Preventive effect of prunes (Prunus domestica) & raisins (Vitis vinifera) muffins stuffed and their mixture on rats with irritable bowel syndrome

By

Afaf-Haniem M. Ramadan
Nutrition & food science, Department of Home Economics, Faculty of Specific Education, Mansoura University, Egypt

Fatma M. Elzamzamy
Nutrition & food science, Department of Home Economics, Faculty of Specific Education, Mansoura University, Egypt

Fayza M. EL-Ezaly
Nutrition & food science, Department of Home Economics, Faculty of Specific Education, Mansoura University, Egypt

Eman Abd Elnaser
Nutrition & food science, Department of Home Economics, Faculty of Specific Education, Mansoura University, Egypt

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*Afaf-Haniem M. Ramadan** *Fatma M. Elzamzamy*

Fayza M. EL-Ezaly *Eman Abd Elnaser*

Abstract:

This study focused on identifying the preventive effect of prunes (Prunus domestica) & raisins (Vitis vinifera) mixture and muffin stuffed with them on rats injured with irritable bowel syndrome. Rats male albino weighing 170 ± 15 g (n=30), were randomly assigned to five groups of 6 rats per each.

The 1st group fed basal diet was served as a normal control (-ve), and the remaining four groups were classified into (+ve) control group (fed on basal diet only), groups of 3, 4, and 5 were fed basal diet containing 20% of (normal muffins (N), or best muffin formula or dried fruit mixture). Irritable bowel syndrome was induced from the beginning of experiment by using 50 µl mustard oil (1% in 70% ethanol) by intra colonic administration.

The results indicated that the best muffin formula recorded the highest total phenols content (427.60 mg/100g), and total antioxidant activity (45.44%) comparing to normal muffin. Irritable bowel syndrome rats group fed the best muffin formula showed significant increase in some nutritional parameters as weight gain, food intake and feed efficiency ratio comparing with (+ve) positive control group, decreased levels of free radicals (malondialdehyde (MDA) and hydrogen peroxide (H2O2), while increasing in antioxidant enzymes such as, glutathione peroxidase (GSH), superoxide dismutase (SOD) and catalase activity (CAT). Furthermore, the same group recorded significant decreases in anti-inflammatory factors namely, (C-reactive protein (CRP), Interleukin-6 (IL-6) and

* Nutrition & food science, Department of Home Economics, Faculty of Specific Education, Mansoura University, Egypt

Cyclooxygenase 2 protein (COX2), and significant increase serum serotonin hormone comparison with positive group at $p<0.05$. On the other hand, a significant decrease in anti-inflammatory indicators and a significant increase in the hormone serotonin were observed in all groups Protictive with muffins stuffed with dried fruits or dried fruit mixture

In conclusion, muffin stuffed with dried fruits such as raisins and prunes which are baked dough products are considered a functional and protective food that is promising in protecting the gastrointestinal system from inflammation, especially irritable bowel syndrome.

Key words: Raisins, Prunes, Muffin, Irritable Bowel Syndrome and Experimental Rats

INTRODUCTION

Irritable bowel syndrome (IBS) is a widespread illness of the gut-brain connection, at any given moment, affects 5%–10% of people globally (**Oka *et al.*, 2020**). IBS has a significant negative influence on a person's quality of life, the healthcare system, and society in terms of financial expenditures (**Black and Ford, 2020**). According to **Canavan *et al.*, (2014)**, the cost of direct care is expected to range from £90 to £316 per patient per year in the UK and to be close to $1 billion per year in the USA (**Everhart and Ruhl, 2009**). As advised by guidelines for the care of IBS, costs may be minimized by making an early, positive diagnosis of IBS after judicious use of clinical investigations (**Vasant *et al.*, 2021; Lacy *et al.*, 2021**). The idea that an organic cause for symptoms has not yet been found may be reinforced by repeated normal studies, even though thorough testing may provide patients and clinicians comfort. Improving the overall quality of care for patients with irritable bowel syndrome (IBS) and helping patients better accept their diagnosis and get treatment quickly should be possible with a standardized approach that stresses early diagnosis and targeted inquiry. (**Lacy *et al.*, 2018)**.

Within minutes of administration, oil of mustard (OM), a strong neural activator, induces both hyperalgesia and allodynia (**Kimball *et al.*, 2005**). Mustard oil administered peripherally intracolonically also induces

acute mucosal inflammation, which heightens reflexive and behavioral reactions to colorectal distension. Furthermore, central sensitization in somatic pain is facilitated by elevated phosphorylation of cyclic adenosine monophosphate-responsive element-binding protein (**Miletic *et al.*, 2002; Lu *et al*., 2007**).

Customers' favorite bakery item is muffins because of their delicious flavor and delicate texture. But because they are poor in dietary fiber and heavy in fat and sugar, muffins have a low nutritional density (**Heo *et al.*, 2019**). In response to the widely acknowledged benefits of maintaining a healthy diet, the food industry has created new products that have attributes that promote health. Specifically, dietary fiber is essential for maintaining health (**Lebesi and Tzia, 2011**). Consuming dietary fiber, such as that found in prunes and raisins, is crucial for preventing irritable bowel syndrome.

Prunes (which are also called dried plums (*Prunus domestica* L.) have drawn more attention between functional foods and plant-derived chemicals formula for their effects on health, and these results have recently been summarized (**Wallace, 2017 and Hooshmand *et al.*, 2016**). Prunes are a rich source of B, Cu, vitamin K, and phenolic compounds, including chlorogenic acids, phenolic acids, and flavonoids, which have antioxidant properties (**Stacewicz-Sapuntzakis, 2013; Treutter *et al.*, 2012**). Consmption of prunes has long been associated with its history that they are beneficial to digestive health (**Del Caro *et al.*, 2004**). Prunes include more dietary fiber, vitamins A and K, and total oxygen radical absorption capacity than prune juice. Furthermore, compared to fresh plums, prunes contain greater total phenolics (**Stacewicz-Sapuntzakis, 2013**). Overall, eating prunes may potentially change the gut flora, which could have an impact on one's health (**Noratto *et al.*, 2014; Voreades *et al.*, 2014 and Anhe *et al.*, 2015**). According to **Muller-Lissner *et al.*, (2005**), the general public believes that prunes (dry plums) have a laxative effect and have long been used as a treatment for constipation. The high dietary fiber content of prunes is thought to be responsible for their purported effects. Although the sugar alcohol sorbitol and phenolic chemicals, primarily chlorogenic and neochlorogenic acids, are poorly absorbed by the small intestine and pass

undigested into the colon, prunes and their extracted juice include other ingredients that may support gastrointestinal health. Prunes are a good treatment option for improving GI function through increasing fiber intake.

