



## THE SAFETY OF USING IRRADIATED SORGHUM (SORGHUM BICOLOR), USING ALBINO RATS FED ON THEM

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### ABSTRACT

Sorghum, the world's fourth major cereal in terms of production, is a staple food crop of millions of poor in semi-arid tropics of the world. The objective of this study was to investigate the safety of using X- ray, Gamma ray and UV light treated sorghum (*Sorghum bicolor* (L.) Moench), by testing the renal and liver function parameters of Albino rats fed on the second generation yielded seeds. Original sample of sorghum seeds was brought from the local market, cleaned manually, divided into six groups and put in clean Petri dishes. Four groups were treated with low and high doses of X-ray and gamma ray, one group was treated with the UV light, while the last was the control. Fifteen Wister Albino rats were weighed and distributed randomly in six groups according to their irradiated sorghum feed. After 60 days, blood samples of the Albino rats were collected from the retroorbital sinus. The blood serum was separated using centrifuge. Renal function and liver function parameters were determined. The results showed that, the mutant sorghum seeds were safer to be used (no significant differences in renal and liver functions on the experimental Rats). Similar studies on other crops should be run.

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### INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is a cereal crop that is usually grown under hot and dry conditions. It is found in the Africa and Asian regions, however, the world sorghum production is still dominated by the USA, India, Nigeria, China, Mexico, Sudan and Argentina (FAO, 1995). Sorghum, the world's fourth major cereal in terms of production and fifth in acreage following wheat, rice, maize and barley (US Grains Council, 2005). The yield and quality of sorghum produced worldwide is affected by a wide array of biotic and abiotic constraints (ICRISAT, 2004; Nadia et al., 2009).

Sorghum is used for human food, (Dirar, 1991). Also for fodder and feed for animals, (Miller, 1996) and building material, fencing, or for brooms (Doggett, 1988). In the USA, used for the syrup made from the sweet juice. In Europe, used for livestock feed. In USA and Europe, for the renewable fuels industry and into the gluten-free food market (Berenji, 1991; Berenji and Dahlberg, 2004). In Africa and India, for bread, porridge or gruel, and as popped grain and beer.

Sweet sorghums used in sugar production. Traditionally, used in alcoholic and non-alcoholic beverages (Berenji and Dahlberg, 2004). Tannin sorghums, used as antioxidant that are comparable to blueberries (Waniska, 2000). There is growing evidence that some of these sorghums have high anti-inflammatory and anti-colon cancer activities. Special sorghums have been identified with very high levels of the rare 3-deoxy anthocyanins that have unique color stability and potential health applications (Dykes and Rooney, 2006). Sorghum is debated for biofuel, ethanol, and biogas production (Dahlberg et al., 2011).

#### Nutritional value (per 100 g (3.5 OZ) of the dried seeds)

Energy	1,418 KJ(339 kcal)
Carbohydrates	74.63g
Dietary fiber	6.3g
Fat	3.30g
Protein	11.30g

Source: NRC (1996); Leder and Schusterne (2000).

X-rays cause damage to living tissue (break molecular bonds and create highly reactive free radicals such as H and OH), which in turn can disturb the molecular structure of the proteins and especially the genetic materials (Acharya, 1977).

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In molecular biology and genetics, mutations are changes in a genomic sequence: the DNA sequence of a cell's genome or the DNA or RNA sequence of a virus. They can be defined as sudden and spontaneous changes in the cell. Mutation can affect organisms on structure, function, fitness, protein sequence or on inheritance ability (Burrus and Waldor, 2004; Aminetzach *et al.*, 2005). Mutations are caused by radiation, viruses, transposons and mutagenic chemicals, as well as errors that occur during meiosis or DNA replication. There are two classes of mutations: spontaneous mutations (Sawyer *et al.*, 2007); induced mutations which can be caused by chemicals (e.g Hydroxylamine and other agents), radiation (Kozmin *et al.*, 2005) or Viral infections (Pilon *et al.*, 1986).

Genetically modified organisms (GMOs) are living organisms whose genomes have been modified as a result of gene technology, resulting in the introduction, removal or alteration of a specific characteristic or trait (Nelson, 2001).

