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Comparative evaluation of the treatment of induced ulcer and diabetes with yoghurt and drugs in rats

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Abstract

Stomach ulcer is a break in the lining of the stomach, mostly the first part of the small intestine and occasionally the lower oesophagus. With a gastric ulcer the pain may worsen with eating. Diabetes on the other hand is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period. The effects of yogurt on ulcer induced and diabetes induced in rats was evaluated. The induction of ulcer was done using acetic acid, while diabetes was done using Streptozotocin following standard procedures. The rats were fed with the yogurt (Rev up unsweetened yoghurt) for three (3) weeks, taking the samples of the stomach and pancreas weekly for histopathological examination. The results showed that the 99% acetic acid induced severe gastric ulcer after 72 hrs while Streptozotocin induced type 1 diabetes (235 mg/dl from 90 mg/dl) after 96 hrs. The administration of the yogurt caused cell infiltrations of the stomach wall, distinct ductile without aberrant superior or inferior diverticular of the stomach interlobular lining and well-formed stomach blood vessels. The yoghurt on the other hand caused little positive effect on the pancreas globules after three weeks of administration. The results suggests that consuming unsweetened yoghurts (containing probiotics) may improve glucose metabolism to a degree while it has more gastro-protective effect on ulcer. Therefore, while yoghurt may be recommended for ulcer treatment, it may not be for diabetic patients.

Keywords: Induced ulcer, diabetes with yoghurt, drugs in rats

1. Introduction

Yogurt is made by the fermentation of lactose (milk sugar) by bacterial enzymes. This process is anaerobic, meaning that it occurs in the absence of oxygen. Lactose is a compound sugar, made up of the two simple sugars; glucose and galactose. During the making of yogurt, the lactose is broken down by the lactase enzyme (produced by bacteria) into these two components (Park 2017) ^[15]. The beneficial microorganism in yogurt include; *Lactobacillus*: is a genus of Gram-positive, facultative anaerobic or microaerophilic, rod-shaped, non-spore-forming bacteria (Makarova *et al.*, 2006) ^[26]. *Lactobacillus* species are normally a major part of the vaginal Microbiota. They form biofilms in the vaginal and gut Microbiota, allowing them to persist during harsh environmental conditions and maintain ample populations (Salas *et al.*, 2016) ^[17]. *Lactobacillus* exhibits a mutualistic relationship with the human body as it protects the host against potential invasions by pathogens, and in turn, the host provides a source of nutrients (Martin *et al.*, 2013) ^[11]. Some species commonly used in yogurt production includes; *L. bulgaricus*, *L. acidophilus*, *L. casei*, *L. animalis*.

Streptococcus thermophiles: also known as *Streptococcus salivarius* subsp (Tannock *et al.*, 2005) ^[22], is a fimbriated, non-motile, gram-positive bacterium, and a fermentative facultative anaerobe of the viridians group that does not produce endospores (Courtin *et al.*, 2003) ^[3]. Some of the benefits of yogurts include: (1) According to Keyzet, Yogurt can help promote hair growth by getting rid of dandruff, balancing the pH of the scalp and preventing frizz depending on the added ingredients (Olive oil or Coconut oil). (2) It is also used on the face to clean and tighten pores. After all, this treat includes lactic acid - an alpha hydroxyl acid (AHA), which is milder than other exfoliate but still rejuvenates your skin. (3) Yogurt with other ingredient (like crushed Walnuts) can be useful in treating the feet; by relieving dry patches under the feet, giving a soft and moisturized feel. (4) It can also be used as a teeth whitening agent because of it calcium and phosphorus content.

(5) According to researchers from the Jean Mayer U.S. Department of Agriculture Human Nutrition Research Centre on At Tufts University Yogurt with active cultures may help certain gastrointestinal conditions, including: Lactose intolerance, constipation, colon cancer, inflammatory bowel disease, H. pylori infection. (6) Yogurt With Active Cultures May Discourage Vaginal Infections.