Raisins include considerable levels of fructooligosaccharides (FOS), including inulin, and are a great source of both soluble and insoluble fiber. In terms of "source of dietary fiber," raisins rank quite well with 3.0 g of dietary fiber per 90 calories in a 1 ounce serving (28 g). Grapes have extremely little FOS compared to raisins' high FOS concentration (**Camire and Dougherty, 2003**). The prebiotic effect of this inulin is very significant, and it also has other advantages for human health (**Anderson and Waters, 2013**). Prebiotics are a class of dietary substances that are distinguished by their capacity to encourage the development of particular beneficial (probiotic) gut bacteria (**Kelly, 2008**). Numerous studies have proven that inulin-type fructans have a prebiotic effect. Bifidobacteria are the typical target microorganisms, and eating causes significant increases in their population. Numerical increases of 0.5–1.0 $\log^{10}$ are frequently observed. This represents a significant change in the gut microbiota's makeup toward one that is "healthier" (**Kolida and Gibson, 2007**). The frequent eating of raisins has the potential to decrease blood pressure because of their high level of dietary fiber, potassium, and phytonutrients. Regular consumption of raisins also boosts blood antioxidant capacity and reduces fasting serum LDL (low density lipoprotein) cholesterol, triglycerides, and oxidized LDL cholesterol (**Anderson and Waters, 2013**). Phenolic antioxidants, which are present in raisins, support their potential health advantages. The maximum phenolic content and antioxidant capacity were found in golden raisins (**Parker *et al.*, 2007**).

This study was conducted to shed light on the antioxidant activity of a mixture of dried fruits (prunes and raisins) and different muffin formulae and their treatment effects on rats with irritable bowel syndrome.

MATERIAL AND METHODS

Materials:

Dried fruits as prunes (*Prunus domestica*) & raisins (*Vitis vinifera*), and other materials for muffin which listed in Table 1 namely; wheat flour, oat flour, milk, eggs, sugar, corn oil and baking powder were obtained from local market in Mansoura city, Egypt. Mustard seeds were obtained from the Crops Research Institute, Agriculture Research Center, Giza, Egypt.

Chemicals*:* All the used kits and chemicals of analytical grade were purchased from Al-Gomhorya Company for Trading Medicines and Medical equipment, Mansoura, Egypt and Cuprizone was obtained from Lab chemical, Egypt

Animals: Thirty adult male albino rats (*Sprague dawely*) weighing 170 ± 15 g were obtained from the Agricultural Research Center, Giza, Egypt. Biological experiment adhered to the strict protocols established by the international standards for the care and use of laboratory animals. Ethical norms when handling animals were followed.

Diet: The basal diet was prepared according to modification of **NRC (1995)** as follows: 20% Casein, 10% sugar, 5% corn oil, 2% vitamin mixture, 10% mineral mixture, 0.3% DL-methionine, cellulose (3%), and the remained is 49.7 % corn starch.

Methods:

Preparation of Mustard oil (Induction material): Mustard seeds were obtained from local market in Mansoura city, Egypt. The oil extract of mustard seed was produced using petroleum ether in continuous extraction in a Soxhlet reflux device according to earlier works (**Reinhold, 1992; Ojiako and wanjo, 2006**), at the Drug Department in Faculty of Pharmacy Mansoura University. The petroleum ether was totally evaporated following the extraction process by heating the oil and petroleum ether mixture continuously. The desired sample was the oil that remained after the petroleum ether evaporated.

Muffin formulae preparation: The components of the normal muffin formula (N) and the muffin formula were tabulated in Table (1). The ingredients of the regular muffin formula (N) were used to prepare muffins according to **Nicol (1995).**

Table (1): Preparation of Muffin formulae:-

Ingredients	*** Muffin (N)**	****Muffin formula**
Wheat flour (g)	157.5	150
Oat flour(g)	-	52.5
Milk (g)	31.0	31.0
Eggs (g)	123	123
Sugar (g)	105	105
Corn oil (ml)	64	64
Baking powder (g)	9	9
prunes and raisins (%)	-	40 % (1:1)

*Muffin (N): normal muffin formula (wheat flour)

**Muffin formula: muffin (oat and wheat flour) + 40% (prunes and raisins) (1:1)

Induction of bowel syndrome rats:

Induction of IBS-D in an experimental rat's model through mustard oil administration of 50 µl mustard oil (1% in 70% ethanol) OM can induce a rapid, acute and transient colitis (1-5 days after administration of inflammation mediator) according to **Kimball *et al*., (2005)**.

Experimental design:

The thirty male rats used in this experiment were housed in metallic cages under healthy environmental conditions for acclimatization. Water and diet were provided ad-libitum. They were divided into 5 groups (6 rats each), following one of them remained on the basal diet only which served as normal control (- ve) as group (1). The induced rats with IBS were then divided into three protective groups:

Group 2: Induced of IBS and fed on basal diet as positive control (+ve).

Group 3: Induced of IBS group protective by fed on basal diet containing 20% of normal muffins (N).

Group 4: Induced of IBS group protective by fed on basal diet containing 20% of 20% of muffin formula

Group 5: Induced of IBS group protective by fed on basal diet containing 20%of dried fruit mix. (prunes and raisins (1:1W/W).

