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Research Article

Effects of Diets Containing Seeds of Soybean (*Glycine max)* on Healing of Acetic Acid Induced Gastric Ulcers in Rats

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Abstract

Soybean-based foods are a known to be good sources of phytochemicals which are of immense health benefit. The effect of soybean incorporation into rat diet on healing of experimental gastric ulcers was investigated. Male Wistar rats (120-140g; n=5) rats were fed for six weeks on normal rat diets but specially composed to contain 25%, 50% and 75% by weight of raw Soybean (SB) diet respectively fed for six weeks respectively. Thereafter, gastric ulcer was induced using acetic acid and feeding continued till the end of the experiment. Daily body weights were monitored, Basal gastric acid secretion, ulcer score and histological evaluations were carried out on days 3, 7 and 10 post ulcer induction. Results were subjected to one-way ANOVA analysis, presented as mean ± SEM. Body weights of SB diet fed rats were significantly increased compared with ulcer alone all through the experiment. Basal acid output significantly reduced as healing progressed in the 50% and 75% SB diet fed group when compared with the group without SB. Incorporation of SB into diet increased ulcer healing rate, with the 75% SB diet group having the highest rate of 68% (day 3) and 92.1% (day 10). 25% SB diet afforded 50% (day 3) and 47.5% (day 10) healing respectively. There were no visible lesions but moderate preponderance of mucous cells in gastric tunics were observed in both the 50% and 75% SB diet. Soybean diet may be beneficial in peptic ulcer management.

Key Words: Soybean diet, gastric ulcer healing, Basal Gastric secretion, parietal and mucous cell counts

INTRODUCTION

The gastrointestinal tract is saddled with the task of ingestion, digestion and absorption of food, drugs and water for energy supply necessary for normal body system functioning. As a result, it is exposed to many harmful substances which alter the integrity of the gastric mucosa. The normal gastric mucosa integrity is achieved as a result of a balance between the protective and aggressive factors (Najm, 2011). A tilt in favour of the aggressive factors (Berardi *et al*., 2008, Calam *et al*., 2001) leads to gastric ulceration. Consequent to adverse and undesirable side effects of most effective synthetic anti-ulcerogenic drugs/medications, there has been a massive search in the diet therapy and naturopathic communities for the best and most balanced natural sources in form of food with possible anti-ulcerogenic or ulcer-healing activities (Reis, 2003).

Phytochemicals from functional foods are fast becoming subjects of interest. These non-nutritive plant chemicals are being promoted in the prevention or treatment of diseases. Soybean (Glycine max (L) Merril) belongs to the class of legume native to East Asia and is of the isoflavone group of flavonoids, (Stopper *et al*., 2005). Contained in soyabean are notable amounts of phytic acid, alpha-linolenic acid and isoflavones; upon analysis, soybean has been found to contain about 50-55% Genistein, 4-45% Daidzein and 5-10% Glycitein of total isoflavones contained in it (Murphy *et al*., 1999). Soyabean is considered by many agencies to be a source of complete protein (IITA, 1990, Henkel *et al*., 2000) since they contain significant amounts of essential amino acids in the appropriate quantity. Soybeans have been reported to be rich in the B-vitamin complex, particularly in folic acid, a proof of its reported hematinic properties (Alada *et al*, 2014). Olaleye *et al*., (1999) had earlier observed that processed soya bean exhibited better hematinic properties when compared with the raw form. Several health benefits have been reported for soya bean and its constituents. For example, Pinitol from soybeans has been shown to reduce postprandial blood glucose in patients with type 2 diabetes mellitus (Kang *et al*, 2006). This is in addition to the anti-obesity and hypolipidemic effects reported for black soybean anthocyanins (Kwon *et al*, 2007).