Diabetes is a group of metabolic disorders in which there is high blood sugar levels over a prolonged period (WHO, 2014) [25]. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications. Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycaemic state, or death (Kitabchi *et al.*, 2009) [8]. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes (WHO, 2013) [24]. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced (Shoback *et al.*, 2011) [19]. Stomach ulcer is a break in the lining of the stomach, first part of the small intestine or occasionally the lower oesophagus. An ulcer in the stomach is known as a gastric ulcer while that in the first part of the intestines is known as a duodenal ulcer. In 2014, a total of 422 million adults were reported worldwide to have diabetes, the complications of diabetes can lead to heart attack, stroke, blindness, kidney failure and lower limb amputation. Managing these diseases can be very expensive and the use of medications can lead to uncomfortable side effects. This research will give more exposure and help decide if yogurt can be a cost-effective alternative to treat these diseases rather than expensive medications that have prominent side effects.

Materials and Methods

Environment of Study

The study was conducted in Elizade university laboratory, Ilara-Mokin, Ondo state, Nigeria.

Materials

Materials used for this research include Cages, measuring cylinders, conical flask, petri dishes, chloroform, foil paper, cotton wool, paper tape, distilled water, cover slips, slides, specimen bottles, dissecting tools, gloves.

Animals and Feeding

Albino rats were obtained from an animal house in Akure, Nigeria. They were maintained in a customised cage with a mesh top to allow for ventilation at room temperature, the cages were cleaned regularly (twice daily) to prevent infections. Rats' diet consisted of cereals and cereal by-product (rich in starch and proteins) and definitely water *ad libitum*.

Experimental design

A total of twenty rats were used for the assay. They were divided into five (5) groups consisting of four (4) rats per group. The groups and their designations are as follows:

Group 1- Diabetes induced rats treated with yogurt; Group 2- Diabetes induced rats treated with drug; Group 3- Ulcer induced rats treated with yogurt; Group 4- Ulcer induced rats treated with drug; Group 5- control group.

Confirmatory tests

The lactic acid bacteria present in the yogurt used was

isolated and confirmed using MRS agar, microscopy and biochemical tests according to Cheesbrough (2014).

Gram staining test

A drop of distilled water was placed on a clean, grease free slide and a loop full of the organism was added onto the slide and done according Cheesbrough (2014).

Induction of Diabetes

Experimental diabetes mellitus was induced by a single intra-venous injection of Streptozotocin (STZ) dissolved in 0.1M citrate buffer in the adult rats under anaesthetics. After which diabetes was induced in four days.

Induction of Ulcer

The rats were denied food for 24 hrs prior to ulcer induction but had access to water. After which ulcer was induced by oral application of 0.2 ml of pure acetic acid. The animals were returned to their normal diet.

Administration of Treatment

Treatments including yogurt and about 0.5ml of reference drugs for diabetes (Melanov) and ulcer (Polygel) were orally administered to the rats by gavage daily.

Biochemical studies

To monitor the glucose level in diabetic rats, the rats were placed under anaesthetics and about 0.5 ml of blood was collected into an Ethylene Diamine Tetra-acetic Acid (EDTA) bottle from the tail vein and with the use of glucometer the sugar level was determined.

Histopathological test

Each week one rat from each group was sacrificed, from the diabetic group the pancreas was harvested while the stomach for the ulcerated group was harvested and the organs were maintained in specimen bottles containing 20% formalin. The samples were first of all fixed in fixative liquid, trimmed, embedded in paraffin wax, sectioned and stained. Staining was done with the use of Haematoxylin and Eosin and excess stain was removed by rinsing.

Results

Observation of general condition of the experimental rats

Various observation were made on the activities of both groups (Diabetic and ulcerated group). After induction of ulcer; writhing, drastic decrease in body weight, poor intake of food, and fatigue was observed in the rats, while in the diabetic group increased production of urine as compared to the control group was noticed. Table 1 shows that streptozotocin induced diabetes few days after induction. The initial blood sugar after before treatment was as high as 235 mg/dL. An elevation in the blood sugar of the rats can be observed in the first two groups, while the control group maintained blood sugar in the normal range. The group treated with yogurt compared to the group treated with drug didn't show significant reduction in the blood sugar level but there was a little significant variation in the sugar level after treatment compared to the sugar level after diabetes induction.

Table 2 shows the elevation and reduction in weight of various experimental group. In the first group the weight is seen to have decreased gradually through the week, the

second group shows an increase in the weight, in the third group it was observed that the body weight decreased drastically during the first week of induction and gradually increases through the week, the fourth week showed inconsistency in weight, whereby the weight increased and decreased regularly and finally at the third week there was a drastic reduction in weight is observed compared to the initial weight. The control group however shows increased weight gain from the first week through the third week.