For another after 29 days from the experiment, by administration of 50 µl mustard oil (1% in 70% ethanol) after one day of that, the rats were sacrificed and after one day of that, the rats were sacrificed. Part of blood was drawn from the portal vein and their plasma was separated, according to Drury and Wallington (1980) Samples were kept in a deep freezer at -20°C until they were used for various biochemical analyses. The study's animal protocols were approved by the Research Ethics Committee at Mansoura University, Egypt's Home Economics Department, Nutrition and Food Science, under animal protocol code No. (R/10). All animals used in the experiments were cared for in accordance with the standards for the Care and Use of Laboratory Animals in Neuroscience and Behavioral Research

Antioxidant parameters of different muffin formulae: Total phenol was determined according to **Slinkard and Singleton, (1977)** and the results were expressed as Gallic acid equivalent (mg Gallic acid/g dried extract) and **Total antioxidant** was determined as the methodology described by **Prieto *et al.* (1999**).

Some Nutritional Parameters in rats: weight gain, feed intake and feed efficiency ratio: According to **Chapman *et al.*, (1959**), feed intake (gm.) was assessed every day, and rats weight (gm.) was recorded weekly to identify the gained weight during the study period of 30 days. Body weight gain for each animal was determined according to **Zali Chedjeu *et al.*, (2021)** using the following formula: Final weight – Initial weight, while equation of Feed efficiency ratio (FER) = weight gain (g) / Feed intake (g)

Biochemical analysis:

Serum Antioxidant enzymes: Enzymatic CAT activity was measured according to method of **Claiborne (1985)**, while conformation of

Superoxide dismutase (SOD) activity according to **Nandi and Chatterjee (1988),** GSH-Px was measured according to **Gross *et al.*, 1967 and Necheles *et al.*, (1968)**.

Blood: Malondialdehyde (MDA) measured calorimetrically according to the method of **Satoh (1978**, while H2O2 hydrogen peroxide was measured according to (**Aebi 1984**).

Anti-inflammation markers: Interleukin-6 (IL-1β levels) was assessed and quantified according to the method of **Fristiohady *et al.*, (2020**). While, Cyclooxygenase 2 protein (COX-2) level was measured by **Zanjani *et al.*, (2014).** C- reactive protein level (CRP): was measured depending on the method of **Ben *et al.*, (2019**).

Hormons: Serotonin or 5-HT was analyzed according to **Flora *et al.*, (2016**).

Statistical data analysis:

The data was presented as a mean with standard deviation (mean ± SD) and examined using one way analysis of variance (ANOVA), by the computer program Co State, version 6.30. According to **Gomez and Gomez (1984**), and the means of the groups were compared using the least significant difference (LSD) statistic test and Duncan's test.

RESULTS AND DISCUSSION

Total phenolic compounds and antioxidant activity of formula muffin:

Total phenol and antioxidant activity of muffin (N), muffin formula and dried fruit Mix (prunes and raisins). are presented in Table 2. As for the content of total phenol, it was found that muffin formula contained higher amount of total phenol (427.60 mg/100g) followed by muffin (N) (371.27 mg/100g) and dried fruit Mix. (prunes & raisins) had 214.14 mg/100g.

Data shows that the highest value of total antioxidant activity was recorded for muffin formula (45.44%) followed by dried fruits Mix. (39.26%), however muffin (N) recorded the lowest activity level (37.18%). The high content of total antioxidant activity might be related to muffin formula.

Williamson and Carughi (2010) formula the health benefits of raisins and found that the main phenolic constituents were flavonols, quercetin, kaempferol, caftaric, and coutaric acids. According to **Breksa *et al.*, (2010**), the phenolic content and antioxidant capacity of the 16 raisin samples varied from 316.3 to 1141.3 mg of gallic acid per 100 g of dry weight and 7.7 to 60.9 mol Trolox/g DW, respectively. The range of phenolics content in green Indian raisin samples was between 0.808 and 4.631 mg/g, according to **Adsule *et al.*, (2012)**. In the same line **Mehta *et al.*, (2014)** indicated that dried plum had high phenol content (1.05 mg/100mg). According to **Benmeziane-Derradji *et al.*, (2019)**, plums are a dried fruit that is high in antioxidants and has a variety of other nutrients. The strong antioxidant content of raisins helps to keep dermal follicles youthful and to restrain the oxidative damage to developing cells.

Table (2): Total phenolic compounds and total antioxidant activity of formula muffins and dried fruits mix.

Variable / Groups	Muffin (N)	*Muffin formula (1:1)	**Dried fruit Mix.	LSD at 0.05
Total phenolic.Com. mg/100 g	371.57±1.110 b	427.60±2.076a	214.14±1.229c	**1.26**
Total antioxidant activity (%)	37.18±0.439c	45.44±0.127a	39.26±0.075b	**0.65**

Mean±SD values in each column having different superscript (a, b, c, …..) are significant at $p<0.05$

* Muffin formula (40% prunes + raisins 1:1)

*Dried fruit mix (prunes+ raisins 1:1)

Protective effect of dried fruits Mix. and different muffin formulae on irritable bowel syndrome (IBS) rats.

Effect of dried fruits Mix and different muffin formulae on some nutritional parameters as body weight gain, food intake and feed efficiency ratio (FER) of (IBS) rats.

Weight gain, food intake and feed efficiency ratio of IBS rat composition of muffin (N) and muffin formula intake are presented in Table 3.

Results show that a significant increase was observed in Protectived groups rat (+ve) in weight gain, feed intake and FER relative to the normal group. While weight gain showed significant increase at ($p<0.001$) between Protectived groups rat fed on muffin formula and dried fruit M ix. comparing to the +ve control group.

Regards feed intake (g), results show significant increases in Protectived groups rat fed on fed muffin formula and dried fruit Mix. compared to the (+ve) control rats group. While, Protectived groups rat fed on muffin (N) had no significant differences with (+ve) control rats.

