

## The impact of fortify flat bread with Arabic gum (*Sengalia Senegal*) on chronic renal failure in experimental rats

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### ABSTRACT

This research aims to study the effect of preparation flat fortified bread (FFB) by replacing 25% and 50% of Arabic gum (AGM) on the chemical composition of the product (FFB) and its effectiveness on potassium bromate (KBrO<sub>3</sub>) (100 mg / kg of weight Body) that caused renal toxicity in albino male rats through nutritional indicators, some chemical and biological analyzes of liver and kidney function, antioxidant enzymes and histology of kidney, in rats used in the experiment. Thirty rats (Sprague Dawley Strain) weighing (130 ±150g) were randomly divided into five groups, each consisting of six rats classified (n=6), the first group was kept as negative control group (G1)-ve, while the other four groups were injected with KBrO<sub>3</sub> to induce toxicity in various experimental rats. One of those groups was fed on basal diet only and served as positive control group (G2) +ve. The third group (G3) was fed on basal diet and treated with flat bread FB (100% WF) control, the fourth and fifth (G4&G5) groups were fed on basal diet containing FFB with AGM (25% & 50%) respectively. Results revealed that rats consumed FFB with AGM (25% & 50%) showed significantly increased in FER and weight gain compared with positive group, biochemical analysis data indicated that a significantly decrease at p< 0.05 in blood lipids profile (TC, TG and LDL), while significantly increased at p< 0.05 in liver function (ALT and AST), and kidney function (protein, uric acid and creatinine) compared to positive group +ve. The previous results were also supported by microscopic examination of the renal tissues of groups infected with renal inflammation and treated with bread supported by different percentages of Arabic gum (25% and 50%).

**Keywords:** Flat bread, AGM, Renal Toxicity, KBrO<sub>3</sub>, Antioxidant Parameters.

### Introduction

Kidney is the organ that filter waste products from the blood. It is also involved in regulating blood pressure, electrolyte balance, and red blood cell production in the body it receive blood through the renal artery. Kidney disease occurs when the nephrons inside the kidneys are damaged Urologic Diseases Information Clearinghouse (NKUDIC), (2012). Chronic kidney disease (CKD) is a modern day epidemic and has significant morbidity and mortality effects (Sager *et al.*, 2014).

Arabic gum (*Sengalia senegal*) AGM is extracted from exudates of Acacia seyal trees. It is using in Middle Eastern nations broadly utilized as a part of the treatment of patients with ceaseless kidney infection and end-organize renal illness (Al Majed *et al.*, 2002). AGM considered an edible, gummy, dried exudate rich in non-viscous soluble fibers taken from the mature trees of Acacia senegal and Acacia seyal (Yebeyen *et al.*, 2009). The physical properties of AGM recognized as quality parameters incorporate total ash, moisture, volatile matter and internal energy. AGM is a natural product complex mixture of hydrophilic carbohydrate and hydrophobic protein components (FAO, 1995). AGM is comprises of a blend of polysaccharides in addition to oligosaccharides glycoproteins, calcium, magnesium and potassium. AGM has been displayed to adverse affect on electrolyte balance and vitamin D in mice, and to cause hypersensitivity in humans (Ahmed, 2007).

Flat bread is made throughout most of the world. Examples are tortilla, chapati, pita, parotta, yufka, tandoori roti, sangak, balady, barbari, taftoon, lavash, ciabatta, baati, bafla, phulka, kulcha and gyro bread (Ali and Hanaa, 2016). Flat bread is characterize lower specific volumes, high crust and crumb ratio than pan bread. The unleavened flat breads have shorter fermentation period in comparison to pan bread. They have different production conditions coming from higher baking temperature (350- 425°C) and shorter baking time (1- 10 min.) (Pahwa *et al.*, 2015).

