. It might be related to the stimulation of bioenergetic processes in the gastric epithelium under the influence of *Piper*.

الخلاصة

أجريت هذه الدراسة في البيت الحيواني التابع إلى كلية الطب البيطري/جامعة البصرة . لمحاولة استحداث القرحة المعدية بواسطة الأسبرين في إناث الأرانب لمعرفة ما تعكسه القرحة المعدية من تأثيرات سلبية على المعايير الدمية والكيموحيوية والتغيرات في التراكيب النسيجية للكبد و المعدة وكذلك تناولت هذه الدراسة إمكانية تأثير المستخلص المائي لنبات الفلفل الأسود بجرعة (500ملغم/كغم من وزن الجسم) في تثبيط القرحة المعدية.

أستخدم في هذه التجربة (24) أنثى أرنب محلي تتراوح أوزانها ما بين(1250-1500غم) وبمعدل عمر (7شهر) قسمت

عشوائيا بالتساوي إلى أربعة مجاميع (6 أرنب/ مجموعة).
المجموعة الأولى سيطرة تم تجريعها (3 مل من المحلول الملحي الفسيولوجي)والمجموعة الثانية تم تجريعها (400 ملغم /كغم من وزن الجسم عقار الأسبرين) والمجموعة الثالثة تم تجريعها (400ملغم /كغم من وزن الجسم من عقار الأسبرين+ 500ملغم /كغم من وزن الجسم من مستخلص المائي للفلفل الأسود) ة إما المجموعة الرابعة فقد تم تجريعها (500ملغم /كغم من وزن الجسم المستخلص المائي للفلفل الأسود واستمرت المعاملة لمدة شهر.
وجد إن الأسبرين سبب تآكل وتحطم الطبقة المخاطية المعدية واحدث مجموعة من التغييرات النسجية المرضية التي تضمنت تلف الطبقة السطحية والنزف وارتشاح الخلايا الالتهابية مع زيادة عمق االوهدات المعدية فضلا عن تنكس الخلايا الجدارية والرئيسة والتي ظهرت بشكل واضح قرب الطبقة العضلية المخاطية. بالإضافة إلى تأثر المعايير الدمية والكيموحيوية بالأسبرين إذ سبب نقص في المعايير الدمية مثل(RBC,WBC, PCV, Hb, DWBC).وكذلك تأثر المعايير الكيموحية إذ سبب ارتفاع معنوي في مستوى البليروبين والكرياتنين و انزيمات AST,ALT.ولم تتاثير هذه المعايير بالمستخلص المائي للفلفل الأسود. واثبت الفلفل الاسود امكانية تثبيط القرحة المعدية ولوحظ تحسن بالمعايير المذكورة أعلاه في المجموعة التي أعطيت الفلفل الأسود بالإضافة إلى الأسبرين.

Introduction

Peptic ulcers are open sores that develop on the inside mucosal lining of the digestive tract, specifically, the initial portion of the small intestine (duodenum), esophagus, and stomach. Peptic ulcers develop when the balance between the digestive acids and the protective mucosal layer is disrupted. In healthy individuals, the digestive tract is coated with a mucous membrane that protects the underlying tissue against the highly corrosive digestive acid; however, if the amount of acid is dramatically increased, or the pH of the acid is significantly reduced, or the mucus membrane layer becomes too thin or dry, the acid damages the tissue and ulceration ensues. Thus, gastric ulcers are a type of peptic ulcer that affects the stomach lining due to an imbalance between gastric acid and the gastric mucosa.

Non-steroidal anti-inflammatory drugs (NSAIDs) associated with gastric ulceration occurs in 30% of users that led to hospitalization and is also associated with high mortality [1]. Plant extracts are attractive sources of new drugs and have been shown to produce promising results in the treatment of gastric ulcers [2].The risk of symptomatic ulcers for aspirin and non aspirin (NSAIDs) was elevated throughout treatment. These findings suggest that NSAIDs might not only complicate but also originate clinically relevant peptic ulcers[3].

Aspirin also known as acetylsalicylic acid and it is a salicylate drug, often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever and antiinflammatory medications[4]. Low doses of aspirin are also recommended for the prevention of stroke and myocardial infarction in patients with diagnosed cardiovascular disease [5]. Aspirin use has been shown to increase the risk of gastrointestinal bleeding, although some enteric coated formulations of aspirin are advertised as being gentle to the stomach. In one study enteric coated aspirin did not seen to reduce this risk [5]. Aspirin induced ulcer has been used as a model for the evaluation of antiulcerogenic agent [6].

Recently there has been a renewed interest in improving health and fitness through the use of more natural products. Spices are an important part of the human diet which has been used to enhance the flavor, color and aroma of food. In addition to boosting flavor, spices are also known for their preservative and medicinal value [7].