One of the earliest reports of the benefits of Soya bean on the gastrointestinal tract integrity was that of Nafstad (1967) who showed that inclusion of soybean meal in the pig ration markedly reduced the number of ulcers. This was further corroborated by Borge and Nafstad (1969) in the same species. Fung (1975) gave Soya bean milk orally in doses of 150 ml second-hourly (7 a.m. to 11 p.m. daily) to 10 Chinese subjects with proven gastric ulcer for two weeks and concluded that the effect of soya bean milk on gastric ulcer healing was not significant. However, McArthur *et al* (1988) reported that Soy protein meals stimulate less gastric acid secretion and gastrin release than beef meals, suggesting a beneficial effect of the

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legume. Alada et al (2005) also reported the antiulcer potential of Soya bean diet using indomethacin model of gastric ulceration in rats.

The present study was designed to investigate further, the effect of soya bean-based meal on experimental gastric ulcers, with special interest on the healing of ulcers in rats.

MATERIALS AND METHODS

Preparation of Soybean diet:

Soybean diet was prepared according to the method described by Bolarinwa and Evbuomwen (1990) and Olaleye *et al.*, (1999) to contain 25%, 50% and 75% soybean incorporated into the rat feed. The control and ulcer groups were fed normal rat cubes (0% raw soya-bean diet).

Animals and Experimental design:

Seventy-five male Wistar rats obtained from the Faculty of Basic Medical Science's animal house, University of Ibadan were used. They were allowed to acclimatize to laboratory conditions of temperature (23±2ºC), relative humidity of 45%-55%, 12-hour light and dark environmental cycle for a period of 2 weeks. They were thereafter housed in experimental cages under standard conditions of the Animal house at the Department of Physiology, University of Ibadan and fed with standard rat cubes (food) and water *ad libitum*. The animals were housed in the Animal House of the College of Medicine, University of Ibadan in well-ventilated cages. All procedures on animal handling were in accordance with guidelines of the University of Ibadan Ethical Committee which conformed to the Guide for the Care and Use of Laboratory Animals by the National Institute of Health. The rats were randomly divided into five groups of fifteen animals each as shown in Table 1 Groups A_1 and A_2 were control rats.

Table 1
Animal grouping and treatment

Group	Treatment
A_1	Control, received rat chow and distilled water daily. No Soyabeans, No ulcer
A_2	Control, received rat chow and distilled water daily. No Soyabeans, with acetic acid- induced ulcers
B	25% Soyabeans diet (for 6 weeks) with acetic acid- induced ulcers
C	50% Soyabeans diet (for 6 weeks) with acetic acid- induced ulcers
D	Soyabeans diet (for 6 weeks) with acetic acid-induced ulcers

Determination of Ulcer Scores: Rats from each group were sacrificed at post-induction days 3, 7 and 10 after carrying out basal gastric acid secretion. The severity of damage done to the inner surface (mucosal lesions) was determined by examining the glandular part for a gross ulcer score using $\pi/4 \times a \times b$ mm^2 , where a and b are the long and short axis of the ulcerated area (Onasanwo *et al.,* 2000)

Acetic acid-induced gastric ulcer.

Chronic ulcer was induced based on the method originally described by Takagi *et al*. (1969) with some modifications by Wang *et al.,* (1989) and Elliott *et al.,* (1995). Briefly, the animal was anesthetized using ketamine (60mg/kg) – xylazine (5mg/kg) cocktail, after which laparotomy was done to carefully expose the stomach. Gastric ulcer was induced by injecting 0.04 mL of 60% acetic acid solution into the gastric serosa for 30 seconds after which it was withdrawn, the stomach was thoroughly cleaned with normal saline, returned back into the viscera and then sutured back (skin).