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## Materials and Methods

### Materials:

- Arabic gum (*Sengalia senegal*) AGM were obtained from the Agricultural Research Center, Dokki, Giza, Egypt., wheat flour (72% extraction), active dry yeast and salt were obtained from local market of El-Mansoura city, Egypt.
- Potassium bromate (KBrO<sub>3</sub>) was an odorless white powder, purchased from El-Gomhoria Co., Cairo, Egypt.
- Thirty male albino rats (Sprague Dawley Strain) weighing (130 ±150g per each) were obtained from National Research Center, Dokki, Egypt.

### Methods:

#### Preparation of fortified flat bread (FFB):

Flat bread was preparing by mixing wheat flour (72% extraction), salt, water and activated yeast to form dough according to Hosoney, (1988). Fortified flat bread (FFB) were made by wheat flour (72% extraction) substituted with AGM ( 25% & 50%), dough was formulated, fermented, and baked on an Aluminum trial ( 350-425 C° at 5 -10 mints ) according to method describe by Pahwa, *et al.* ( 2015 ),the final product were packaged in polyethylene bags and stored in frozen at-18c until using and analysis .

#### Chemical analysis of raw materials:

Moisture, protein, fat, ash, and fibers were determined according to the method outlined in A.A.C.C. (2005). Total carbohydrates were determined by difference as mentioned by Abd El-Latif, (1990).

#### Experimental design

All albino rats were feeding the basal diet for two weeks prior to commencement of the experiment for adaptation. Fed on basal diet contain of corn starch (497g/kg), suger (100g/kg), cellulose (30g/kg), casein (200g/kg), corn oil (50g/kg), bend of mineral (100g/kg), bend of vitamins (20g/kg) and colin (3g/kg).according to NRC, (1995). Then they were divided into five main groups (6 rats each) and fed the experimental diets for thirty five days. The first group (G1) was kept as negative control group and fed on the basal diet only, while the other six groups (G2, G3 , G4, and G5) were injected intraperitoneally into rats with LD50 of KBrO<sub>3</sub> in a dose of 100 mg/kg body weight according to Atssahin *et al.* (2006), to induce toxicity in various animal studies. One of these groups fed on basal diet only and served as positive control group (G2). The third group (G3) fed on standard diet and treated with control flat bread (100% wheat flour). The fourth and fifth (G4 and G5) groups were fed on basal diet containing FFB with 25% & 50% AGM respectively. During the experimental period, the quantities of diet, which were consumed and / or wasted, were weighed daily. In addition, rat's weight was recorded weekly, to determine feed intake and body weight gain.

#### Biochemical Analysis:

Serum on ALT (alanine aminotranferase) and AST (aspartate aminotransferase) enzymes activity were decorator accordance to Bergmeyer and Horder (1980). Total protein was decorator accordance to Henry (2001). Serum creatinine, uric acid and urea were determined by Young (2001), Fossati *et al.*, (1980) and Patton and Crouch (1977). Serum (TC), (TG), (HDL-c) was measured according to Cohn *et al.* (1988) and Fossate and principe, (1982). (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) were calculated by Friedewald *et al.*, (1972). Total antioxidants capacity (TAC), Superoxide dismutase (SOD) and nitric oxide (NO) were determined according to Cao, *et al.* (1993), and Nagi *et al.* (2010).

### Statistical Analysis:

Totally the gained data were statistically evaluated by SPSS computer software. The calculated occurred by analysis of variance ANOVA and follow up test LSD by SPSS ver .11 accordance to Abo Allam (2003).

### Results and Discussion

The data in Table (1) presented that the control flat bread 100% WF contain moisture, protein, fat, ash and carbohydrate were (28.8, 16.8, 0.66, 0.8 and 52.9% ) respectively ,while flat beard fortified (FFB) with 25% AGM contain moisture, protein, fat, ash and carbohydrate were (29.2 ,18.68, 0.48, 1.9 and 49.6%) respectively. Fortified flat beard (FFB) with 50% AGM contain moisture, protein, fat, ash, fiber and carbohydrate were (18.5, 22.5, 0.65, 1.4 and 56.8%) respectively. Pečivová, *et al.*, (2011) revealed that the estimation of dry issue in the example was diminishing in examination with the control test by expanding expansion of AGM from acacia tree. The contrast between the most reduced expansion AGM and the control test isn't significant.