Black pepper (*Piper nigrum, Piperaceae*) is derived from the fruit and it is one of the most widely used among spices. It is valued for its distinct biting quality attributed to the alkaloid “piperine” (a pungent alkaloid which represents the active principle of black pepper)[8]. Black pepper was shown to have certain medicinal properties (a): By increasing the bioavailability of other anti-tumourigenic spices, black pepper dramatically increases their potency and effectiveness against cancer. (b): Black pepper contains several powerful antioxidants and is thus one of the most important spices for preventing and curtailing oxidative stress. (c): Black pepper exhibits immunomodulatory properties and is capable of boosting the number and the efficacy of white cells, thereby assisting the body to mount a powerful defense against invading microbes and cancer cells, (d): Piperine increases the bioavailability of valuable photochemicals present in other spices

and can boost the activity of biochemically active compounds contained in green tea, curcumin and a variety of other spices by up to several hundred percent, depending on the molecule concerned. It does this via two principal mechanisms. First, it promotes the rapid absorption of certain chemicals from the gastrointestinal tract, protecting them from being broken down by chemicals in the intestinal lumen and by enzymes that occur in the cells lining the intestines. Secondly, once the compound has entered the blood stream, piperine provides protection against oxidative damage by liver enzymes. In this way black pepper enables us to reap optimum benefits from the medicinal phytochemicals found in other dietary spices [9, 10, and 11].

Spices have long been recognized for their digestive stimulant action. Several spices are also employed in medicinal preparations against digestive disorders in traditional medicine. Earlier reports on the digestive stimulant action of spices are largely empirical; only in recent years, this beneficial attribute of spices has been authenticated in exhaustive animal studies. Animal studies have shown that many spices induce higher secretion of bile acids which play a vital role in fat digestion and absorption. When consumed through the diet also spices produce significant stimulation of the activities of pancreatic lipase, amylase and proteases. A few of them also have been shown to have beneficial effect on the terminal digestive enzymes of small intestinal mucosa. Thus, the digestive stimulant action of spices seems to be mediated through two possible modes: (*i*) by stimulating; the liver to secrete bile rich in bile acids, components that are vital for fat digestion and absorption, and (*ii*) by a stimulation of enzyme activities that are responsible for digestion.

Clinical studies on Black pepper revealed significant increases in parietal secretion, pepsin secretion and potassium loss [12]. In mice, Black pepper was reported to protect against the audiogenic seizures and convulsions produced by N-methyl-DL-aspartate and maximal electroshock [13]. These reports are contrary to the reported folkloric use of Black pepper as a carminative, stimulant and stomachic[14-16]. The aim of the present study to evaluate the effect of aqueous extract of *Piper nigrum* on aspirin induced gastric ulceration.

Materials and Methods

Preparation of *P. nigrum* Extract:

Dried fruits of *P. nigrum* were purchased from local market, then powdered in an electric grinder. (50 g) of dried fruits *P. nigrum* powder were extracted by (500ml) distal water for 16 hours by reflex. Afterward, the solvents were filtered using filter days with shaking. The extract were filtrated by Buchnner funnel and using filter papers(Wattman No.-185. The extract were dried under pressure in rotary evaporator at 50c◦. The extract was then dried in vacuum drier (to obtain yields7.5g equal (15%) and stored at (- 4c◦) until used[17].

Experimental Animals.

The animals were maintained under standard conditions of temperature (24 ± 2), humidity (60%) and light (12 hr dark, 12 hr light). Experimental animals were kept in individual cages, provided with ration composed fodder in addition to green alfalfa (Medicago *sativa*) and tap water *ad libitum* and given a prophylaxis drug against coccidiosis (Amprollium 1g/L of drinking water). To induce gastric ulceration twenty four rabbits were randomly divided into main four equal groups, every group included (6) rabbits. Gastric lesions induced by necrotizing agents. First group as control each rabbit in the control group was drenched 3ml of normal saline daily by using gastric tube for 30 days. Second group was drenched (400 mg /Kg B.W. single dosage) of aspirin dissolved in 3ml of normal saline in non fasted rabbits daily by using gastric tube for 30 days. Third group was drenched (400 mg /Kg B.W. single dosage) of aspirin plus (500 mg /Kg B.W. single dosage) of aqueous extract of *Piper nigrum* in non fasted rabbits daily by using gastric tube for 30 days. Fourth group was drenched500 mg /Kg B.W. single dosage) of aqueous extract of *Piper nigrum* non fasted rabbits daily by using gastric tube for 30 days[18].

Animals were sacrificed after treatment with ulcerogenic agents. The stomach was excised and opened along the greater curvature. After washing with normal saline, the gastric lesions were quantified using a binocular magnifier. The ulcers were scored according to the method of [19].

Stomach and liver were removed and were fixed in 10% formal saline in specimen bottles for: Routin histological studies using haematoxylin and eosin staining techniques. Following fixation, the tissues dehydration through ascending grades of alcohol 70% alcohol for a day followed by90% alcohol overnight and finally two changes of absolute alcohol the following day. After dehydration the tissues were treated with xylol (70% xylene/ 30% absolute alcohol) and followed by infiltration in three changes of paraffin were at 60ºC for two days, using an oven. Lastly, the tissues were transferred into an embedding medium (fresh paraffin wax), followed by blocking. Sections of about 5 microns thick were cut using a rotary microtome and stained with hematoxylin and eosin[20].

Evaluation of degree of ulceration:-

was expressed in terms of ulcer score which is calculated by dividing the total number of ulcers in each group by number of rabbits in that group [21]. The degree of ulceration was also expressed as ulcer index (U.I.) and calculated by multiplying ulcer score x 100 [22]. Preventive index was calculated according to the method of [23].

$$\text{Preventive index} = \frac{\text{U.I control} - \text{U.I. treated x 100}}{\text{U.I. treated group}}$$

$$\text{\% of ulceration} = \frac{\text{number of ulcerated rabbit} - \text{number of non ulcerated rabbit x 100}}{\text{Number of rabbit in group}}$$

Studied Parameters

Determination of Hematological Parameters: (RBC) and (WBC) counts were determined by the improved Neubauer haemocytometer method, hemoglobin (Hb) concentration was measured by acid haematin method, the packed cell volume (PCV) was determined using the microhaematocrite method, differential lecukocyte count was used to determine the distribution of the various white blood cells and erythrocyte parameters, i.e. mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated; [24].