Receiving Protectived groups rat on muffin formula and dried fruit mix caused a significant increase in feed intake comparing with the Protectived groups rat fed on (+ve) at ($P<0.001$). It could be noticed that groups protective with muffin formula was the most suitable for IBS rats.

As for FER, results showed that the highest FER was recorded for IBS group fed muffin formula, comparing to the positive group (+ve).

Table (3): Effect of muffin (N), muffin formula and dried fruit Mix. on body weight gain, feed intake and feed efficiency ratio (FER) of IBS rats.

Groups \ Variable		Initial weight (g)	Final weight (g)	Weight gain (g)	Weight gain (%)	Feed intake	FER%
Control (-ve)		166.33 ±6.43a	224.00 ±5.57a***	57.67 ±10.97a***	34.87 ±8.04a***	22.40 ±0.56a***	0.092 ±0.015a***
Control (+ve)		167.00 ±4.58a	186.67 ±4.51c	19.67 ±8.39c	11.87 ±5.24c	18.67 ±0.451c	0.037 ±0.015d
Protectived groups	**Muffin (N)**	171.67 ±6.51a	194.67 ±5.69c	23.00 ±2.00c	13.43 ±1.54c	19.47 ±0.57c	0.042 ±0.004cd
	***Muffin formula (1:1)**	163.33 ±7.37a	211.00 ±6.25b**	47.67 ±6.66a***	29.30 ±4.94ab**	21.10 ±0.62b***	0.081 ±0.011ab**
	****Dried fruits Mix.**	170.67 ±8.02a	206.00±6.56 bc**	35.33 ±2.08b**	20.76 ±2.02bc*	20.60 ±0.66b***	0.061 ±0.005bc*
LSD at 0.05		**N.s**	**10.52**	**12.07**	**8.91**	**1.05**	**0.020**

Mean±SD values in each column having different superscript (a, b, c, …..) are significant at *p<0.05, ** p<0.01, *** p<0.001

* Muffin formula (40% prunes + raisins 1:1)

**Dried fruit mix (prunes+ raisins 1:1)

Because of their unusual nutritional composition, raisins are consumed all over the world and may provide some special health advantages. Raisins provide vital minerals, dietary fiber, including fructooligosaccharides, and are low- to medium-energy rich. According to **Fulgoni *et al.* (2017)**, one snack serving of raisins (43 g) provides 129 kcal, 0.2 g total fat, 1.6 g dietary fiber, 25 g total sugar, 14 mg magnesium, 322 mg potassium, and 0.8 mg iron. In addition, raisins contain a wide range of phytochemicals, such as hydrocinnamic acids (cathenic acids and coutaric), resveratrol, epicatechins, phytoestrogens (daidzein and genestein), and flavonoids (quercetin, kaempferol, catechins, and rutin) (**Karadeniz *et al.*, 2000 and Williamson and Carughi, 2010**). So, it is perfect source of calories as mentioned by **Fulgoni *et al.*, (2017**), adult consumers of raisins

consumed significantly more calories and carbohydrate but less fat compared to their respective non-consumers. It is clear that dried plum contains a number of bioactive substances that have the potential to change metabolism (**Bushinsky *et al.*, 1997**). The same results by **Almajwal and Elsadek, (2015) and Ghorbanian *et al.*, (2018**) suggested that eating raisins on a daily basis may lead to weight gain, which may be the result of eating more overall. Prunes served as a snack before a meal were examined by **Farajian *et al.* (2010)** for their short-term effects on satiety and calorie intake in normal-weight people. Also, they showed that consuming prunes instead of bread products as a preload before a meal led to decreased energy consumption during subsequent meals, such as lunch and dessert (910 Kcal 233 on prunes day versus 971 Kcal 249 on bread product day).

Protective effect of muffin (N), muffin formula and dried fruit Mix. on free radical in serum (MDA and H_2O_2) of IBS rats

Data in Table (4) shows free radical (H_2O_2 and MDA) in Protectived groups rat fed muffin (N), muffin formula and dried fruits Mix.

Significant differences were observed between all rats groups in H_2O_2 and MDA levels. The (+ve) recorded the highest significant increase in H_2O_2 and MDA levels.

Protectived groups rat fed on muffin (N), muffin formula and dried fruits Mix. significantly decreased H_2O_2 and MDA levels comparing to the positive control (+ve). Protectived IBS groups rat with muffin (N), muffin formula and dried fruits mix significantly decreased H_2O_2 and MDA levels comparing to the (+ve) control.

Results cleared that H_2O_2 recorded (0.06 ±0.015) with IBS group rats given muffin (N), (0.03 ±0.010) with protectived groups rat fed on received muffin formula and (0.05±0.010) with protectived groups rat fed on given dried fruit Mix. From the mentioned data, it was found that protectived groups rat received muffin formula was the nearest to the normal control followed by group rats given dried fruit Mix. then, the muffin (N).

Regarding to the data, its cleared that protectived groups rat given muffin (N)-recorded 41.43 ± 2.155 for MDA. While the protectived groups rat received muffin formula recorded 23.93±4.952 and protectived groups rat given dried fruit Mix., results were 28.60±3.959, while the muffin formula was the most effective for lowering MDA level comparing to the positive control, which recorded 46.39 for MDA.

From the previous results, it could be observed that all formula treatments improved the antioxidant status by decreasing free radicals levels, especially with the addition of dried fruit mix which caused a significant improvement in H_2O_2 and MDA levels comparing with the (-ve) control group. The protectived groups rat given muffin formula and dried fruit mix recorded the lowest H_2O_2 and MDA levels comparing with the positive control rats group. It could be noticed that groups protectived with muffin formula was the most suitable for irritable bowel syndrome rats and control free radical (MDA and H_2O_2) activity comparing to the positive control.