**Table 1:** Chemical composition of control and fortified flat bread (FFB) with (AGM)

Samples	Moisture %	Protein %	Fat %	Ash %	Carbohydrate
Control flat bread 100% *WF	28.8	16.8	0.66	0.8	52.9
FFB by 25% AGM	29.2	18.68	0.48	1.9	49.6
FFB by 50% AGM	18.5	22.5	0.65	1.4	56.8

Values are the means of 4 independent determinations; fresh matter basis \*WF: Wheat Flour

Data in Table (2) showed body weight gain, feed intake and feed efficiency ratio in all treated groups with FFB of (25% &50) AGM. The untreated group (positive control) showed significant decrease in weight gain, feed intake and feed efficiency ratio (FER) at  $p < 0.05$  comparing with normal control group. Also the non-treated group (+ve) showed a significant decrease in feed intake and feed efficiency ratio (FER) at  $p < 0.05$  compared with all treated groups. Treated group were feeding on bread with 100% WF(G3) showed significant decrease in feed intake at  $p < 0.05$  comparing with normal control group and positive group but showed significant increase in weight gain and FER comparing with normal control group and positive group . Treated group feeding on bread with 25% AGM (G4) showed significant increase in weight gain and FER at  $p < 0.05$  compared with normal control and positive group, while it showed significant decrease in feed intake at  $p < 0.05$  compared with normal and positive group.

**Table 2:** The effect of fortified flat bread (FFB) with AGM on nutritional parameters and body weight (g) of rats groups

Groups Variables	G1 (-ve)	G2 (+ve)	G3	G4	G5
Weight gain (g)	22.49 ± 7.25 b	22.33± 7.25 b	28.16± 9.22 c	36.67± 11.02 <sub>a</sub>	36.83± 11.25 a
Feed intake (g)	17.66± 3.14 a	15.33± 4.81 ab	13.17± 4.03 b	13.66± 3.61 b	13.83± 3.54 b
FER	0.038± 0.004 c	0.014± 0.004 a	0.047± 0.004 c	0.026± 0.003 b	0.026± 0.003 b

Each value represented the mean ± SD, n=6 .Mean values in each column having different superscript (a, b, c,..) are significant by different and vice versa .

Treated group feeding on flat fortified bread (FFB) with 50% AGM (G5) showed significant decrease in feed intake and FER at  $p < 0.05$  compared with normal control group. On the other hand it showed significant increase in weight gain at  $p < 0.05$  compared with normal group. on the other hand

it showed significant increase in weight gain and FER at  $p < 0.05$  compared with positive group, this result was agreement with Nasir (2010) who reported that the dietary fiber AGM favorably affects lessening of body weight gain, which could be utilized as a part of turn as prophylactic or treatment of weight and the improvement of metabolic syndrome.

Data in Table (3) showed cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL) and high density lipoprotein cholesterol (HDL) of renal oxidative stress rat groups treated by FFB with AGM. The untreated group (positive control (+ve)) showed significant increase in triglyceride, cholesterol low density lipoprotein cholesterol (LDL) but clearly moral depressed at  $p < 0.05$  in high density lipoprotein cholesterol (HDL) of renal oxidative stress rat groups treated by FFB with AGM compare with negative control (-ve). Also the non-treated group (+ve) showed a significant increase in cholesterol, triglyceride (TG) and low density lipoprotein cholesterol at  $p < 0.05$  but showed significant decrease in (HDL) comparing with all treated groups.

