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Effect of feeding rats on gamma irradiated flour infected with Tribolium confusum

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► ***Original article***

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ABSTRACT

Background: Flour is one of the most important food sources, therefore it must be kept healthy and clean. As a potential pest control modality, we aimed to examine whether flour irradiation is a feasible tool to eradicate *Tribolium confusum* adults from infected flour considering probable health effects of infested flour consumption. ***Materials and Methods:*** unirradiated and irradiated flour were infested with 70 or 100 *T. confusum* adults/25g and stored for 6 and 12 weeks, the number of *T. confusum* progeny, antifeedant activity and biochemical changes (Aspartate Aminotransaminase (AST) and Alanine Aminotransaferase (ALT) activities) in adults were estimated. In addition the effect of feeding rats on bread made from theses infested flour was studied. ***Results:*** the obtained results revealed that flour irradiation caused disturbance in the production and physiology of *T. confusum* adults feed on it. In addition, the gained data exposed a significant decline in rats' weight and alterations in some biochemical aspect (glutathione content, glutathione-S-transferase, malondialdehyde, gammagluamyl transferase, AST, ALT) which were more pronounced in those fed on irradiated flour infected with *T. confusum*. As well as, there was a disturbance in the complete blood picture (white blood cells, the lymphocyte count, red blood corpuscles and hemoglobin). Moreover, rats fed on bread made from irradiated or unirradiated infected flour exhibited pathological damages in their liver sections. ***Conclusion:*** It is concluded that irradiation of flour disturbs the physiological process in *T. confusum* so it may serve as a good preservation tool and does not significantly affect the rats.

Keywords: *Tribolium confusum, gamma radiation, rat, biochemical studies.*

INTRODUCTION

Since Beetles are the most destructive insect pests found in stored grains and milled products (flour, bean, cereals, spices, pasta, and many other products), the adults and larvae of beetles are the cause of serious economic losses as they contaminate the product, lower its nutritive value, and generate favorable conditions for the growth of mold and other fungi. Some fungi secrete toxic metabolites such as mycotoxins [1], have various lethal effects (carcinogenic and immunosuppressive activity) on human and animal health [2]. In addition, beetles are capable of producing benzoquinones (BQ), and provide grains of distinguishing, unpleasant odor which turn to pink color [3-5]. Although, IARC [6] did not categorize BQs as a carcinogenic substance, it can simply enter the blood causing inhibition of the protease enzymes and discouraging bone marrow generation in mice, which lead to cellular death. Moreover, 1,4-BQ has been reported to cause skin erythema and tissue necrosis. In addition, Lis *et al.* [7] sated that

1,4-BQ has a carcinogenic effect on cells. Additionally, McCord [8]; Apel and Hirt [9] conveyed that toxicity causes production of reactive oxygen species (ROS) "superoxide anion O_2^{o-}, hydroxyl radical OH^0 and hydrogen peroxide H_2O_2" which boosts lipid peroxidation that leads to tissues' injury by oxidizing membranes molecules' and breaking of DNA strand.

Several studies proved that continuous feeding on flour infested with insect boosted the hazard of many diseases (cancer and kidney failure). Biscuits made of infested flour induced the formation of heptacellular carcinomas in 22% of the experimental toad (*Bufo regularis*) and also induced liver, spleen and breast tumors in 35% of the experimental Swiss albino mice [10]. Also severe malfunctions were observed in the liver and kidney of rat as fed on insect infested flour for 4 weeks [11].

Irradiation technology is a convenient tool for storing products [12]. One of the privileges of the irradiation process is that it is a cold technology that kills insect pests and microbes, without affecting the nutritional content, quality and freshness of the product and without leaving residue [13].

Consequently, the aim of the study was to assess the effect of preserving flour by gamma irradiation on the re-infestation with *Tribolium confusum*. Also, to investigate the biochemical changes and liver malformation in male albino rats fed on bread prepared from irradiated flour infested with *T. confusum*.

MATERIALS AND METHODS

Insect rearing

Tribolium confusum were reared at *28 ±2C* and 65 ±5 R.H. on wheat flour in the Natural Control Laboratory, Natural Products Research Department, and National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt.