When free radicals produced by reactive oxygen species start to harm cells through their chain reactions, antioxidants appear to be crucial in avoiding structural damage. According to **Lakshmi *et al.*, (2014),** supplementing Al-IBS rats with 400 mg/kg of *V. viniferous* extract caused the MDA levels to significantly decrease. Also, according to **Ghorbanian *et al.*, (2018**), rats in the raisin group that received raisins for 90 days showed significantly higher blood serum antioxidant levels than the control rats. Additionally, compared to the control group, the raisin group's average MDA concentration was considerably lower. These findings support the study's goals and the hypothesis that oral administration of raisins mixed with muffins to rats boosted their blood serum antioxidant capabilities and decreased oxidative stress.

Fiber and vitamin C are found in dried plums. The antioxidants carotenoids, flavonoids, anthocyanins, and quercetin are also abundant. As scavengers, carotenoids can combat diseases brought on by free radicals. Dried plums contain mostly beta-carotene and just a little amount of beta-

cryptoxanthin as carotenoids. Flavonoids, in addition to beta carotene, have antioxidant properties. The ethanolic extract of plum fruit may operate as an antioxidant by preventing rats' liver MDA levels from rising as a result of an excess of fat. Antioxidants can stop the lipid peroxidation process that free radicals can start in liver damage brought on by a high-fat diet. Free radicals can cause this damage (**Roomi *et al.,* 2013; Birwal *et al.,* 2017 and Vlaic *et al.,* 2018**). According to **Priyadi *et al.,* (2023)**, the control and treatment groups' liver MDA levels differ from one another. The MDA levels were lowest in the negative control group, and the highest in the positive control group, which was produced by a high-fat diet. The liver MDA levels rose with increasing dosages of the ethanol extract of plum when compared between treatment groups. Though, the amount was still less than the liver MDA levels in the positive controls.

Table (4): Effect of muffin (N), muffin formula and dried fruit Mix. on free radical (MDA and H_2O_2) of IBS rats.

Groups / Variable	Control (-ve)	Control (+ve)	Protectived groups			LSD at 0.05
			Muffin (N)	*Muffin formula	**Dried fruits Mix.	
H_2O_2 (mmol/L)	0.02± 0.006 ^{c}***	0.09±0.01 0a	0.06± 0.015b**	0.03± 0.010c***	0.05± 0.010b***	**0.02**
MDA (nmol/L)	14.83± 1.168d***	46.39±1.9 29a	41.43± 2.155b	23.93± 4.952c****	28.60± 3.959c***	**6.28**

Mean±SD values in each column having different superscript (a, b, c, …..) are significant at *p<0.05, ** p<0.01, *** p<0.001

* Muffin formula (40% prunes + raisins 1:1)

**Dried fruit mix (prunes+ raisins 1:1)

Protective effect of muffin (N), muffin formula and dried fruit Mix. on antioxidant enzyme of IBS rats:

Data concerning (GSH, SOD and CAT) of IBS rat groups protective groups with muffin (N), muffin formula and dried fruit Mix. are shown in Table (5), showed that protective group (+ve) control showed a significant

decrease (P<0.001) in GSH, SOD and CAT was observed compared to the normal group (-ve control).

Receiving protective groups rat fed on muffin (N), muffin formula and dried fruit Mix. caused a significant increase (P<0.001) in GSH, SOD and CAT comparing to (+ve) control.

Results show that protectived groups rat given muffin (N) recorded 0.54±0.072 mmol/L for GSH level, while protectived groups rat given muffin formula recorded 0.97±0.086 mmol/L and protectived groups rat fed on dried fruit Mix. recorded 0.83±0.095 mmol/L for the mentioned parameter. So, it could be observed that the protectived groups rat received muffin formula was the most effective to improve GSH level followed by group given dried fruit mix. then the muffin.

As for the SOD level, it was found that muffin (N) recorded 80.00±8.032 U/ml and group given muffin formula recorded 148.43±16.638 U/ml, while group protective with dried fruit mix scored 129.23±18.750 U/ml. From the mentioned data, protective group given muffin formula realized the best SOD level followed by group given dried fruit Mix. then muffin (N).

From Table (5), CAT level recorded 1.60±0.228, 3.24±0.325 and 2.92±0.438 U/L for protective groups given muffin (N), muffin formula and dried fruit mix. group, respectively.

It could be noticed from the previous results that feeding muffin (N), muffin formula and dried fruit mix improved all serum GSH, SOD and CAT of protectived groups rat, especially muffin formula which caused a significant (P<0.001) improvement in GSH, SOD and CAT comparing with the (+ve) control group.

Antioxidants function by blocking the production of the molecules and free radicals that damage cells (**Iwata *et al.*, 2010**). These beneficial health effects have been linked to the plum's high phenol content, which has been shown to have antioxidant characteristics (**Yu *et al.*, 2009; Noratto *et al.*, 2009 and Pawlowski *et al.*, 2014**). Chlorogenic acid (5-O caffeoylquinnic acid), cryptochlorogenic acid (4 Ocaffeoylquinnic acid),

caffeic acid, and p-coumaric acid are some of the other hydroxycinnamates that are present (**Nakatani *et al.*, 2000 and Rothwell *et al.*, 2013**). The high free radical scavenging abilities of caffeic acid and chlorogenic acid isomers have been demonstrated (**Nakatani *et al.*, 2000**), potentially indicating a significant bioactive role in vivo. This finding was supported by **Lea *et al.*, (2008**), who also noted that the synergistic impact of the plum extract's total phenolic content markedly boosted its antioxidant activity. The high phenolic content of these compounds has been primarily credited with these compounds' antioxidant properties (**Ko *et al.*, 2005**), by reducing the production of reactive oxygen species, also demonstrated that after 30 minutes of consumption, nine distinct fruit juices, including plum juice, exhibited significant antioxidant effects in human plasma. Additionally, Excretion of hippuric acid, a possible indicator of total polyphenol consumption and metabolite, and antioxidant capacity in urine, and malondialdehyde excretion, a biomarker for oxidative stress, were all found to increase threefold after consumption of Queen Garnet plum juice by **Netzel *et al.*, (2012**). A study by **Hong *et al.* (2021)** looked at the impacts of eating dried plums on cardiovascular disease risk factors. They found that SOD activity increased significantly in the 50 g/day group after 6 months compared to baseline, and oxidative stress, antioxidant capacity, and total antioxidant capacity all increased significantly in the 100 g/day group. These results indicate that oxidative stress indicators are reduced with no discernible dose dependence when 50-100 g of dried plums are consumed daily**.**