Table 1: Shows the glucose level of experimental rats

Groups	Week one	Week two	Week 3
Diabetes induced treated with yogurt	220±6 mg/dl	216±6 mg/dl	212±9 mg/dl
Diabetes induced treated with drug	205±5 mg/dl	180±4 mg/dl	142±6 mg/dl
Control	90±4 mg/dl	92±5 mg/dl	95±6 mg/dl

Table 2: Shows the variation in weights of experimental rats

Groups	Initial weight before induction (g)	Weight in week 1 (g)	Weight in week 2 (g)	Weight in week 3 (g)
A	120.40±2.63	115.15±3.81	114.28±2.17	110.23±1.56
B	134±2.18	137±3.03	138.41±2.94	139.23±2.60
C	130.50±5.02	105.10±3.48	101.91±1.12	107.82±1.05
D	128±3.15	121.15±1.30	124.02±2.65	121.11±2.29
Control group	120.18±5.42	122.43±4.05	128.12±2.20	130.84±4.54

A. Induced diabetes treated with yogurt; B- Induced diabetes treated with drug; C- Induced ulcer treated with yogurt; D Induced ulcer treated with drug

Table 3: Shows the results for occult blood test

Groups	2 days after induction	Week 1	Week 2	Week 3
Induced ulcer treated with yogurt	+	+	-	-
Induced ulcer treated with drug	+	+	+	-
Control	-	-	-	-

Positive= + Negative= -

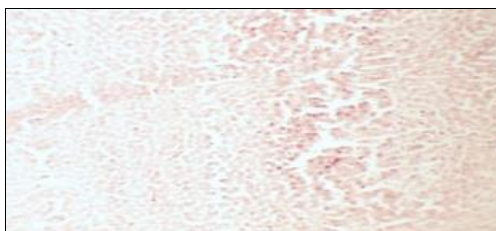


Fig 1: Depicting the histopathology of a normal rat stomach *i.e*

The results of the histopathological analysis on the concerned organs of the induced experimental rats showed various pathological features. These features include presence of cell infiltrations, inflammatory cell infiltrations, washing away of stomach sphincter muscles, formation of ulceric vacuoles, traces of dot haemorrhage along the stomach lining spread across the anterior and posterior wall of stomach sphincter, distinct ductile without necrosis on healing granular semi-saccularis, pancreatic tissue with cell infiltrations and washing away of posterior vacuole and diffused globular masses in the anterior part of the tissue.

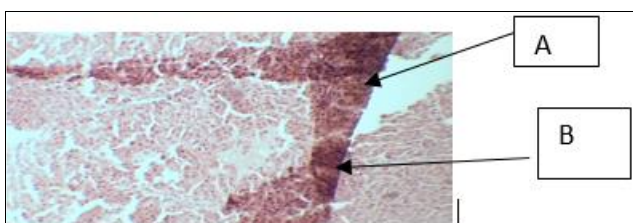


Fig 2: Sample from ulcer induced rat treated with yogurt

Table 3 shows the results obtained from occult blood test. Two days after induction occult blood test yielded positive result for both the first and second group because of haemorrhage that occurred internally. In week one, also both groups tested positive for occult blood test, in the second week group one showed signs of healing as haemorrhage was not detected and in the third week both groups were negative for occult blood test.

Figure 1 depicting the histopathology of a normal rat stomach *i.e.* rat that hasn't been induced with neither diabetes nor ulcer. In fig 2 there is infiltration of cells in the stomach wall. There are however patches of washed splinter {A}. There's also traces of dot haemorrhage along the stomach lining spread across the anterior and posterior wall {B}. There are areas with distinct stomach wall muscles showing signs of ulcer healing.