Determination of Biochemical parameters: of blood plasma: total protein and its fractions with biuret method, glucose with enzymatic method using Analco reagents, cholesterol, creatinine and bilirubin; [25] Activity of blood plasma enzymes: aspartate amino transferase (AST) and alanine aminotransferase (ALT) by kinetic method with NADH and tris buffer. The activity of enzymes was determined at 37ºC using bioMerieux reagents; [26].

Statistical Analysis :

All values were calculated as means ±S.D. and p values <0.05 and 0.01 were considered significant using analysis of variants and were analyzed statistically by using ANOVA test using SPSS program[27].

Results

1-Effect of Aspirin and Piper nigrum on Haematology Parameters in Female Rabbits.

The results in the Table (1) revealed that the effect of 400 mg/kg B.W. of aspirin, 500mg/kg B.W. of Piper nigrum on haematological parameters of female rabbits after treatment for 30 days. Treatment of rabbits with 400 mg/kg BW of aspirin caused significant ($p<0.05$) reductions in RBC, WBC, Hb, PCVand MCHC relative to their respective controls, Piper nigrum group and aspirin + Piper nigrum group; while hematmetric indices significant increased ($p<0.05$) and the (MCV and MCH) values relative to their respective controls, Piper nigrum group and aspirin + Piper nigrum group. While The results observed treatment in group (aspirin + Piper nigrum) also caused significant ($p<0.05$) reductions in RBC, WBC, Hb, PCVand MCHC relative to their respective controls, Piper nigrum group and hematmetric indices significant increased ($p<0.05$) and the (MCV and MCH) values relative to their respective controls, Piper nigrum group only.

Table(1):Effect of Aspirin and *Piper nigrum* on Haematology Parameters in Female Rabbits

Groups / Parameters	N	Control (Normal saline)	Aspirin (400mg/kg)	Aspirin+*Piper nigrum*	*Piper nigrum* (500mg/kg)
RBC×10^6	6	6.27±0.42 A	3.54±0.30 C	4.90±0.23 B	6.62±0.36 A
WBC×10^3	6	5.42±0.24 A	3.28±0.51 C	3.95±0.30 B	5.66±0.33 A
Hb g/dl	6	11.86±0.43 A	8.73±0.24 C	10.80±1.34 B	12.67±0.47 A
PCV %	**6**	39±0.70 A	31.5±2.25 C	36±1.30 B	40.25±.93 A
MCV fl	**6**	63.21±4.19 C	89.67±11.43 A	73.64±5.17 B	60.93±3.36 C
MCH pg	**6**	18.96±1.18 C	24.90±2.11 A	22.07±2.82 B	19.09±1.61 C
MCHC %	**6**	30.42±1.09 AB	27.84±2.20 B	30.12±4.75 AB	31.27±1.68 A

N=number of animals, A,B,C= differences between groups, P≤0.05 vs. control.

2-Effect of Aspirin and *Piper nigrum* on Differential Leukocyte Count in Female Rabbits

The results in Table(2) revealed that value of the percentage of Leukocyte in differential count. The results showed that significant increase (P≤0.05) in neurophiles and monocyte in group treated with aspirin and aspirin + *Piper nigrum* compared with control and *Piper nigrum* groups but caused decrease significant (P≤0.05) in lymphocyte in group treated with aspirin compared with control, aspirin + *Piper nigrum* and *Piper nigrum* groups.

The results showed that significant increase (P≤0.05) in eosinophils and basophils in group treated with *Piper nigrum* group compared with control, aspirin and aspirin + *Piper nigrum* groups.

Table(**2**):Effect of Aspirin and *Piper nigrum* on Differential Leukocyte Count in Female Rabbits

Groups / Parameters	N	Control (Normal saline)	Aspirin (400mg/kg)	Aspirin+*Piper nigrum*	*Piper nigrum* (500mg/kg)
Neutrophils%	6	44.16±2.85 B	48.50±1.04 A	46.85±1.16 A	43.16±1.16 B
Eosinophils%	6	3.5±0.03 B	2.4±0.06 C	1.7±0.02 D	4.1±0.12 A
Basophils	6	0.5±0.01 B	0.1±0.02 D	0.2±0.01 C	0.81±0.01 A
Lymphocyte %	**6**	44.19 ±1.23 A	38.47±2.43 B	42.00±5.8 A	44.36±0.03 A
Monocyte%	**6**	8.00±0.4 B	10.6±2.68 A	9.78±1.1 A	7.89±0.53 B

N=number of animals, A,B,C= differences between groups, P≤0.05 vs. control.

3- Effect of Aspirin and *Piper nigrum* on Biochemical Parameters in Female Rabbits.

The results in Table(3) revealed that the effect of 400 mg/kg B.W. of aspirin, 500mg/kg B.W. of Piper nigrum on biochemaical parameters of female rabbits after treatment for 30 days. The results showed that significant increase (P≤0.05) in total protein, globulin, AST, ALT activities, creatinin and bilirubin in group treated with aspirin compared with control, aspirin + Piper nigrum and Piper nigrum groups but caused decrease significant (P≤0.05) in cholesterol in group treated with aspirin compared with control.