**Table 3:** The effect of fortified flat bread (FFB) with AGM different levels on lipid profile of rats groups

Groups Variables	G1 (-ve)	G2 (+ve)	G3	G4	G5
TC (mg/dl)	163.16± 15.26 d	281.00± 27.64 a	189.16± 18.77 c	219.33± 25.31 c	233.66± 24.54 b
TG (mg/dl)	134.66± 11.09 d	223.17± 26.08 a	156.33± 16.54 c	175.67± 19.43 b	196.50± 20.04 b
LDL (mg/dl)	86.33± 17.25 d	188.33± 28.61a	111.50± 14.79 c	146.66± 21.04 b	151.83± 23.60 ab
HDL (mg/dl)	49.50± 22.16 a	27.66± 8.80 d	40.66± 19.21 b	37.00± 16.41 c	42.16± 19.47 ab

Each value represented the mean  $\pm$  SD, n=6. Mean values in each column having different superscript (a, b, c,...) are significant by different and vice versa

Treated group flat bread with 100% WF showed significant increase in cholesterol, triglyceride, and low density lipoprotein cholesterol at  $p < 0.05$  but clearly moral depressed at  $p < 0.05$  in high density lipoprotein cholesterol compare with normal control. On the other hand, all treated group fortified flat bread (FFB) with (25% & 50%) AGM showed significant increase at  $p < 0.05$  in cholesterol TC, TG, LDL while showed significant decrease in HDL at  $p < 0.05$  in compare with positive control (+ve), when compared to normal control rats group while there were significant increases in cholesterol TC, triglyceride TG, low density lipoprotein LDL. Johan, (2012) reported that the consolidated usefulness of AGM is preference over other consumable biopolymers that don't demonstrate antioxidant activities. Jaafar, *et al.* (2016) reported that the addition of AGM significantly ( $P < 0.05$ ) decreased in plasma total cholesterol, low-density lipoprotein cholesterol and triglyceride concentrations, fixations, while extended high-thickness lipoprotein cholesterol compared to the diabetic groups.

Data in table (4) showed liver and kidney function parameters and oxidative stress rat groups treated by FFB with AGM. The untreated group (+ve) showed significant increase in AST & ALT at  $p < 0.05$  comparing with negative control group.

Also the non-treated group (+ve) showed a significant increased in AST and ALT at  $p < 0.05$  comparing with all treated groups. Treated group flat bread with 100% WF and FFB with AGM( 25% & 50%) showed significant increase at  $p < 0.05$  in AST and ALT but showed significant decrease at  $p < 0.05$  in total protein compare with negative group(-ve). While it showed significant decrease in AST and ALT at  $p < 0.05$  compared with positive group. Elshama *et al.* (2014) registered that the Arabic gum dose (500 ml of 10% AGM) and showed low levels of AST and alkaline phosphatase reflects a protecting effect of AGM on the liver and the bile duct cells.

The untreated group (+ve) showed significant increase in creatinine and uric acid comparing with normal group. Also the non-treated group (+ve) showed a significant increase in creatinine and uric acid at  $p < 0.05$  comparing with all treated groups. Treated group by flat bread with 100% WF and FFB with AGM( 25% & 50%) showed significant increase in creatinine and uric acid at  $p < 0.05$  compared normal group(-ve). These results in parallel with Elshama *et al.*, (2014) who demonstrated

that AGM effective in improving liver and kidney functions. In addition Azzaoui *et al.* (2013) who observed that pomegranate extract has strong antioxidant properties against gentamicin induced nephrotoxicity.