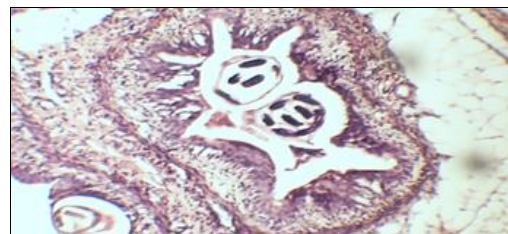


Fig 3: Tissue sample from ulcer induced rat treated with drug taken in first week

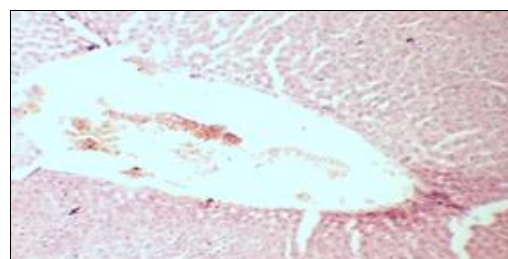


Fig 4: Tissue sample from ulcer induced rat treated with yogurt taken in second week

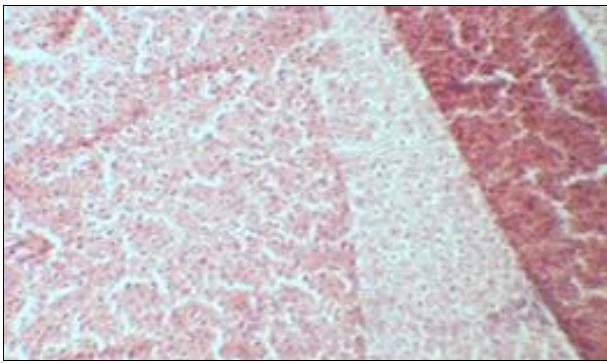


Fig 5: Tissue sample from ulcer induced rat treated with drug taken in second week

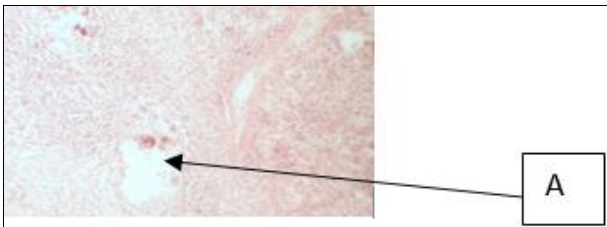


Fig 6: Tissue sample from ulcer induced rat treated with yogurt taken in third week

The stomach is well formed with cell infiltrations and no trace of ulcerated splinter. There's proper formation of the lamina propria and muscularis in fig 5. Well-formed stomach wall with trace of healing in the ulcer region and side haemorrhage {A}. No necrosis nor haemorrhage in the anterior part of the stomach in fig 6.

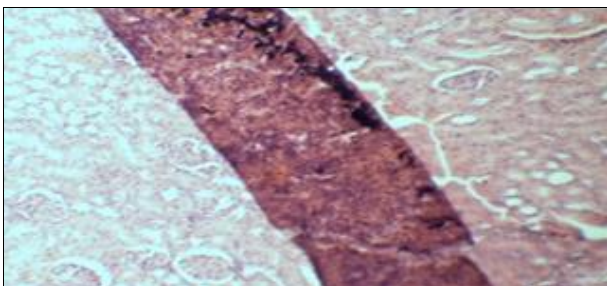


Fig 7: Tissue sample from ulcer induced rat treated with drug taken in third week

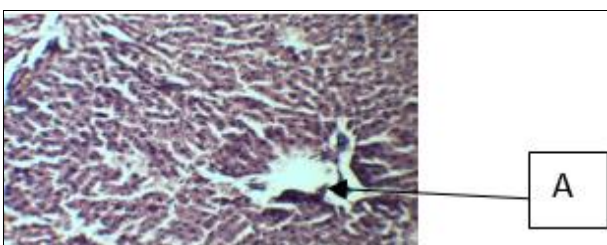


Fig 8: Histopathology of pancreas collected from yogurt treated rat in week one

There is complete healing/properly formed lamina propria can be observed and no haemorrhage in fig 7. Figure 8 shows pancreatic tissue with cell infiltrations and washing away {A} of posterior vacuole. There is no necrosis nor haemorrhage.