The results showed that significant decrease (P≤0.05) in glucose, cholesterol in group treated with Piper nigrum and aspirin + Piper nigrum groups compared with control and aspirin while AST, ALT activities, creatinin and bilirubin insignificant (P≤0.05) compared control.

Table(3):Effect of Aspirin and *Piper nigrum* on Biochemical Parameters in Female Rabbits

Groups / Parameters	N	Control (Normal saline)	Aspirin (400mg/kg)	Aspirin+*Piper nigrum*	*Piper nigrum* (500mg/kg)
Glucose mg/dl	6	125.15±4.02 A	123.97±9.52 A	112.53±4.89 B	105.50±3.61 C
Cholesterol mg/dl	6	89.43±1.46 A	86.49±2.07 B	84.20±2.53 B	78.80±2.26 C
Total Protein g/L	6	5.27±0.13 B	5.47±0.14 A	5.31±0.09 B	5.24±0.05 B
Albumin g/L	6	3.34±0.22 NS	3.24±0.07 NS	3.16±0.01 NS	3.24±0.18 NS
Globulin g/L	6	1.97±0.27 B	2.23±0.07 A	2.15±0.02 AB	2.22±0.20 A
AST U/L	6	15.50± 2.42 B	24.83±5.49 A	15.33±3.38 B	13.16±2.92 B
ALT U/L	6	12.00±1.67 B	21.16±3.37 A	14.16±3.76 B	10.83±2.63 B
Creatinin mg/dl	6	0.88±0.05 B	1.03±0.18 A	0.88±0.06 B	0.81±0.02 B
Bilirubin mg/dl	6	0.19±0.02 BC	0.24±0.025 A	0.22±0.013 AB	0.21±0.021 B

N=number of animals, A,B,C= differences between groups, P≤0.05 vs. control.

1-Grossly Changes of Stomach

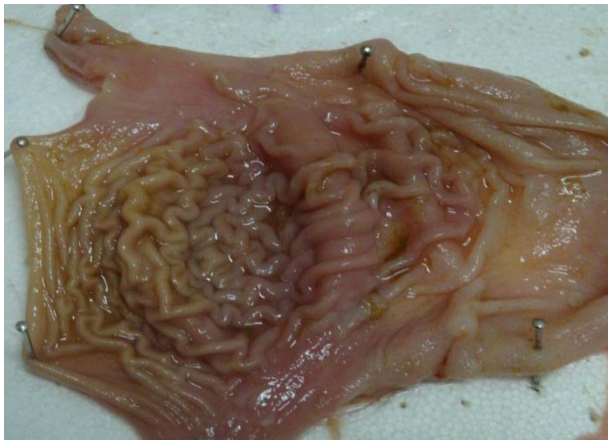

Fig.1: Gross appearance of the gastric mucosa in female rabbits. Showing normal gastric mucosa (Control group).

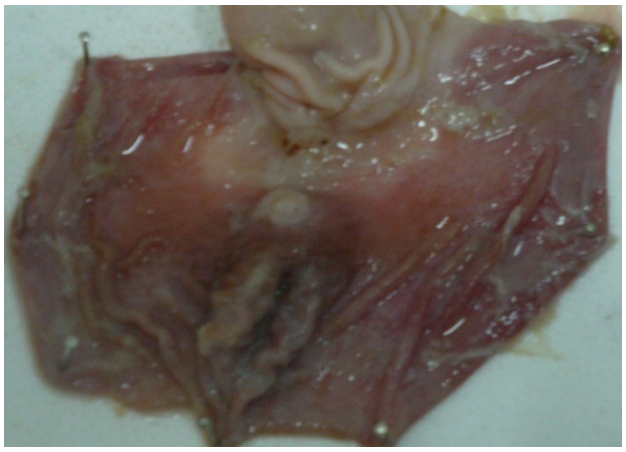

Fig.2: Gross lesion of gastric mucosa of female rabbits treated with aspirin. Showing sever damage are seen in the gastric mucosa.

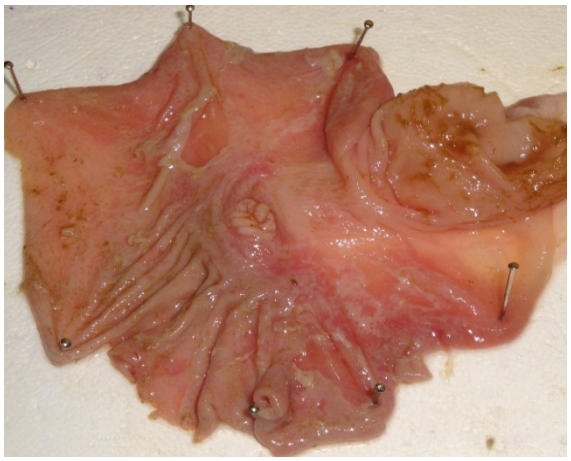

Fig.2: Gross lesion of gastric mucosa of female rabbits treated with aspirin. Showing damage in gastric mucosa.