Gamma-irradiation

Gamma irradiation of flour was carried out at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt using an Indian Gamma cell (Ge 4000 A). The dose rate was 1.296 KGy/h during the experimental periods.

Flour treatment

Wheat flour was introduced to 3KGy for sterilization, afterwards it was infected by 70 or 100 of *T. confusum* adults/kg then baked as bread and presented to rats to feed on it for three months.

Experimental Procedures

Feeding T. confusum

25g of flour was put in small jars (8 cm × 12 cm). Then 70 and 100 adults of *T. confusum* were weighed and introduced into each jar. The jars were wrapped with muslin cloth and fixed by rubber strips; the control was a jar without insects. The jars were stored at 28±2°C and 65±5%R.H for 6 and 12 weeks. Each treatment was replicated three times. At the end of each tested period, the *T. confusum* were weighed and counted. The flour weight was also taken.

The number of living or dead adults, the number of larvae, weight loss and anti-feeding activity, growth rate and efficiency of conversion of ingested food (ECI) were determined.

The following equation was used for calculating the anti-feeding activity [14]

Nutrition signals were estimated using equations 1and 2 as described by Waldbauer [15].

$$\text{Growth rate (G.R.)} = \frac{G}{TA} \quad (1)$$

$$\text{Consumption index (C.I.)} = \frac{F}{TA} \quad (2)$$

Where:-

F= weight of ingested food (mg)

G= weight of insect through the tested period (mg)

T= duration of tested period (in days)

A= mean weight of insect through the tested period (mg) =

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$$\frac{\text{Initial weight of insects + final weight of insects}}{2}$$

The ECI was estimated as the capability of insect to transfer the ingested food to the body tissues with the use of equation 3:

$$\frac{\text{Weight gained (mg)}}{\text{Weight of food ingested (mg)}} \quad (3)$$

The percent reduction was computed according to the equation 4 as follows:

$$\% \text{ Reduction} = \frac{\text{Control - treated}}{\text{Control}} \times 100 \quad (4)$$

Assay of transaminase enzymes (ALT and AST) in T. confusum

After 6 weeks of the adults feeding, 1gm *T. confusum* adults of each treatment was homogenized by mortar in 1ml saline and centrifuged in Centurion Scientific cooling centrifuge (Centurion Scientific Ltd, UK) at 2376g and 5°C for 5 min. the supernatants were used for colorimetrically estimation of Alanine Aminotransferase (ALT) and Aspartate Aminotransaminase (AST) using assay kits from Biodiagnostic®, Egypt, according to the Reitman and Frankle [16]. The absorbance was calculated using T60 UV-VIS spectrophotometer (PG Instruments Limited, UK).

Experimental design with rats

4 weeks old male rats of *Sprague Dawley* species of about (50 - 55 gm) were used in the experiments. Rats were maintained in well ventilated cages, at 25±3°C and 62±2% RH, with light/dark cycle and were allowed fresh water ad libitum during the tested period.

Animals were divided into four groups of 6 rats in each:

Group (1): rats feeding on bread made from 1kg unirradiated flour/ no adults *T. confusum* infection.

Group (2): rats feeding on bread made from 1kg irradiated flour/ no adults' infection.

Group (3): rats feeding on bread made from 1kg irradiated flour/ 70 adults' infection.

Group (4): rats feeding on bread made from 1kg irradiated flour/ 100 adult's infection.

Animal ethical consideration

All animal experiments were conducted following the 3Rs principles for animal experimentation (Replace, Reduce and Refine) and is organized and operated to the CIOMS and ICLAS International Guiding Principles for Biomedical Research Involving Animals 2012. This study has been approved by research ethics committee, NCRRT (Reg. No. 26/A18).

Sample preparation and biochemical analysis

Rats were sacrificed after 1.5 and 3 months of feeding on bread. Blood samples were collected by piercing their hearts by sterilized syringes; quantity of blood was taken as whole blood on EDTA and used for white blood cells (WBCs), lymphocyte count, red blood corpuscles (RBCs) and hemoglobin concentration (Hb%) in heparinized tubes using blood counter 2800 Mindray. The rest of the blood sample was collected in dry clean tubes, left to coagulate then centrifuged in Centurion Scientific cooling centrifuge (Centurion Scientific Ltd, UK) at 855g for 10 min. and the serum was separated for estimation of gamma glutamyltransferase (GGT) activity. Liver tissue was rapidly excised, rinsed in saline solution cut two pieces one part kept in 10% formalin for histopathological studies. The other part of the liver was homogenized (10% w/v) in distilled water using Teflon homogenizer and centrifuged at 9503g 5°C for 15 min; the supernatant was used for the biochemical assays.