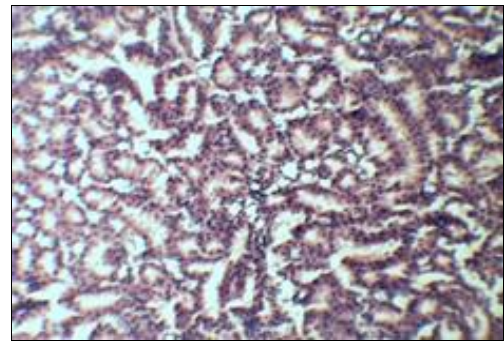


Fig 9: Histopathology of pancreas collected from drug treated rat in week one;

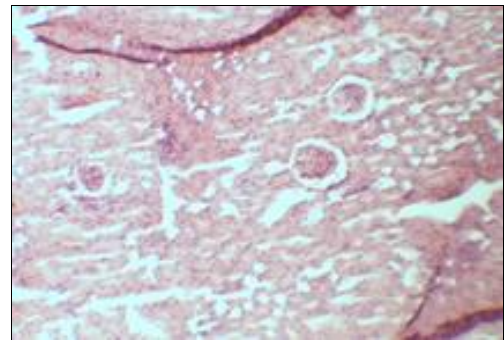


Fig10: Histopathology of pancreas collected from yogurt treated rat in week two;

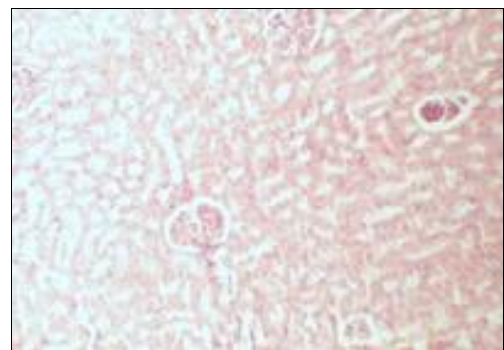


Fig 11: Histopathology of pancreas collected from drug treated rat in week two

The pancreatic section consists of well-formed cells with infiltrations and no formation of vacuole in fig 9. The figure shows poorly formed pancreatic cells with infiltrations and diffused globular masses in the anterior part of the tissue in fig 10 and Well-formed tissue with cell enlargement that looks like dot haemorrhage.

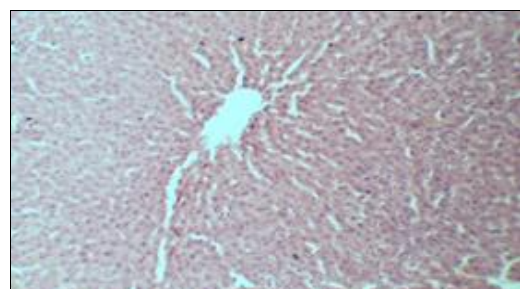


Fig 12: Histopathology of pancreas collected from yogurt treated rat in week three.

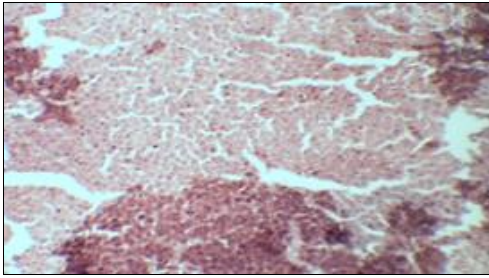


Fig 13: Histopathology of pancreas collected from drug treated rat in week three

Pancreatic cells shows large central vacuole (arrow pointed) with few inflammatory cells. Cell infiltration can also be observed and there's sign of regeneration of cells fig 12 and the stained pancreatic section shows drainages, severe haemorrhage and necrosis fig 13.

Discussion

Results obtained from the confirmatory test carried out on the isolates from yogurt showed that it contained live microorganism (bacteria). These bacteria were probiotics of the *Lactobacillus* species, and it conformed to their identity with the laid down procedure by Hassan *et al.*, (2014) [27]. According to Makarova *et al.*, (2003), *Lactobacillus* species are Gram positive rods incapable of producing spores and are catalase positive, as observed in this research.

In table 1, the blood glucose level was elevated to 235 mg/dl compared to the normal 90 mg/dl of the control group after the induction of diabetes. Sugar level of the diabetic group treated with yogurt reduced a little from 220±6 mg/dl to 212±9 mg/dl from the time of induction to the third week of treatment. This may be due to the fact that probiotics appear to slow down digestion of carbohydrates in foods thereby preventing blood sugar spikes, according to Health day, (2016).