Gastric acid secretion studies

Gastric acid secretion studies were carried on five rats per group on post-induction days 3, 7 and 10 using the continuous perfusion technique of Ghosh and Schild (1955), modified by Amure and Ginsburg (1964). Rats anaesthetized with a mixture of 4:3 ketamine HCl- xylazine were surgically prepared for *in situ* stomach perfusion. A tracheal cannula was inserted via an incision on the neck to ensure normal breathing throughout the course of the experiment. An abdominal incision through the *linea alba* was made to expose the stomach and a semi-transection made at the junction of the pylorus with the duodenum. A pyloric cannula was inserted and tied to collect gastric contents. An orogastric cannula was inserted for perfusion of pre-warmed (at temperature 37^0C) 0.9% normal saline (pH 7.00) at a rate of 1ml/minute using a perfusion pump. Gastric acid was collected via the pyloric cannula at 10 minutes intervals. The acidity of each 10 minutes effluent collected was assayed by a titration method described by Olowokorun (1975) and expressed as the basal acid secretion (mEq/L/10min).

Histology

A small section of the stomach was collected from rats from each group after examination/ assessment of ulcer severity and preserved in 10% phosphate buffered formalin. Tissue processing was carried out by autotechnic on 5µm thick sections were prepared and mounted on slides thereafter stained with haematoxylin and eosin (H&E). Stained sections were morphologically evaluated and the microphotograph was taken.

Statistical analysis

The Mean, Standard Deviation (S.D) and Standard Error of Mean (S.E.M) of all values were calculated. The result was presented as Mean ± S.E.M and one-way Analysis of variance (ANOVA) was used to compare differences among variables. The difference between the groups was taken to be significant at $P< 0.05$.

RESULTS

Effect of Soybean diet on body weight profile.

All animals had increased body weight in throughout the six-week feeding period. The mean body weight of the control animals increased from 88.67±5.74g to 143.67±8.33g after six weeks (38.28% increase). For the animals given 25% SB diet the mean body weight increased from 110.5±0.76g to 169.17±7.89g after six weeks (34.68% increase). For the animals given 50% SB diet the mean body weight increased from 109.0±0.76g to 156.83±5.05g (30.50% increase). For the animals given 75% SB diet the mean body weight increased from 101.17±3.09g to 142.33±14.83g (28.92% increase). As shown in Fig. 1, significant decreases in percentage weight gains were observed in the SB diet fed groups when compared with the control animals.

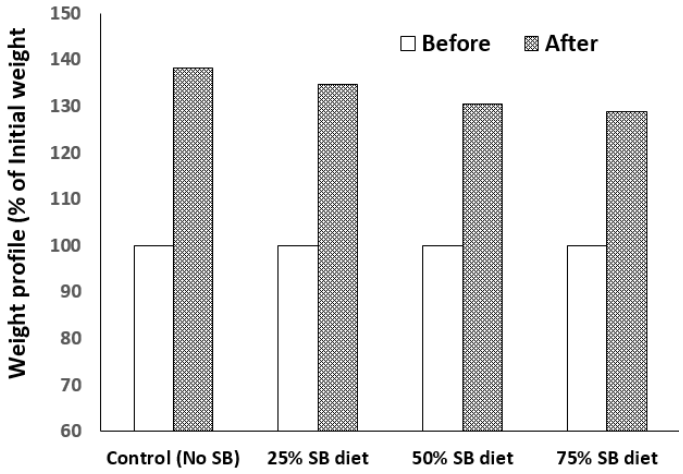

Figure 1
Profile of body weight changes in control and Soybean diet- fed rats. Each bar represents percentage changes over the initial body weight after six weeks of feeding.

Effect of raw Soybean diet on acetic acid-induced gastric ulcer healing.
Results of this experiment showed that acetic acid produced round ulcer in the stomach of all the animals. There was a significant increase in ulcer scores of the ulcerated group that were not given SB diet when compared with all the other experimental groups on days 3, 7 and 10. However, there was no visible sign of ulcer in the normal control group without acetic acid-induced ulcer.

In the control rats with acetic acid-induced ulcer, total ulcer area was 293 ± 43.43mm^2 three days post induction (Fig. 2). Ulcerated area was reduced to 139.8 ± 15.03mm^2 (52.29% reduction) and 40.06 ± 3.60mm^2 (86.33% reduction) on day 7 and day 10 respectively. Significant improvement in ulcer healing was recorded in the soya bean diet fed rats with 76.29, 74.75% and 70.21% reduction in ulcerated area 7 days after ulceration for the 25% SB, 50% SB and 75% SB diet respectively.