Estimation of Liver enzymes

Both AST and ALT activities were colorimetrically determined using assay kits from Biodiagnostic®, Egypt, according to method described by Reitman and Frankel [16].

Assessment of oxidative stress

Malondialdehyde (MDA) was estimated according to the technique of Ohkawa *et al.* [17] using Biodiagnostic® kit, Egypt.

Determination of antioxidants levels

Glutathione content (GSH) was specified

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using a commercial kit from Biodiagnostic®, Egypt according to the method of Beutler *et al.* [18]. Liver total Glutathione-S-transferase (GST) activity was assessed according to scheme of Habig *et al.* [19]. Gamma glutamyltransferase (GGT) activity was evaluated using Biodiagnostic® Company, Egypt kit based on the model of Persin and Vanderslika [20].

T60 UV-VIS spectrophotometer (PG Instruments Limited, UK) was used for the biochemical studies.

Statistical analysis

The experimental design permitted identifying the variance among groups ranging from 15-20% with an SD 9% of mean. Power (β) needed to detect alteration of the same degree to that detected at 1.5 and 3 months was calculated by post hoc power calculations with the smallest sample range N=6/group has β= -0.8 with an error =α-0.05 using Mini tab 18 software.

Data of experiments on rat were statistically evaluated by analysis of variance (F) followed by Tukey Pairwise Comparisons test to examine the significant differences between the treatments. While the 2 sample *t-test* was conducted to compare between the *T. confusum* experiments using statistical Minitab program.

RESULTS

When irradiated (Sterilized) and unirradiated flour was infested with 70 insect /25 g and left for 6 weeks the number of insects changed through this period of time. In unirradiated flour, the alive adults were 536.67, 133.33 dead adults and 240 larvae. These numbers changed after 12 weeks to become 226.67, 203.3 and 283.33 for alive adults, dead adults and larvae respectively. In the case of irradiated flour, the number of insects changed to 213.33, 316 and 146.67 for alive adults, dead adults and larvae respectively. Data showed that when unirradiated and irradiated (sterilized) flour was infected with 100 adult insects /25g and left for 6 and 12 weeks, the number of insects were 86.67, 13.33 and 185 for 6 week and 506.67, 253.33 and 313.33 after 12 weeks for alive adults, dead adults and larvae respectively. Contrarily, the number of alive and dead adults and larvae under this treatment were 83.33, 16.67 and 124.67 for 6 weeks and 496.67, 313.33 and 330 for 12 weeks respectively (table 1).

Table 1. Changes in number of adult *T. confusum/* and their progeny when feeding on irradiated and non-irradiated flour for 6 and 12 weeks.

Parameters / Group	6 Weeks			12 Weeks		
	No. Alive Adults	No. Dead Adults	Larvae	No. Alive Adults	No. Dead Adults	Larvae
70 adult *T. confusum*/25g						
Un irradiated flour	536.67± 37.5	133.33 ±66.67	240± 15.27	226.67 ±24	203.3± 18.55	283.33 ±17.63
irradiated flour	213.33± 14.52*	316± 17.63	146.67 ±26.03	183.3± 29.62	310± 30.55	93.33± 8.81*
100 adult *T. confusum*/25g						
Un irradiated flour	86.67± 8.81	13.33± 8.81	185± 17.5	506.67 ±33.82	253.33 ±84.12	313.33 ±24.03
irradiated flour	83.33± 3.33	16.67± 3.33	124.67 ±1.45	496.67 ±18.55	313.33 ±13.33	330±1 1.54

Values represent the mean of percentages of 3 replicates for each group. *Statistically significant at p<0.05 between groups of each insect number (2 sample *T-test*).