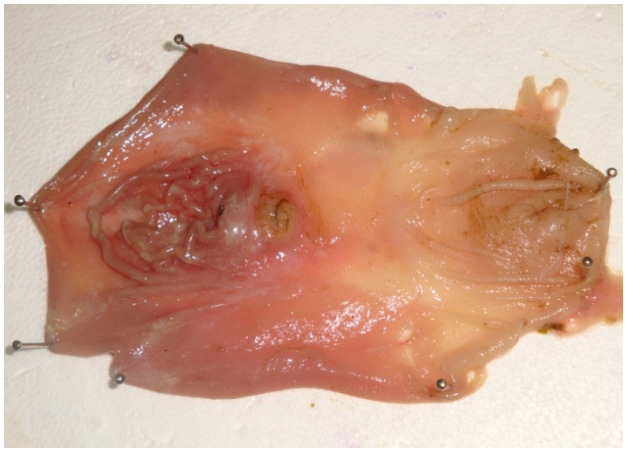

Fig.3: Gross lesion of gastric mucosa of female rabbits treated with aspirin and *Piper nigrum*.

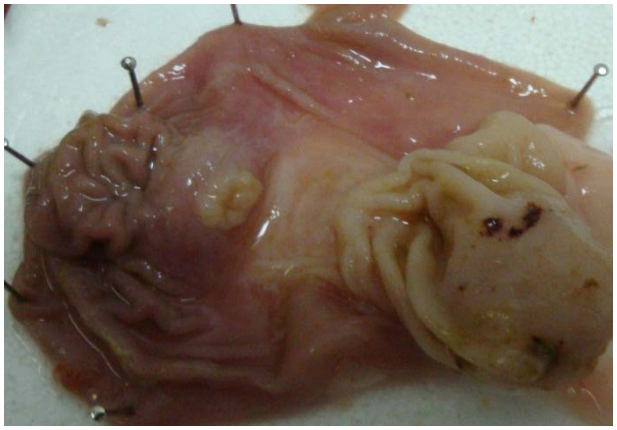

Fig.4: Gross appearance of the gastric mucosa of female rabbits treated only with *Piper nigrum*. Showing normal in gastric mucosa.

Histological Changes :-

1-Stomach:-

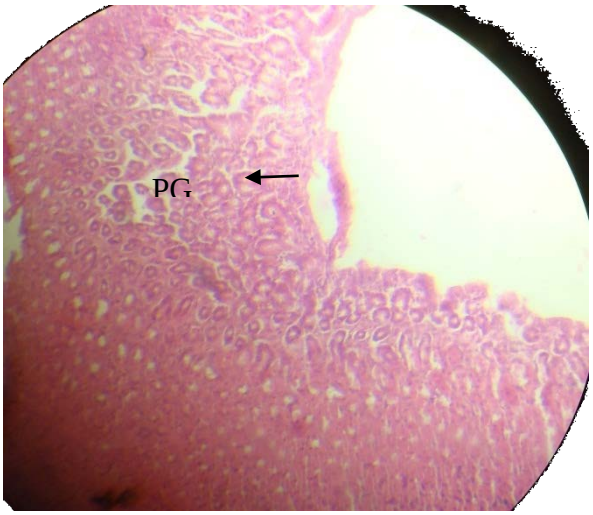

Fig.5: Histological section of gastric mucosa of control rabbits. Showing pyloric glands(PG) composed of parietal cells, chief cells and mucous cell H&E. 100X.

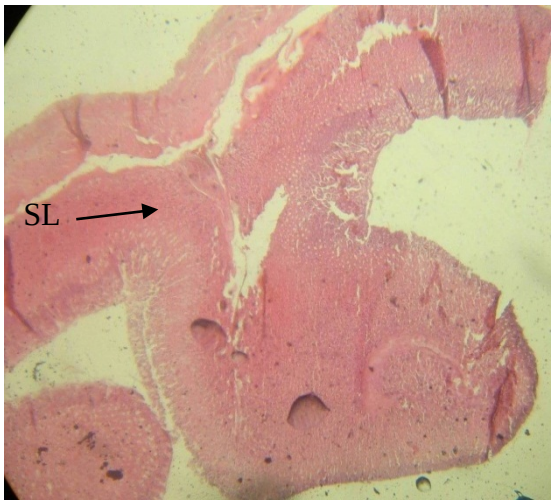

Fig.5: Histological section in stomach pyloric region of control rabbits. Showing mucus deposition on surface layer (SL). H&E.40X.

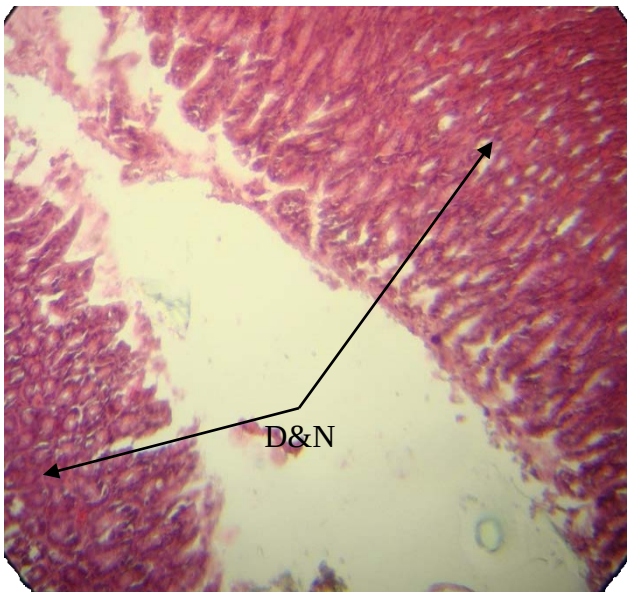

Fig.6: Section in gastric mucosa of rabbits treated with aspirin. Showing severe disruption to the surface epithelium and necrotic lesions penetrate deeply into mucosa (D&N)and the degenerated gastric pits (GP), stain with .H&E. 400X.