In the drug treated group, sugar level reduced from 205±5 mg/dl to 142±6 mg/dl. This shows that the drug had more effect on the reduction of blood sugar than the yogurt. This result is similar to the result obtained by Hassan *et al.*, (2012) [28] in which a particular drug used to treat diabetes caused a reduction in the sugar level of experimental rats. This may be due to the fact that the drugs are well purified and directly targeted at the pancreas.

The mechanism by which streptozotocin (STZ) brings about its diabetic state in animals includes selective destruction of pancreatic beta cells thereby causing the cells to be less active, leading to poor sensitivity of insulin for glucose uptake by tissues according to Elisa *et al.*, (2009). The pancreatic sections stained with Haematoxylin and Eosin in this research showed that streptozotocin caused severe destruction of the pancreas. For example, the group induced with diabetes and treated with yogurt showed pathological damages such as poorly formed pancreatic cells with infiltrations and diffused globular masses in the anterior part of the tissue.

According to Omoya *et al.*, (2017) [29], pancreatic cells with infiltrations and diffused globular masses in any animal will definitely make it difficult for such animal to utilize any form of sugar for metabolic activities. Although, there was no necrosis nor haemorrhage in any of the pancreas slide. Alfarag, (2009) stated that the washing away of vacuoles in pancreatic sections shows that the significant content of the pancreas is gone and such pancreas will not be optimal in its

metabolic role. This probably explained the reason why the yogurt may not be effective in the treatment of diabetes as seen in this research.

It was observed that the weight of the rats in the first group decreased from 120.40±2.63 g to 110.23 g±1.56 g at the third week (table 2). The second group however showed an increase in body weight of 134±2.18 g to 139.23±2.60 g from week one through week three. In the third group, a drastic decrease in weight was observed *i.e.* from 130.50±5.02 g to 107.82±1.05 g which may be as a result of loss in appetite and poor food intake. In the fourth group, an initial weight of 128±3.15 g which reduced to 121±2.29 g was observed, the decrease in weight in the third week compared to that of the second week could be as a result of their response to the drug, while the increase in weight in other group is as a result of improved appetite.

Occult blood test was positive in the two groups induced with ulcer few days after induction and during week one, as the week progresses it was observed that occult blood test was negative in all groups. This may be because of the healing effect exerted by the probiotics in the yogurt on the ulcerated areas in the gastrointestinal tract. There is no report or studies on the use of occult blood test in evaluating the healing of ulcers in rats. However, the fact that it was negative by the third week may be an indication that healing has taken place.

The results obtained from this research therefore supports the use of yogurt as an alternative for treating ulcer rather than drugs. For instance in the first week of treatment using yogurt infiltration of cells in the stomach wall were observed which is an indication of fast reproduction of the stomach cells or sphincter to replace the ulcerated part. There was also traces of dot haemorrhage along the stomach lining spread across the wall. However there were areas with distinct stomach wall muscles showing signs of ulcer healing. But in the third week of yogurt treatment there was obvious regeneration of cells which showed healing, this is because the probiotics (*Lactobacilli*) may have acted as a protective layer in the area of lesions, thereby preventing further inflammation of cells that may result from acid damage (Poonam *et al.*, 2013) [16].

Diabetes induced by Streptozotocin (STZ) injection provided symptoms like excessive urine production and weight loss, while Acetic acid-induced ulcer produced symptoms like; loss of appetite, fatigue and death. According to the data, the increased levels of glucose sugar in STZ-induced diabetic rats were slightly lowered by administration of yogurt, weight gain was observed in the ulcerated group but the most active groups were the control group and the ones treated with yogurt. This research has shown that probiotics can exhibit some kind of protective mechanism on the stomach lining which can be explored further in treatment of some ailments.

The results gathered from this research suggests that consuming unsweetened yogurts (containing probiotics) may improve glucose metabolism to a degree, but is more effective in treating gastrointestinal complications. Probiotics should therefore be introduced into most convenience foods or produced in large scale in form of capsules especially for ulcer patients.

I therefore recommend yogurt (Containing probiotics of the species *L. acidophilus* and *L. plantarum*) for ulcer treatment, but not for diabetic patients because of the little positive effect observed in this study. Also, further research should

be carried out on the use of yogurt as an alternative to drugs because there may be no adverse effects on its use unlike drugs.

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