Raisins is rich in antioxidant as polyphenol and phenolic acid chemicals. Free radicals are eliminated from the body by natural polyphenols, which also stimulate antioxidant enzymes, chelate metal catalysts, lessen -tocopherol radicals, and inhibit oxidases (**Oboh and Rocha, 2007**). According to **Aljarari and Bawazir (2019**), raisin led to a significant increase in cortex and hippocampus GSH, GSSG, and SOD levels compared to positive control group. This could be primarily as a result of raisins' antioxidant and free radical-scavenging abilities (**Tagliazucchi *et al.*, 2013**). According to **Lakshmi *et al.*, (2014**),

supplementing Al-treated rats with *V. viniferous* extract (400 mg/kg) caused a noticeably higher level of antioxidant enzymes, such as CAT and GR. Additionally, *V. vinifera* extract included a variety of substances, including vitamins, organic acids, resveratrol, sugars, proanthocyanins, tannin, mineral salts, and flavonoids. Additionally, *V. vinifera* extract included a variety of substances, including organic acids, mineral salts, vitamins, resveratrol, proanthocyanins, sugars, tannin, and flavonoids. In oxidative stress models, several compounds have been shown to function as antioxidants.

Table (5): Effect of muffin (N), muffin formula and dried fruit Mix. on antioxidant enzymes (GSH, SOD and CAT) of IBS rats.

Groups \ Variable		GSH (mmol/L)	SOD (U/ml)	CAT (U/L)
Control (-ve)		1.35±0.065a***	203.07±18.800***a	3.85^{a}±0.115a***
Control (+ve)		0.47±0.015c	51.49^{d}±6.906b	1.38^{c}±0.190c
Protectived groups	**Muffin (N)**	0.54±0.072c	80.00^{c}±8.032c**	1.60^{c}±0.228c
	***Muffin formula**	0.97±0.086b***	148.43±16.638b***	3.24±0.325b***
	****Dried fruit Mix.**	0.83±0.095b***	129.23±18.750b***	2.92±0.438b***
LSD at 0.05		**0.15**	**27.84**	**0.57**

Mean±SD values in each column having different superscript (a, b, c, …..) are significant at *p<0.05, ** p<0.01, *** p<0.001

* Muffin formula (40% prunes + raisins 1:1)

**Dried fruit mix (prunes+ raisins 1:1)

Protective effect of muffin (N), muffin formula and dried fruit Mix. on anti- inflammation parameters of IBS rats:

Results concerning anti inflammation (CRP, IL-6 and COX2) of protectived groups rat received muffin (N), muffin formula and dried fruit Mix. in Table 6.

Regards anti inflammation (CRP, IL-6 and COX2), results show that Protectived groups rat fed on (+ve control) recorded the highest CRP, IL-6 and COX2 levels (3.01±0.230 mg/L, 33.93 ±1.123 Pg/ml and 2.20±0.148 ng/ml), respectively as compared to the normal rats group which recorded

the lowest anti inflammation levels (1.43±0.208 mg/L, 9.67±1.457 Pg/ml and 1.24±0.115 ng/ml for CRP, IL-6 and COX2), respectively.

Feeding IBS rats with formula products decreased the CRP as anti-inflammation parameters. Muffin (N) recorded 2.93±0.153 mg/L, muffin formula recorded 1.90±0.200 mg/L and dried fruit mix recorded 2.23±0.306 mg/L.

As for the IL-6 level, data in the same Table revealed a significant decreased in the IBS group received muffin (N), muffin formula and dried fruit mix which recorded 32.40±2.700, 17.7±3.302 and 23.03±4.565 Pg/ml, respectively. The most effective treatment for lowering IL-6 level was muffin formula followed by dried fruit mix then the muffin (N) as compared to the (+ve) control.

COX2 recorded 2.20±0.148 ng/ml for rats group given muffin (N). While their level was, 1.67±0.105 ng/ml for the rats group given muffin formula. On another hand, dried fruit Mix. group recorded 1.78±0.160 ng/ml. This data indicates that the most powerful influence was for muffin formula, followed by dried fruit Mix. then the muffin (N).

Overalls, protectived groups rat given muffin (N), muffin formula and dried fruit Mix. significantly improved ($P<0.001$) the inflammation levels as compared to the positive control group. The best results achieved in the rats group received muffin formula followed by dried fruit Mix. with no significant different between them relative to the +ve control

Raisins are one of the dried grape products. Due to their high level of polyphenolic chemicals, particularly proanthocyanidins, they provide a variety of health advantages, including anti-inflammatory properties. In contrast to the control group, CRP, TNF-, and IL-6 were not upregulated in visceral fat when grape seed extract was added to high-fat proinflammatory diets for 19 weeks (**Terra *et al.*, 2009 and Terra *et al.*, 2011**). The anti-inflammatory cytokine adiponectin was also elevated in visceral fat as a result of grape seed extract administration. The plasma levels of TNF- and CRP were also reduced, pointing to a systemic decrease in inflammation with the addition of grape seed extract. Zinc and selenium, two additional