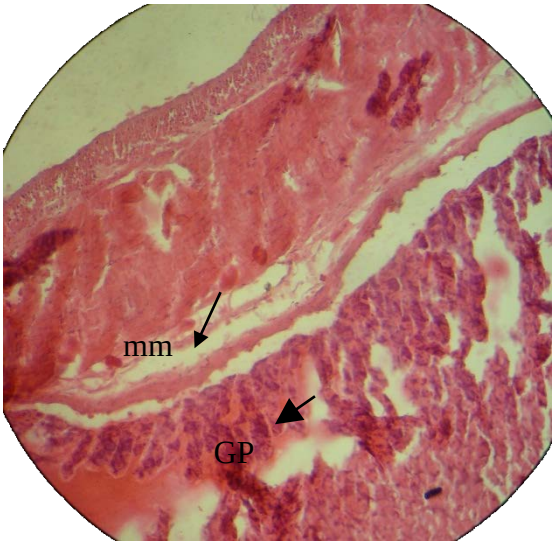

Fig.6:Section in gastric mucosa of rabbits treated with aspirin. Showing the degenerated gastric pits (GP)with infiltration of inflammatory cells near the muscularis mucosa(mm) .H&E. 100X.

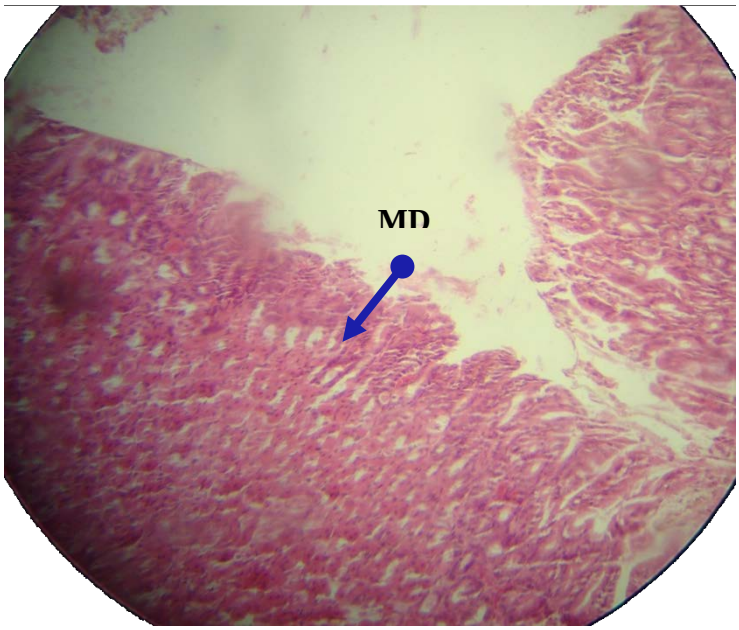

Fig.7: Histological section of gastric mucosa of rabbits treated with aspirin + *Piper nigrum*. showing mild disruption (MD) of the surface epithelium mucosa are present but deep mucosal damage is absent. H&E. 400X.

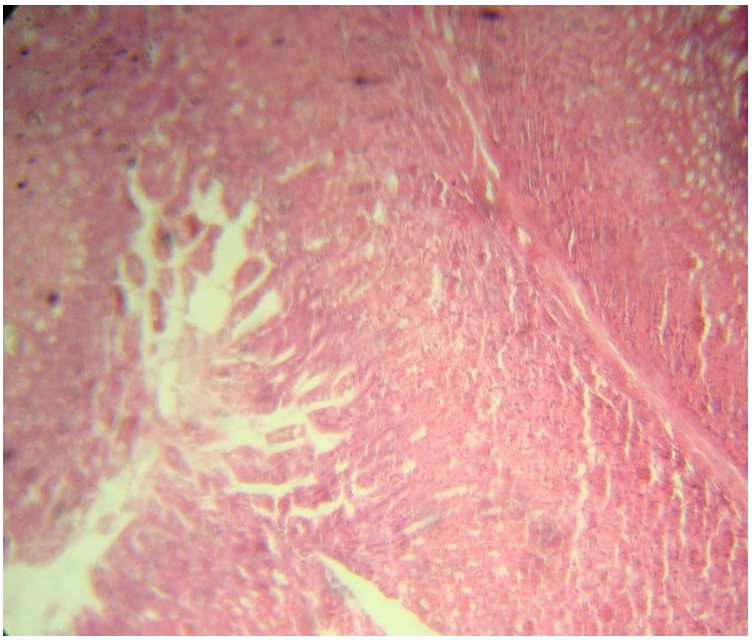

Fig.8: Histological section of gastric mucosa of rabbits treated with *Piper nigrum*. There is no disruption to the surface epithelium with no edema and no leucocytes infiltration of the submucosal layer, stained with H&E. 400X.