## MATERIALS AND METHODS

### *Sorghum samples*

A sample of sorghum seeds (*Sorghum bicolor*) was brought from the local market, cleaned manually, divided into six groups and put in clean Petri dishes. Four groups were treated with low and high doses of X-ray (LX (33.4 sec.) and HX (200.2 sec.), respectively) using X-ray device, and gamma ray (LG (200 CGY) and HG (800 CGY), respectively) using Co-60 device. One group was treated with UV light for 30 minutes, while the last group was used as a control (C).

### *Sowing of sorghum samples*

The six groups of sorghum seeds were planted routinely with considerable distances between them in a designed 40X100 m<sup>2</sup> agricultural area at the Experimental Farm, Faculty of Agricultural Sciences, University of Gezira,. Each group (1X10 m<sup>2</sup>) was divided into four rows (the distance was 25 cm between rows and it was 20 cm between the plants in the same row). The distance between the groups was about 35 m (Figure, 1). The second generation was sown in July, the third generation in mid-October, while the fourth generation was sown in February. The soil was prepared mechanically and the irrigation was derived directly from the main canal in the Farm, once a week. No fertilization nor pesticides were added throughout the experiment period (for the second mutant generation).

### *The physiological activity of the Albino rats fed on mutant sorghum*

#### *The experimental Albino rats*

Fifteen Wister Albino rats (4 weeks old) were weighed and distributed randomly in five groups according to their weights and their feed. The mean weight of each Albino rat was measured twice during the experimental period (60 days); before and after experimental. The experimental rats were then placed in clean separate cages. Each rat was fed on 12 g/day of wheat bran and the flour of each mutant sorghum seeds, in addition to control (at a ratio of 1:1). Each feeding component was mixed with a considerable amount of water to made compact paste, which was then cut into small pieces, compressed and let to dry separately. Each sorghum cake-piece was weighed before being fed to the Albino rats at 12

g/day for two months. No other food or additives were added throughout the experiment period.

### *Sampling blood for hematological analysis*

At the end of the experimental period (60 days), blood samples of the Albino rats were collected from the retroorbital sinus (a system of dilated venous channels at the back of the orbit). A microhematocrit tube was along the inner corner of the eye (Plate, 1) beside the eyeball. After collecting the blood the tube was withdraw and a gentle press was applied on the closed eyelids to stop any bleeding.

About 2.5 ml of blood was poured in clear container containing the anticoagulant EDTA so as to avoid clotting. Sysmex KX 21N model was used for counting blood cell. The collected blood samples were put on the hematology mixer machine to determine the blood cell: white blood cells, red blood cells, platelets, PCV, MCH, MCHC, MCV and neutrophils and lymphocytes. The analysis was executed when the instrument was set its ready status (the tube setting was performed manually).



Plate 1 Sampling of blood from rats

### *Renal and liver function test*

After the experiment period (60 days), three ml of blood was collected by heparinized tubes. The plasma of the collected blood was separated after centrifugation at 2500 rpm for 5 minutes. Renal function parameters were creatin, urea, Na<sup>+</sup> and K<sup>+</sup>. These tests in addition to the liver function parameters (protein, albumin, bilirubin, ALP, ALT, and AST) were run at the Blood Bank Laboratory, Wad Medani Teaching Hospital, according to (Spinreact, 2013; BioSystem, 2014).

### *Statistical Analysis*

Simple descriptive statistic (means) were compared to the standards values

## RESULTS AND DISCUSSION

### *The physiological activity of the Albino rats fed on mutant sorghum*

#### *Renal function (RF)*

Table (1) showed the effects of different irradiated sorghum seeds on the renal function parameters (RF) of albino rats fed on it. The creatin (in mg/dl) was 0.70 in HX, 0.88 in UV, while in LG and HG samples it were 0.65 and 0.60,

respectively. The UV sample showed slight increase, while gamma ray showed slight decrease, whereas HX sample was normal compared to control (0.70 mg/dl).