elements found in raisins, aid in the renewal of skin cells (**Schuster *et al.*, 2017**). Free radicals and oxidative damage are risk factors for the development of tumors, cancer, and ageing. Nutritional antioxidants in raisins are crucial for protecting cells from the damage caused by free radicals. It's interesting to note that grape seed extract prevented weight growth despite the high-fat meals being fed ad libitum. In another study, **Zern *et al.*, (2005**) discovered that giving 24 pre- and 20 postmenopausal women raisins from grape powder supplements (36 g daily for 4 weeks) improved their levels of oxidative stress and inflammatory cytokines. The effects of supplementing with raisins from grape powder on inflammatory and antioxidant biomarkers in non-diabetic hemodialysis (HD) patients were examined by **Janiques *et al.*, (2014**) in different research. Compared to the placebo group, patients who took raisins from grape powder had higher GSH-Px activity and lower C-reactive protein levels in this research. According to these results, non-diabetic HD patients may benefit greatly from using grape powder as an antioxidant and anti-inflammatory medication. The production of IL-1 and IL-6 in supernatants from Lipopolysaccharide-activated peripheral blood mononuclear cells (PBMCs) was also found to increase with dietary grape powder supplementation (46 g grape powder in 240 mL of water, twice daily for 3 weeks, equivalent to four servings of grapes/day) (**Zunino *et al.*, 2014**). In a different investigation, **Barona *et al.* (2012)** assessed the impact of grape intake on inflammation and oxidation in men with metabolic syndrome: 11 men with high triglycerides and low HDL, and 13 men without dyslipidemia. By raising the anti-inflammatory cytokines IL-10 and adiponectin, grape intake shown positive effects.

Bioactive ingredients found in dried plums have been shown to have anti-inflammatory properties. In order to ascertain if eating dried plums lowers the risk factors for cardiovascular disease, **Hong *et al.*, (2021)** particularly examined inflammation in a dose-dependent way. Interleukin-6 and tumor necrosis factor levels were considerably reduced in the 50 g/day dried plum group at 6 months compared to baseline. These results indicate a dose-independent reduction in inflammatory markers with daily intake of

50–100 g dried plum. Studies have shown that mice fed a 25% dried plum diet for four weeks had lower levels of the blood cytokines TNF- α, MCP-1, IL-1, IL-10, IL-12p70, and IL-13 than mice on a control diet (**Shahnazari *et al.,* 2016**). Additionally, when stimulated by concanavalin A, the splenocytes of OVX mice fed a diet of 15% or 25% dried plum for 4 weeks after OVX produced less TNF- α than splenocytes from OVX mice on a control diet (**Rendina *et al.,* 2012**). These studies show that eating dried plums can affect how many different types of cells secrete cytokines, however it's crucial to remember that dried plums made up a sizable amount of the diet in these trials (15% and 25% by weight). Since the percentage of dried plum in the average human diet will not be 15%, it is crucial that future pre-clinical research employ lower doses of dried plum to examine the effects of dried plum on cytokine production in animals. **Kumar (2009)** revealed that dried plum polyphenols suppress the production of COX-2 and also lowers malondialdehyde, a marker of lipid peroxidation. According to, **Van Every (2021),** dried plum consumption was associated with a decrease in lipopolysaccharide stimulated IL-8 and IL-6 secretion from peripheral blood mononuclear cells. In contrast to what we expected, dried plum intake had no effect on the blood levels of CRP in osteopenic or osteoporotic postmenopausal women. Measures of CRP were also analyzed in three prior clinical studies on dried plum use. While two additional studies observed reductions in CRP in the serum of women who ate dried plum for three months (**Hooshmand *et al.,* 2011 and Chai *et al.,* 2012**) compared to women who ate dried apples, one study showed no changes in serum CRP between groups who ate dried plum and those who did not (**Hooshmand *et al.,* 2016**). CRP in the serum of women who consumed dried plum was different after three months, but at the end of a year of continuous consumption, it was not different from the dried apple consumption group (**Hooshmand *et al.,* 2011**). The fact that CRP is a non-specific indicator of inflammation may be the cause of these contradictory CRP readings (**Pepys and Hirschfield, 2003**). CRP may not be extremely responsive to dietary modifications and can be elevated in situations of acute sickness or injury (**Khor *et al.,* 2018**). BMI and age are anthropometric factors that have a

correlation with CRP (**Timpson *et al.*, 2011 and Wyczalkowska-Tomasik *et al.*, 2016**).

Table (6): Effect of muffin (N), muffin formula and dried fruit Mix. on some anti- inflammation parameters (CRP, IL-6 and COX_2) of IBS rats.

Groups \ Variable		CRP (mg/L)	IL6 (Pg/ml)	COX_2 (ng/ml)
Control (-ve)		1.43±0.208c***	9.67±1.457c***	1.24±0.115***d
Control (+ve)		3.01±0.230a	33.93±1.123a	2.78±0.207a
Protectived groups	**Muffin (N)**	2.93±0.153a	32.40±2.700a	2.20±0.148**b
	***Muffin formula**	1.90±0.200b***	17.73±3.302b***	1.67±0.105***c
	****Dried fruits mix**	2.23±0.306b**	23.03±4.565b**	1.78±0.160***c
LSD at 0.05		**0.45**	**5.97**	**0.31**

Mean±SD values in each column having different superscript (a, b, c, …..) are significant at *p<0.05, ** p<0.01, *** p<0.001

* Muffin formula (40% prunes + raisins 1:1)

**Dried fruit mix (prunes+ raisins 1:1)

Protective effect of muffin (N), muffin formula and dried fruit Mix. on serum serotonin hormone of IBS rats:

Data represented serum serotonin level of IBS rats fed muffin (N), muffin formula and dried fruit Mix. in Table (7).

Significant differences were observed between all rats' groups in serum serotonin level. The positive control (+ve) recorded the lowest significant decrease in serum serotonin level compared to the normal control rat group (-ve). Treating IBS rats with muffin (N), muffin formula and dried fruit mix significantly increased serum serotonin level at P <0.001 comparing to the (+ve) control.

Results revealed that protective rats given muffin (N) recorded 18.57±2.701 ng/ml for serum serotonin. While the Protectived groups rat fed on received muffin formula recorded 37.43±4.743 ng/ml for the

mentioned parameter. Regards protective rats given dried fruits Mix. recorded 32.97±5.652 ng/ml.