No visible sign of ulceration was observed in the 75% SB diet by day 10 post ulcer induction (100% healing) while the 50% SB diet group afforded 96.32% healing rate. There were however, visible ulcers in both the 0% and 25% SB diet fed groups. Representative pictures of the stomach from each group are shown in Plate 1.

Effect of Soybean diet on basal gastric acid secretion, gastric acidity and pH
Table 2 shows the profile of basal gastric acid secretion 3, 7 and 10 days after inducing gastric ulcers using the acetic acid model. Basal gastric acid section in the control rats without ulcer was 0.71±0.04 mEq/L. There was no significant difference in basal secretion in this group throughout the experiment. three days post-ulcer. In the control animals (0% SB diet) with acetic acid induced ulcer, gastric acid secretion significantly rose to 0.86±0.02 mEq/L. Feeding rats with 25%, 50% and 75% SB diet caused a reduction of basal gastric secretion to 0.76±0.01, 0.71 ±0.01 and 0.65±0.03 respectively after 3 days of ulcer induction. Similar patterns of ulcer reduction was observed after 7 and 10 days of inducing ulcer (Table 2).

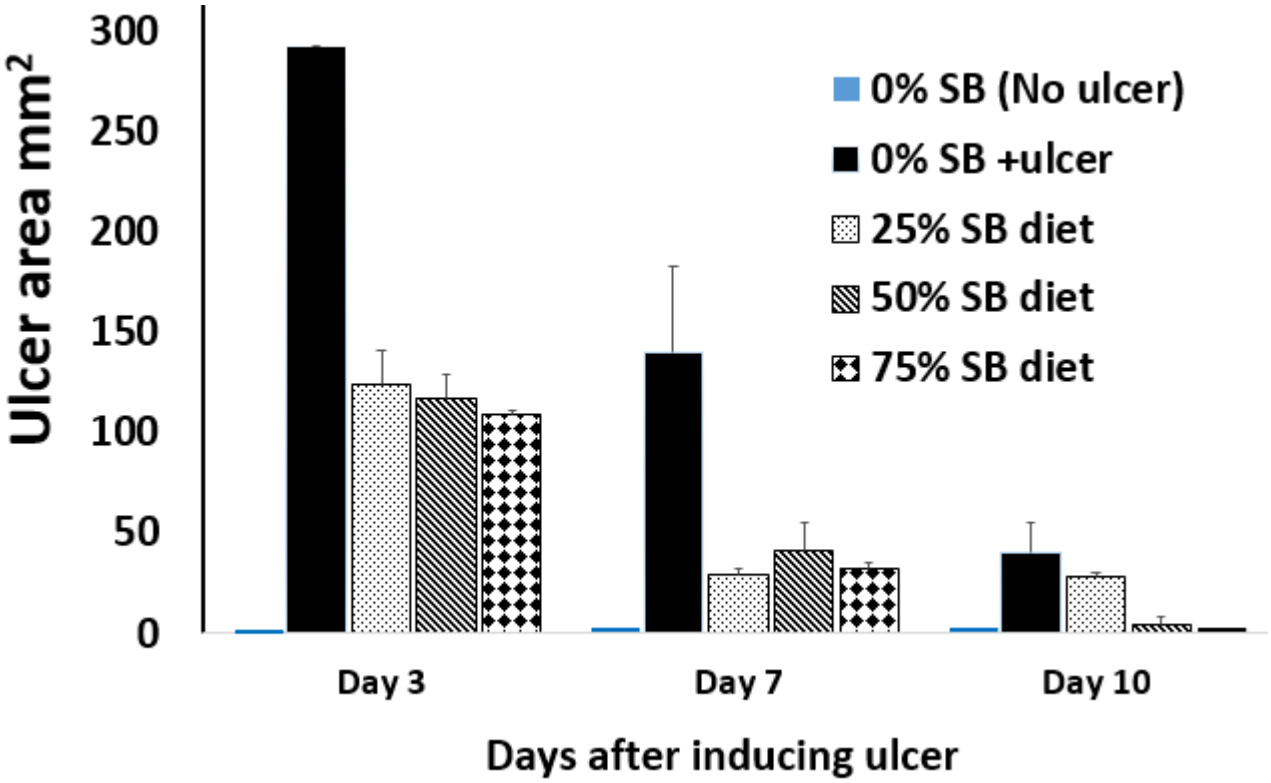

Figure 2
Effect of diets containing seeds of Glycine max (Soybean, SB) on healing of acetic acid-induced gastric lesions in rats. Results are expressed as mean ± S.E.M. (n = 5/group).

Plate 1:
Photomicrographs of the stomach of rats

Table 2
Basal Gastric Acid Secretion, Parietal and Mucous Cell Counts in the induced stomach of rats fed with diets containing seeds of soybean 3, 7 and 10 days after acetic acid induced ulcer.