The table also showed the Urea contents (in mg/dl) in these albino rats. The Urea content was 40.3 in HX (the highest value measured among all samples), 12.0 in UV (the least value measured among all samples), while in LG and HG samples it were 36.0 and 31.3, respectively. All irradiated samples, except UV, showed values higher than the control (24.5 mg/dl).

The Na<sup>+</sup> contents (in mmol/l) was 145.0 in HX (normal compared to control (145.0)), 144.0 in UV, while in LG and HG samples it were 149.0 and 143.0, respectively. Whereas, K<sup>+</sup> contents (in mmol/l) was 5.6 in HX, 5.3 in UV (both were normal compared to control (5.3)), while in LG and HG samples it were 4.9 and 5.9, respectively. The LG sample showed a lowest value among all tested samples.

ANOVA analysis revealed no significant difference in the columns level (samples; f= 1.20 and f-crit= 2.26), i.e. the control and the irradiated samples were statistically similar in their renal function parameters.

This results agreed with Bourre (2005), who found that, no significant difference in the activity of the plasma alanine transaminase between growing male Albino rats fed raw or irradiated full-fat linseed at 2.5, 5, 7.5 and 10 kGy during the experimental period (5 weeks). On top of that, there are no significant differences between AST/ALT ratio for groups of rats fed raw or irradiated linseed and groups kept on casein diet (Hikmat *et al.*, 2010).

**Table (1)** Effects of irradiated sorghum seeds on renal functions parameters (RF) of albino rats

Parameter	Control	HX	UV	LG	HG	Standards (Brzóska <i>et al.</i> , 2003 and Jensen <i>et al.</i> , 1981)
Creatinine (mg/dl)	0.70	0.70	0.88	0.65	0.60	0.25-3.09
Urea (mg/dl)	24.5	40.3	12.0	36.0	31.3	10.8-75.1
Na (m mol/L)	145.0	145.0	144.0	149.0	143.0	120.5
K (m mol/L)	5.3	5.6	5.3	4.9	5.9	5.6-12

#### On the liver function

Table (2) showed the effects of the irradiated sorghum seeds on the liver function parameters (LF) of albino rats fed on it. The Albumin (in g/dl) was 4.2 in HX, 4.3 in each of UV and HG samples, while in LG it was 5.0 (the highest value among control (4.8) and treated samples).

The ALP content (in u/l) in albino rats fed on the irradiated sorghum seeds was 230.0 in HX (the highest among all samples), 88.0 in UV, while in LG and HG samples it were 128.0 and 206.0, respectively. X and gamma treated samples showed considerable increase in ALP contents compared to control (75.5 u/l). The alanine transaminases (in u/l) was 190.7 in HX, 342.0 in UV, 129.0 in LG and 640.7 in HG samples. All irradiated samples showed considerable increase compared to control (141.5 u/l).

The table also showed that, the aspartate transaminases contents (in u/l) was 410.7 in HX , 816.0 in UV, while in LG it was 253.0, whereas in HG sample it was 480.0. All treated samples, except LG, show increased values compared to control (279.0). Bilirubin contents (in mg/dl) were 0.0 in all tested samples. The Total bili contents (in mg/dl) were 0.1 in

control, HX and LG samples, whereas, it was 0.0 in UV, while in HG sample it was 0.13. The total protein content (in g/dl) was 7.6 in each of the HX, and UV samples, while it was 8.8 in LG (the highest value among all samples), whereas, in HG sample it was 7.3. All irradiated samples, except LG, show decreased in value compared to control (8.2 g/dl).

It was clear that, according to the standards, the difference was not great, between control and the irradiated samples in their liver function parameters. There were no difference in the activity of the aspartic and the alanine transaminases in the plasma of growing male Albino rats fed raw or irradiated full-fat linseed at 2.5, 5, 7.5 and 10 kGy during 5 weeks, while the data obviously indicated that, the level of AST and ALT of animals fed casein diet were higher than the levels of those fed raw and processed linseed. On top of that, there are no considerable differences between AST/ALT ratio for groups of rats fed raw or irradiated linseed and groups kept on casein diet.