2-Liver :-

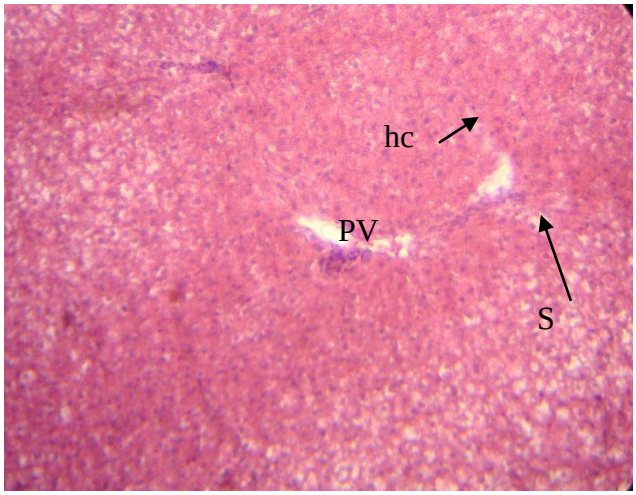

Fig.9: Histological section of liver of control rabbit. Showing normal hepatocyte (hc) normal portal vein (PV), siunsiod (S), stain (H&E)100X.

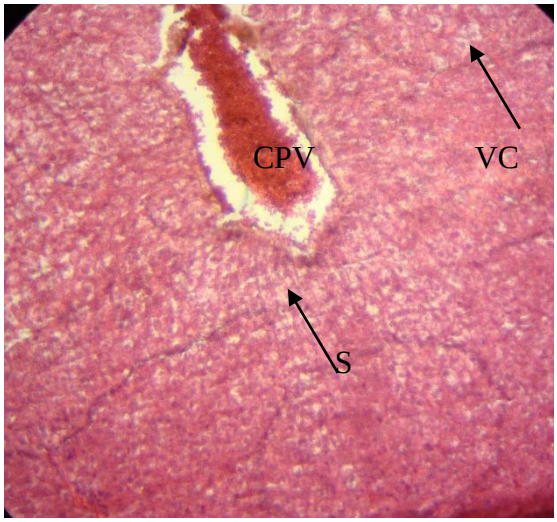

Fig.10: Histological section of liver of female rabbit treated with aspirin. Showing congestion of portal vein (CPV), moderate enlargement of hepatic cells, pyknotic cells as well as some enlargement of hepatic sinusoid(S) and vacuolated cytoplasm of hepatocyte(VC), stain (H&E)100X.

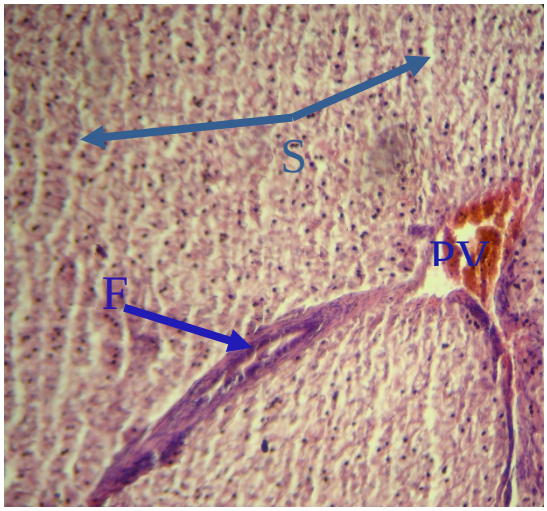

Fig.11: Histological section of liver of rabbit treated with aspirin + *Piper nigrum*. Showing engorgement of the portal vein (PV), fibrosis (F) moderate enlargement of hepatic cells, pyknotic cells as well as some dilated of hepatic sinusoid(S), stain (H&E)400X.

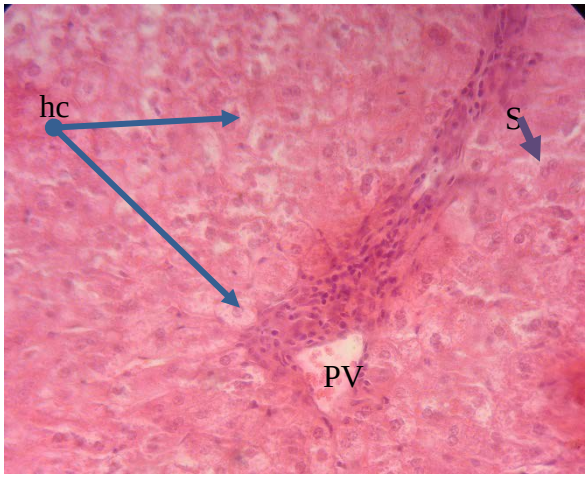

Fig.12:Histological section of liver of rabbit treated with *Piper nigrum*. Showing normal hepatocyte (hc) normal portal vein (PV), siunsiod (S), stain (H&E)400X.

Discussion

Piper nigrum Linn. (Piperacae) known as black pepper. The king of spice is one of the oldest and most popular spice in the world, *Piper nigrum* is an herbal plant. *Piper nigrum* different therapeutic agents including herbal preparations are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucus production. *Piper nigrum* used in folk medicine for stomach disorder[28].