Group/ Treatment	Gastric Basal Secretion (mLs/10mins)			Parietal Cell Count			Mucous Cell Counts		
	Day 3	Day 7	Day 10	Day 3	Day 7	Day 10	Day 3	Day 7	Day 10
Control (No ulcer)	0.71 ±0.04	0.75 ±0.03	0.73 ±0.03	27 ± 1.53	20 ± 0.58	6.0 ± 1	38.67 ± 3.33	45.0 ± 2.0	96.67 ± 4.3
Ulcer alone	0.86 ±0.02[a]	0.84 ±0.01	0.81 ±0.01	25.33 ± 1.67	21.67 ± 0.33	23.0 ± 1.0	42.33 ± 3.7	44.33 ± 1.8	49.33 ± 1.67
25% SB diet + ulcer	0.76 ±0.01	0.68 ±0.05	0.76 ±0.01	21.50 ± 1.5	26.67 ± 0.67	26.67 ± 0.67	75.67 ± 11.33	35.0 ± 1.53	62 ± 11.02
50% SB diet + ulcer	0.71 ±0.01[b]	0.66 ±0.10	0.63 ±0.01[a,b,c]	17.0 ± 1.00	3.00 ± 0	16.0 ± 0.6	65.33 ± 0.67	102.7 ± 0.57	58.3 ± 2.6
75% SB diet + ulcer	0.65 ±0.03[b]	0.49 ±0.90[a,b]	0.50 ±0.03[a,b,c,d]	14.67 ± 1.83	13.67 ± 2.67	16.33 ± 3.3	84.67 ± 8.33	71.33 ± 4.4	67.67 ± 9.7

Values are expressed as Mean ± SEM. Values are significant at p<0.05, significant when compared: a- with control, b-ulcer alone, c-25%, d-50%

Histology

Plates 2 - 4 show the histological analysis of control rats without ulcer as well as those fed 25 – 75% SB diets. In the control rats, there were normal mucosa surface epithelial layer. The mucosa layer shows no infiltration of inflammatory cells. The gastric gland and lamina propria appear normal. The parietal cells appear normal. H&E-stained stomach specimens from the ulcerated rats revealed well-defined ulcers with complete destruction of the mucosal and submucosal layers caused by acetic acid administration.