**Table 4:** The effect of fortified flat bread (FFB) with AGM different levels on liver and kidney function parameters of rats groups

Groups Variables	G1 (-ve)	G2 (+ve)	G3	G4	G5
AST ( $\mu$ /ml)	22.8 $\pm$ 4.31 d	94.8 $\pm$ 17.26 a	50.7 $\pm$ 14.73 c	61 $\pm$ 8.9 b	67.2 $\pm$ 8.71 b
ALT ( $\mu$ /ml)	23.7 $\pm$ 6.16 d	86 $\pm$ 21.13 a	46 $\pm$ 12.6 c	55.6 $\pm$ 16.16 b	64 $\pm$ 17.8 b
T. Protein (mg/dl)	7.24 $\pm$ 1.85 a	3.75 $\pm$ 0.39 c	6.51 $\pm$ 1.14 a	5.82 $\pm$ 1.15 b	5.27 $\pm$ 1.07 b
Uric acid (mg/dl)	3.28 $\pm$ 0.23 d	8.35 $\pm$ 2.33 a	4.20 $\pm$ 1.26 c	5.20 $\pm$ 1.23 b	6.33 $\pm$ 2.16 b
Creatinine (mg/dl)	0.48 $\pm$ 0.05 d	4.00 $\pm$ 1.47 a	1.45 $\pm$ 0.13 c	2.11 $\pm$ 0.19 b	2.90 $\pm$ 0.14 b

Each value represented the mean  $\pm$  SD, n=6 .Mean values in each column having different superscript (a, b, c,) are significant by different and vice versa.

Result in Table (5) revealed that concentration of total antioxidants and SOD for normal control group (-ve) were (3.25 mmol/L and 72.12 U / mL) respectively, but the corresponding levels for positive control group were lower (1.4 m mol/L and 32.24 U/mL respectively). Results found a significant increase in total antioxidants and SOD levels and a significant decrease in NO level in all treated groups by FFB with 100% WF, and FFB with AGM as compared to the untreated group (+ve).

While, all treated group fortified flat bread with (25% &50%) AGM presented significant decrease at  $p < 0.05$  in NO as compared to positive control, while all treated group fortified flat bread with (100% WF, 25% &50%) AGM showed significant no significant at  $p < 0.05$  in NO as compared to negative control (+ve) Treated group fortified flat bread with (100% WF, 25% &50%) AGM showed significant decrease in total antioxidants and SOD at  $p < 0.05$  but showed significant increase in NO as compared to normal control. Treated group fortified flat bread with (100% WF, 25% &50%) AGM showed significant increase in total antioxidants and SOD at  $p < 0.05$  but showed significant decrease in NO as compared to positive control. Antioxidant activity of AGM is referred to its phenolic compounds (Jaafar *et al.*, (2016), have been used as antioxidant agents to prevent various lipid-peroxidation induced damages in different organs. The results at the same trend with finding of Azzaoui *et al.* (2013) reported that the level of MDA returned to normalcy in rats treated with supplemented AGM.

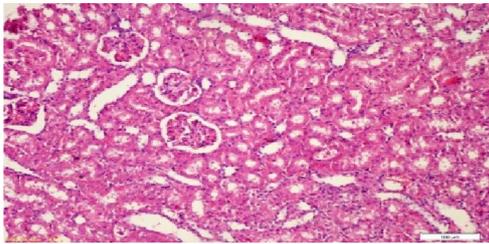
**Table 5:** The effect of fortified flat bread (FFB) with AGM different levels on "total antioxidant capacity (TAC), Superoxide dismutase (SOD) and nitric oxide (NO) of rats groups"

Groups Variables	G1 (-ve)	G2 (+ve)	G3	G4	G5
TAC (mmol/L)	3.25 $\pm$ 0.28 a	1.04 $\pm$ 0.15 d	2.3 $\pm$ 0.16 b	1.03 $\pm$ 0.16 c	3.03 $\pm$ 0.15 ab
SOD (u/mL)	72.12 $\pm$ 15.22 a	23.24 $\pm$ 6.47 d	58.86 $\pm$ 11.35 c	70.13 $\pm$ 14.16 a	66.32 $\pm$ 12.23 b
NO (mmol/I)	3.52 $\pm$ 0.33 c	9.82 $\pm$ 1.44 a	5.63 $\pm$ 1.03 b	4.13 $\pm$ 1.11 c	3.85 $\pm$ 1.05 c

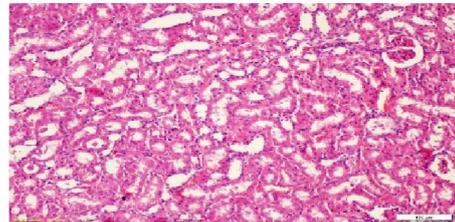
Each value represented the mean  $\pm$  SD, n=6 .Mean values in each column having different superscript (a, b, c,) are significant by different and vice versa.