In this study focuses primarily on the effect of high dose of aspirin on induced gastric ulcer and to determine the grossly and histopathological changes occurred on gastric architecture. Results showed that the grossly and histological changes clarified in gastric mucosa. The effect is more obvious on surface mucosal layer and this may be related to the effect of aspirin which is considered as an aggressive factor. These findings agreed with other studies which showed that oral administration of aspirin produce severe gastric mucosa damage and significantly decreased the gastric output because of so called back diffusion of HCl through the broken barrier[29]. Also the results in this study observed that the aqueous extract of *Piper nigrum* unable to cause any changes in the normal gastric mucosa of rabbit at the dose used for the study. In this study, on lack of any effect of aqueous extract of *Piper nigrum* on the gastric mucosa of normal rabbits, but when used in group(aspirin + *Piper nigrum)*. The aqueous extract of *Piper nigrum* improve the gastric mucosa compared with aspirin group. In recently studied, the investigations found *Piper nigrum* to significantly increase the parietal secretions, pepsin secretion and potassium loss [30]. The results of thisr study on antigastric ulcer activity revealed that aqueous extract of *Piper nigrum* at this dose confers a dose-dependent protection against the gross damaging action of necrotizing agents (sodium chloride and sodium hydroxide) on gastric mucosa of rabbits. The protection against the gastric damage caused by these agents might be related to the inhibition of gastric motor activity [31] and the stimulation of prostaglandin synthesis [32] caused by mild irritants. Thus, it is assumed that these gastric irritants might have activated the defensive mechanism and preserved the integrity of gastric mucosa[33].

These results could be explained by that prostaglandins normally protect the gastrointestinal mucosa from damage by maintaining blood flow and increasing mucosal secretion of mucous and bicarbonate[34]. Synthetic non-steroidal anti-inflammatory (NSAIDS) like aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and block diffusion of H^+ [35]. Aspirin blockade of cyclooxygenase-1 (Cox-1) and (Cox-II) results in reduction of prostaglandin synthesis. The interruption of prostaglandin synthesis results in impairment of mucosal damage repair, thus facilitating mucosal injury[36]. Aspirin and related non-steroidal anti-inflammatory drugs and alcohol can aggravate or interfere with the healing of peptic ulcers. Smoking is also known to slow ulcer healing [37]. In parietal cells, H+ ion secretion is an oxidant process. H^+ ion dissociates from H_2O or H_2CO_3 also in an oxidant process. H^+ is pumped out in exchange with K^+ as an active transport process and Cl- ion in exchange with HCO_3- in basolateral membrane of parietal cells [33]. Cl- ion is then pumped and transported into gastric lumen. These increase oxidant process and increase hydrogen peroxide production. In presence of Cl- and H_2O_2, hydrochloric acid will be formed and this is a very toxic oxidant. This will results in mucosal membrane lipid peroxidation and mucosal soreness and disruption [38]. Lipid peroxidation and lipid derived products have been implicated in pathogenesis of a variety of diseases [39]. Extracts of *piper nigrum* antioxidant phytochemicals that prevent oxidants damage[40].

In this study on the gastric ulcers induced by aspirin revealed aqueous extract of *Piper nigrum* to affect the motor activity and prostaglandin synthesis in the gastric tissue. The data on gastro protective activity is further supported by our observation on the effect of *Piper nigrum* to inhibit the pylorus ligation-accumulated secretions and the related ulcers. The gastric antisecretory activity observed may be due to peripheral parasympathetic blockade,as the Black pepper does not antagonize the acetylcholine induced contraction of the smooth muscle of guinea pig ileum[41,42].

However, in Black pepper-treated Shay rats, the severity of ulcers was significantly reduced and this protection could be due to its effect in reducing the volume and acidity of gastric secretions[43]

The present study confirms the previous observation that suppressants of gastric acid secretion increase the healing of gastric ulcers in both humans and experimental animals [44,45].The exact constituent/(s) responsible for the gastroprotective activity of aqueous extract of *Piper nigrum* is assumed that the inflence of piperine on the endogenous levels of coenzyme Q10 [46]. might be crucial in the protection of gastric ulcers.

Hematological disorders as a result of aspirin ingestion are well documented clinically, and the results of the present study further confirm the association, as anemia accompanied by marked leucocytosis was observed in the rabbits treated with aspirin in chronic doses. Prolongation of bleeding time was one of the first clinically recognized hematological side effects of aspirin administration[39]; anemia, agranulocytosis and leucopoenia are some of the most frequently reported adverse effects of aspirin.

Aspirin caused significant changes in the MCV and MCH values which could be an indication of macrocytic anaemia since increased MCV an MCH values are known to be indicative of macrocytic anaemia. Also, aspirin caused non- significant change in the MCHC value which suggest and absence of hereditary spherocytosis since MCHC values are known to be elevated in hereditary spherocytosis. The significant reduction in TWBC count caused by aspirin suggests that the immune system has been compromised.

Aspirin caused significant change in total protein levels, which probably indicates that the buffering capacity of the blood and body fluid balance have been compromised. The increase in albumin level which suggests that the plasma level of metals, ions, fatty acids, amino acids, bilirubin and enzymes have been compromised by aspirin.

Aspirin caused significant increase in the activity of ALT which the hepato-toxic potential of aspirin. Aspirin also caused significant increase in the activity of AST which hepatic tissue necrosis induction by aspirin and histological changes of liver indicate this result. Aspirin caused significant change in creatinine level which suggests that the structural integrity and functions of the nephrons have been compromised. Also the aspirin effect on thyroid structure and ovaries lead to affected reproductive efficiency in female rabbit. While in group treated with *Piper nigrum* insignificant changes in haematological and biochemical parameters except glucose and cholesterol compared with control related to non toxic *Piper nigrum*. This result agreement with [47].

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