Further details of the histological findings are presented in the plates

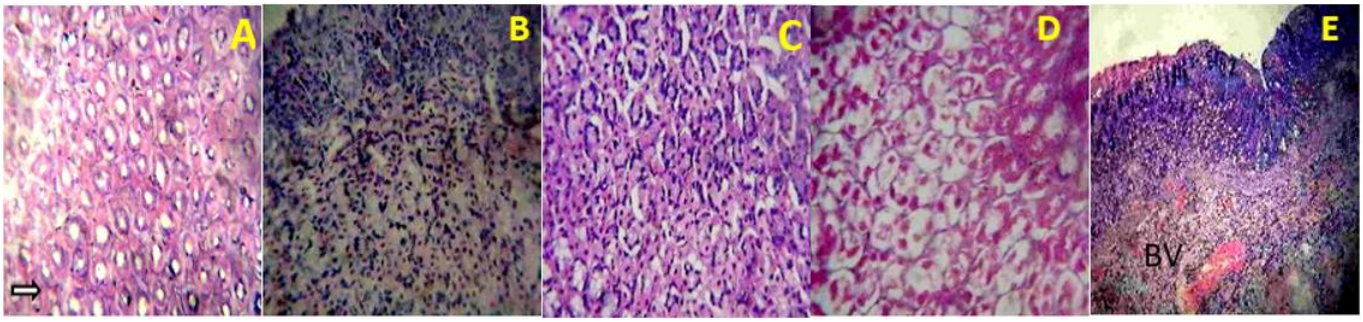

Plate 2

Photomicrograph of a stomach sections by Day 3 showing **A (Control):-** normal mucosa surface epithelial layer. The mucosa layer shows no infiltration of inflammatory cells. The gastric gland and lamina propria appear normal. The parietal cells appear normal. The circular muscle appears normal; B (ulcer alone):- severe haemorrhages in submucosal, extensive moderate erosion of upper mucosa (blue arrow); C (25%/kg b.w Soyabean):- marked congestion of blood vessels in mucosa and submucosal, multiple focal moderate erosions of mucosa with necrosis of a few glands. Moderate inflammatory cellular response (mostly macrophages and neutrophils). D (50%/kg b.w Soyabean):-no visible lession in tunics, moderate preponderance of mucus cells. E (75%/kg b.w Soyabean):- no focus of ulcer seen, moderate increase in neutrophils in submucosal, moderately congested blood vessels (BV), preponderance of mucus cells in upper protion of mucosa, other tunics are normal. (MAG: X100)

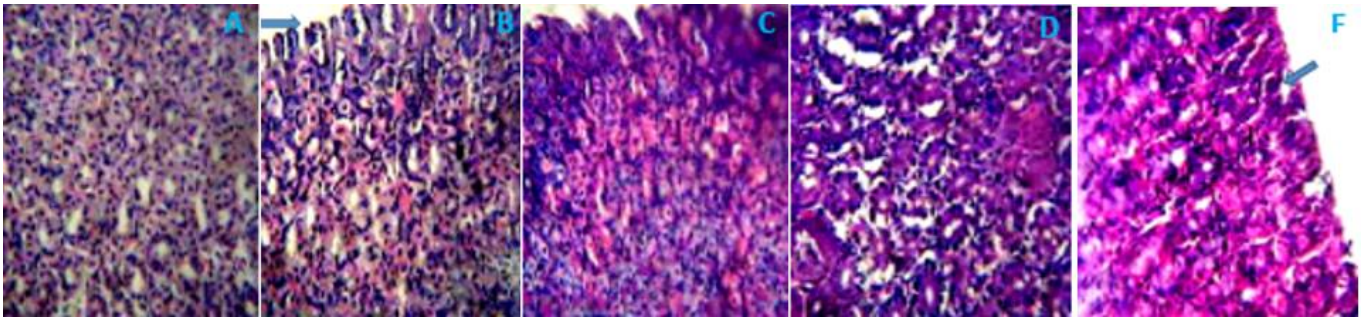

Plate 3

Photomicrograph of a Stomach Sections by Day 7 showing **A (Control)**:- marked congestion of blood vessel in submucosal, other tunics are normal and no ulcer is seen. ; **B (ulcer alone)**:- mild multifocal erosion of surface epithelium (blue arrow), congested blood vessels in lamina propria, a single focus of a necrotic gland, other tunics are normal with no ulcer; **C (25%/kg b.w Soyabean)**:- no visible lession in all tunics. **D (50%/kg b.w Soyabean):-**moderate erosion of surface epithelium (multifocal), intact lamina mucosal (LMM), oderate increase in the number of macrophages and lymphocytes in the submucosal, other tunics are normal with no ulcer seen. **E (75%/kg b.w Soyabean):-** a focus of moderate ulcer (blue arrow) with margins fairly closely apposed. Ulcer extends to submucosal with intense inflammatory **(I)** cellular response in the submucosal, ulcer margins contain regenerating mucosal glands (MAG: X100)