From the previous results, it could be observed that muffin (N), muffin formula and dried fruit mix improved serum serotonin level, especially when protective rats fed on muffin formula and dried fruit Mix. which caused a significant ($P<0.001$) improvement in serum serotonin level comparing with the positive (+ve) control group with no significant difference. It could be noticed that groups Protictive with muffin formula was the most suitable for IBS rats and control reduction in serum serotonin and act as antidepressant comparing to the positive control.

Raisins are a dried type of grape that may be preserved naturally for a considerable amount of time. This enables the fruit to be regularly supplied as an additional source of nutrients throughout the whole year. Because eating grapes, particularly raisins, is frequently advised in Hadiths to promote physical and mental health, particularly to increase happiness. According to **Dadashzadeh *et al.*, (2021**), dried grapes increase plasma levels of the serotonin in the body and ultimately acts as an antidepressant. Resveratrol is a phytoalexin with antioxidant properties and is found in a wide range of foods especially grapes. During the last decade, resveratrol has been shown to possess wide spectrum of pharmacologic properties such as antidepressant (**Zhao *et al.*, 2013**). According to **Ahmed *et al.*, (2014)** animal research using a depression-induced paradigm, rats who were given large doses of resveratrol showed antidepressant-like effects when compared to fluoxetine. This study showed that when trans-resveratrol was administered, the levels of dopamine, serotonin, and norepinephrine rose to the normal range.

Table (7): Effect of muffin (N), muffin formula and dried fruit mix on serum serotonin of IBS rats.

Groups / Variable	Control (-ve)	Control (+ve)	Protectived groups			LSD at 0.05
			Muffin (N)	*Muffin formula	** Dried fruit mix	
Serotonin (ng/ml)	53.40± 3.800a***	18.90± 2.02c	18.57± 2.701c	37.43± 4.743b***	32.97± 5.652b**	**7.95**

Mean±SD values in each column having different superscript (a, b, c, …..) are significant at *p<0.05, ** p<0.01, *** p<0.001

* Muffin formula (40% prunes + raisins 1:1)

**Dried fruit mix (prunes+ raisins 1:1)

In conclusion, muffin stuffed with dried fruits such as raisins and prunes and their interaction which are baked dough products are considered a functional, protective and therapeutic food that is promising in protecting the gastrointestinal system from inflammation, especially irritable bowel syndrome**.**

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التأثير الوقائى للمافن المحشو بالزبيب والقراصيا و الخليط بينهم على الفئران المصابة بمتلازمة القولون

عفاف هانم محمود رمضان، فاطمة محمد الزمزمى ، فايزة محمد الأزلى ، ايمان عبد الناصر*

الملخص العربي:

ركزت هذه الدراسة على التعرف على التأثير الوقائي لخليط القراصيا والزبيب والمافن المحشو بهما على الفئران المصابة بمتلازمة القولون العصبي. تم تقسيم ذكور الفئران الألبينو التي تزن ١٧٠ ± ١٥ جم (العدد = ٣٠) بشكل عشوائي إلى خمس مجموعات مكونة من ٦ فئران لكل مجموعة. كانت المجموعة الأولى التي تغذت على النظام الغذائي القياسى فقط خلال فترة التجربة هى المجموعة الضابطة السالبة(ve-) وتم تصنيف المجموعات الأربع المتبقية إلى مجموعة ضابطة موجبة (ve+) تتغذى على النظام الغذائي القياسى فقط، وتم تغذية المجموعات المكونة من ٣ و٤ و٥ على نظام غذائي قياسى يحتوي على ٢٠٪ من (المافن العادي (N)، أو أفضل خلطة للمافن أو خليط الفواكه المجففة. تم إحداث متلازمة القولون العصبي بعد ٢٩ يوما من بدأ التجربة باستخدام ٥٠ ميكرولتر من زيت الخردل (١٪ في ٧٠٪ إيثانول) عن طريق الإعطاء داخل القولون .

اشارت النتائج إلى أن أفضل خلطة مافن سجلت أعلى محتوى للفينولات الكلية (٤٢٧,٦٠ ملغم/١٠٠جم)، و النشاط الكلى المضاد للأكسدة (٤٥,٤٤٪) مقارنة بخلطة المافن العادية. أظهرت مجموعة الفئران المصابة بمتلازمة القولون العصبي والتي تم تغذيتها بأفضل خلطة مافن زيادة معنوية في بعض المؤشرات الغذائية حيث اظهرت زيادة فى الوزن المكتسب وكمية المأخوذ من الطعام ومعدل كفاءة الغذاء مقارنة بالمجموع الموجبة (ve+)، وانخفاض مستويات الجذور الحرة (المالونديالدهيد (MDA) وبيروكسيد الهيدروجين (H2O2) بينما ارتفع نشاط الانزيمات المضادة للأكسدة مثل الجلوتاثيون بيروكسيديز (GSH) وفوق أكسيد ديسموتاز (SOD) ونشاط الكاتلاز (CAT). كما سجلت نفس المجموعة انخفاضاً معنوياً في العوامل المضادة للالتهابات وهي (بروتين سي التفاعلي). (CRP) وبروتين الانترلوكين ٦ (IL-6) وبروتين السيكلواوكسيجيناز ٢ (COX2) وزيادة معنوية في هرمون السيروتونين في الدم مقارنة مع المجموعة الموجبة عند $p<0.05$. من ناحية أخرى، لوحظ انخفاض معنوي في المؤشرات المضادة للالتهاب ، وزيادة معنوية في هرمون السيروتونين فى جميع المجموعات المعالجة بالمافن المحشو بالفواكه المجففة او مخلوط الفواكهة المجففة .

الخلاصة : يعتبر المافن المحشو بالفواكه المجففة مثل الزبيب والقراصيا وهو من منتجات العجائن المخبوزة غذاءا وظيفيا ووقائيا وعلاجيا واعدا في حماية الجهاز الهضمي من الالتهابات وخاصة المصاحبة لمتلازمة القولون العصبي.

* تخصص التغذية وعلوم الأطعمة، قسم الأقتصاد المنزلى ، كلية التربية النوعية، جامعة المنصورة ، مصر