Results of the histopathological examination of kidney of rats from different experimental groups were illustrated in histopathological images. The obtained biochemical results are confirmed

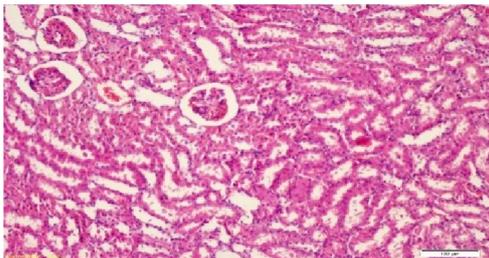
by the histopathological examination. The best results of kidneys were noticed also in groups treated with the higher levels (50%) of AGM fortified flat bread followed (25%) group.



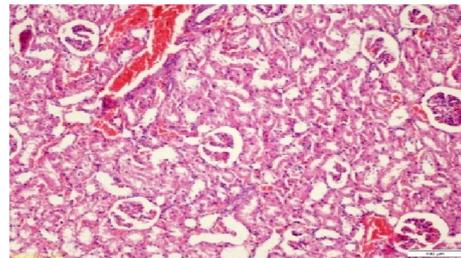
**Pic. 1:** kidney of negative group rat showing normal renal tissue( H and E  $\times 100$ )



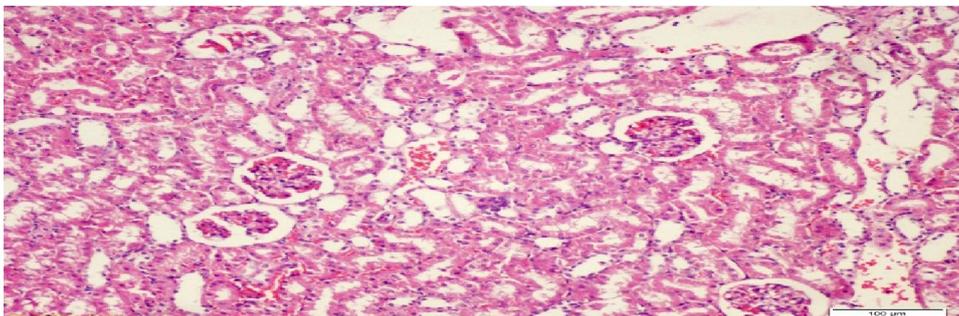
**Pic. 2:** kidney of Disease rat group showing moderated tubular injury with intestinal hemorrhage and inflammatory ( H and E  $\times 100$ )



**Pic. 3:** Kidney of rat treated with 100% WF showing near normal renal tissues with regenerated tubules and absence of intestinal inflammation( H and E  $\times 100$ )



**Pic. 4:** Kidney of rat treated with AGM 25% marked tubular injury and congested capillaries intestinal inflammation( H and E  $\times 100$ )



**Pic. 5:** Kidney of rat treated with AGM 50% Moderate tubular atrophy with congested capillaries and interstitial infiltrate( H and E  $\times 100$ )

## Conclusion

It could be concluded that, addition different percentages of AGM to the diet are effective in nutritional status indicators (gained weight and food efficiency rate), and blood lipids impair some liver and kidney function. This explains the content of AGM from complex sugars, phenolic compounds and some minerals such as calcium, magnesium and potassium, which help to reduce the effects of kidney failure, as a result of reducing the concentrations of creatinine and urea in the blood reduces the changes undesirable from the oxidative stress induced by potassium bromate ( $KBrO_3$ ).

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