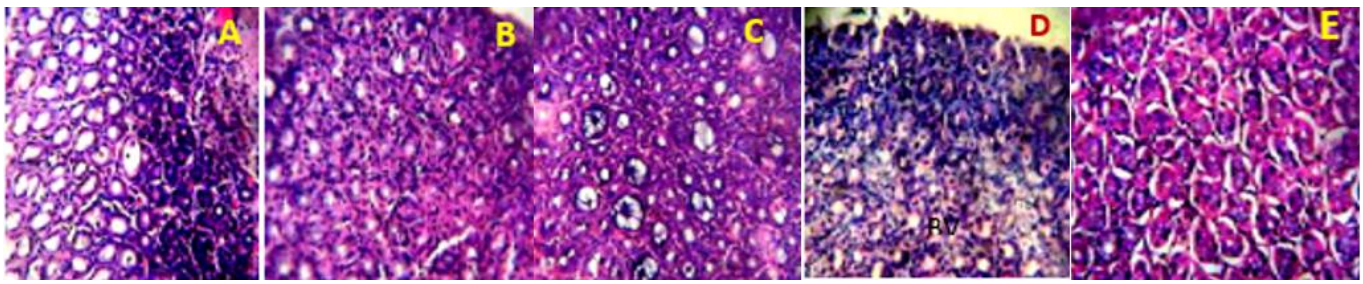

Plate 4

Photomicrograph of A Stomach Sections by Day 10 showing **A (Control)**:- extensive mild erosion of surface epithelium, moderate congestion of blood vessel in mucosa and submucosal, increased mucus glands with no visible lession in other tunics. ; **B (ulcer alone)**:- mild erosion of tips of mucosa and no visible lession in other tunics; **C (25%/kg b.w Soyabean)**:- no visible lession in all tunics except for moderate congestion of blood vessels in mucosa. **D (50%/kg b.w Soyabean):-**no visible lession except for moderate congestion of blood vessels **(BV)** in mucosa and submucosal. **E (75%/kg b.w Soyabean):-** thick mucosa ruggae with mild necrosis of glands at the tips of mucosa. No visible lession in other tunics. (MAG X 100).

DISCUSSION

This study was carried out to investigate the gastroprotective effect of diets containing seeds of soybean (Glycine max) and its healing effect on chronic gastric lesions induced by acetic acid. Our results show that incorporation of Soybean at levels of 50% and 75% into the rat diet significantly enhanced the healing of experimental ulcers.

Although significant increases in body weights were observed at the end of the feeding period, it was generally observed that the body weights of the animals with experimental ulcers showed a decline at the end of the experiment. Nieto (2012) had reported that body weights were negatively compromised during ulcer healing probably due to depletion in the body protein stores in a bit to restored the ulcerated surface. In a study conducted by Niyibituronsa *et al*., (2014), Soybean meal

was found to improve the body weight in malnourished children due to its high protein and amino acid contents (Ghandi 2009; Boye and Ribereau (2011). However, irrespective of the high nutritional status, Sadeghian *et al*., 2015 reported that soyabean had an inverse relationship with body mass index. Observations from this study further buttress the anabolic activities and potentials of soya bean in improving body weight gains