**Table 2** Effects of irradiated sorghum seeds on liver function parameters of albino rats

Parameter	Control	HX	UV	LG	HG	Standards (Young <i>et al.</i> , 2008; Brzóska <i>et al.</i> , 2003)
Albumin (g/dl)	4.8	4.2	4.3	5.0	4.3	3.4-5.4
ALP (u/l)	75.5	230.0	88.0	128.0	206.0	20-140
Alanine aminotrans (u/l)	141.5	190.7	342.0	129.0	640.7	5-35
Aspartate aminotrans (u/l)	279.0	410.7	861.0	253.0	480.0	10-34
Bilirubin (mg/dl)	0.0	0.0	0.0	0.0	0.0	0-0.3
Total Bili (mg/dl)	0.1	0.1	0.0	0.1	0.13	0.3-1.9
Total Protein (g/dl)	8.2	7.6	7.6	8.8	7.3	6-8.3

Plasma enzyme aspartate transaminase (AST) and alanine transaminase (ALT) were used as the biochemical indicators for hepatic damage. Plasma AST and ALT were found to be elevated (P=0.0001) in growing male albino rats received high fat diet (HFD) when compared with those received reference diet (25.538 vs 22.163 and 10.733 vs 7.517 UL-1, respectively). Adding 2% raw or irradiated turmeric powder at 10, 15 and 20 kGy to HFD decreased the hepatic enzyme activities (AST and ALT) of rats maintained on the aforementioned diets for six weeks to be closed to those received reference diet. On top of that, there are no significant differences between groups of rats received 2% irradiated turmeric powder at 10, 15 or 20 kGy in their diets as compared with those received 2% non-irradiated turmeric powder (Hania *et al.*, 2010).

Farag (1987) mentioned that, irradiated different sorts animal feeds up to 50 kGy did not affect the levels of plasma proteins.

#### On blood cell count

The data obtained for evaluation of the effect of the irradiated sorghum seed that were treated with LX, HX, LG, HG and UV light and then fed to the albino rat (as cake) on red blood cells (RBC), white blood cells (WBC) and platelets (PLT) counts were represented in Table (3). The WBC counts (in term of x10<sup>9</sup>/L) were 8.4 in control (the highest value), 7.5 in X ray, 6.3 in UV (the least value), 7.7 in LG and 8.2 in HG groups. The RBC counts (in term of x10<sup>12</sup> /L) were 7.14 in control, 8.53 in LX (the highest value), 7.74 in UV, 6.01 in LG (the least value) and 7.82 in HG. The PLT counts (in term of x10<sup>9</sup>/L) were 513.5 in control, 665.7 in LX (the highest

value), 331.0 in UV (the least value), 407.0 in LG and 356.7 in HG.

It was clear that, the irradiated sorghum seed cake decreased the blood counts in albino rats after 60 days of feeding, except LX treated sorghum.

The comparison also revealed that, there was considerable difference in the blood count parameters, while there was no considerable difference between the tested groups i.e. the blood counts in the albino rats fed on irradiated sorghum were similar to those fed on un-irradiated sorghum. It was clear that, the irradiated sorghum seeds were similar in their effect on blood count as same as control.

**Table 3** Effects of mutant sorghum seeds fed to albino rats on their blood count

Blood Parameter	Control	LX	UV	LG	HG	Standards (Stammers, 1926)
WBC ( $\times 10^3/L$ )	8.4	7.5	6.3	7.7	8.20	9.02-10.20
RBC ( $\times 10^6/L$ )	7.14	8.53	7.74	6.01	7.82	7.8-9.2
PLT ( $\times 10^6/L$ )	0.514	0.666	0.331	0.407	0.357	0.8-1.0

## CONCLUSIONS AND RECOMMENDATIONS

### Conclusions

1. The red blood cells (RBC), white blood cells (WBC) and platelets (PLT) were decreased in count as a result of feeding on the mutant sorghum seeds (except LX group).
2. The mutant sorghum seeds were “comparatively”, safer to be used (they did not make a considerable differences in renal and liver functions), although several obvious variations were noticed between control and treated rats.

### Recommendations

1. More important molecular genetic tests should be run.
2. More concerns should be oriented to sorghum flour.
3. Similar test should be conducted for considerable longer period in order to test the fertility and histological on the experimental rats in addition to lengthen the test period.

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