In this study, 50% and 75% raw Soya-bean diet greatly reduced gastric acid secretion on both days 7 and 10 in the experimental rats. This result is consistent with previous reports about the anti-secretory activities of oven dried Soyabean diet (Alada *et al*., 2005). Meals rich in protein have been documented over the years to have reducing activities on gastric acidity (Saint-Hillarie *et al*., 1960; Williams *et al*., 1976; Brooks 1985; McArthur *et al*., 1988, Mullin *et al*., 2009) which probably might be as a result of the lowered or transient release of the hormone gastrin. Decuypere *et al*., (1981) in his study observed that soluble soya bean protein produced more gastric secretions compared with insoluble soya bean proteins in pig subjects. Brooks (1985) however, suggested that this reduction might be due to the chemical compositions, size and physical characteristics of the protein meal consumed. These observations might have contributed to the dose dependent reductions of gastric acid secretion in this study.

McArthur *et al*. (1988) also buttressed that Soy diets enhances gastric emptying time compared to beef diet meaning less retention or delay or contact time of the diet in the stomach and reduced requirement of the hormone gastrin. These findings by McArthur *et al* (1988) were as a result of Brooks, 1985 postulation. However, it was observed in this study that as SB diets percentage increased, the gastric secretions where further reduced thus confirming one of the postulates by McArthur *et al.* (1988) that concentration may also contribute to the reduction in gastric secretions. Furthermore, raw SB diet was used in the study and has been found to contain trypsin inhibitors (Schneeman *et al*., 1977) which might have also contributed to the observed reductions in gastric acid secretions. Probably, these findings might have summed up for the observed anti-secretory activities of the raw Soya-bean diet which is a rich source of dietary protein.

However, by day 10 post-induction, animals fed with 50% and 75% Soyabean diet had increased gastric ulcer healing indexes. Previously, Alada *et al*., 2005 reported that oven dried Soya-bean diet prevented formation of indomethacin induced gastric ulceration and was dose dependent. Earlier reports have documented the importance of protein and other food classes in the phases of wound healing (Demling, 2009). Dietary protein has been implicated mostly as being essential to the stages of cell proliferation and protein synthesis of ulcer healing (Breslow, 1993, MacKay and Miller, 2003). Likewise, a balance of both the metabolic (Alix *et al*., 2007; Campbell *et al*., 2001) and anabolic (Brewslow 1993; MacKay and Miller 2003) processes of wound healing require ample daily requirement of protein for effective healing to occur (Legendre *et al*., 2008). The observed increased healing rate might be as a result of the ability of soya-bean to contribute to the nutritional status of protein stores and its balanced conversion for energy thereby enhancing its' availability for new cell growth at ulcerated site during gastric ulcer healing.

Histological evaluations of stomach tissues from the different Soya bean diet fed groups revealed reductions in the parietal cell counts but increased mucous cell counts which were dose dependent. Johnson and Chandler 1973; Martins *et al*., 1970 documented in their studies that antrectomy causes reduced production of serum gastrin concentrations as well as decreases in both parietal cell counts and gastric acid secretions. McArthur *et al*. (1988), further suggested that probably chronic consumption of Soyabean diet might produce similar results without antrectomy or change in gastric functions. However, this study confirms the postulations of McArthur *et al*., (1988) as the parietal cells in the SB diet rats were grossly reduced with increasing diet concentrations over the duration of feeding exposure. This probably might be responsible for the progressive and enhanced healing rate in the various SB diet groups.

Soyabeans has also been documented to contain flavonoids (Beil *et al*., 1995, Murphy *et al*., 1999, Takekawa *et al*., (2006)) which have been documented to enhance gastric ulcer healing. It may probably be that these inherent phytochemical compounds worked in synergy with the previously discussed mechanism in enhancing gastric ulcer healing.

In conclusion SB diet enhanced chronic gastrin ulcer healing probably by reducing gastric acid secretion and pH, increased turn-over of protein in the gastric tissue for developing new cells for healing at ulcerated site and marked reduction of parietal cell counts with exacerbated mucous cell counts. These observations were also dose dependent.

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