Percentage of the flour weight loss and anti-feeding activity of the insects after 6 and 12 weeks for 70 insect fed on 25g of irradiated and unirradiated flour showed that weight loss has declined by flour irradiation which recorded 7.53 and 4.14% for un-irradiated and irradiated flour respectively at 6 weeks. Similarly, after 12 weeks, the weight loss in unirradiated flour was 14.09% and decreased to 10.45% in irradiated flour.

In addition, the anti-feeding activity of *T. confusum* after *12* weeks (*15.5%*) was lower (28%) than that noted after 6 weeks (28%). Data also revealed that there was a significant decrease in the percent of weight loss of the irradiated flour after 6 and 12 weeks of 100 adults feeding. The results showed that after 6 weeks the weight loss in unirradiated was 5.48%, which declined to 2.53% in irradiated flour. Furthermore, after 12 weeks the percent of

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weight loss was 17.15 for unirradiated flour and dropped to 6.17 in irradiated flour. On the other hand, the anti-feeding activity of *T. confusum* represented a non-significant increase between 6 and 12 weeks, which exhibited 36.61 and 47.3% for 6 and 12 weeks, respectively (table 2).

Table 2. Weight loss and Antifeedant activity of *T. confusum* feed on irradiated and non-irradiated flour.

Parameters / Groups	6 Weeks		12 Weeks	
	Weight Loss (%)	Antifeedant activity (%)	Weight Loss (%)	Antifeedant activity (%)
70 adult/25g				
70 insect/25g Unirradiated flour	7.53± 0.87	28.6±4.93	14.09± 0.42	15.5±2.82
70 insect/25g irradiated flour	4.14± 0.71		10.45± 0.85	
100 adult/25g				
100 insect/25g Unirradiated flour	5.48± 0.09	36.61±4.53	17.15± 0.059	47.3±3.95
100 insect/25g irradiated flour	2.53± 0.21*		6.17± 0.88*	

Values represent the mean of percentages of 3 replicates for each group. *Statistically significant at p<0.05 between groups of each insect number (2 sample *T-test*).

Table 3 represent the effect of feeding of 70 insect/25g and 100 insect/25g on un-irradiated or irradiated flour on GR and ECI. The results indicated that both parameters decreased in insects fed on irradiated flour and that the reduction was significant in ECI.

The growth rates were 2.48, 2.75 mg/day and declined to 2.28, 2.19 mg/day when 70 or 100 insects were fed on un-irradiated and irradiated flour respectively.

AST (Aspartate Aminotransaminase) and ALT (Alanine Aminotransferase) activities in insects fed on un-irradiated or irradiated flour for 12 weeks. The results revealed a significant increase in both AST and ALT activities in *T. confusum* (*70 or 100 adult/25g*) fed on irradiated flour (table 4).

Table 3. Growth rate and Efficiency of conversion of ingested food after 6 weeks.

Parameters / Groups	70 adult /25g		100 adult /25g	
	Growth rate (mg/ day.mg)	Efficiency of conversion of ingested food (ECI) (%)	Growth rate (mg/ day.mg)	Efficiency of conversion of ingested food (ECI) (%)
Un irradiated flour	2.48± 0.19	0.054± 0.013	2.75± 0.32	0.488± 0.043
irradiated flour	2.28± 0.02	-0.083± 0.023*	2.19± 0.01	-0.413± 0.064*

Values represent the mean of percentages of 3 replicates for each group. *Statistically significant at p<0.05 between groups of each insect number (2 sample *T-test*).

Table 4. AST and ALT activities in T. confusum fed on irradiated and non-irradiated flour after 12 weeks.

Parameters / Groups	70 adult/25g		100 adult/25g	
	AST activity (U/ml)	ALT activity (U/ml)	AST activity (U/ml)	ALT activity (U/ml)
Un irradiated flour	37.56±2	36.79±1	20.35± 0.9	17.95± 0.4
irradiated flour	68.34± 1.6*	61.67± 0.5*	34.54± 1.4*	35.39± 1.3*

Values represent the mean of percentages of 3 replicates for each group. *Statistically significant at p<0.05 between groups of each insect number (2 sample *T-test*).

When rats in different groups were fed on bread prepared from irradiated or unirradiated flour which was infected with 70 or 100 adult insects for 1.5 or 3 months rats' weights significantly increased in group 2 and group 3 at 1.5 and 3 months compared to control, while no significant difference was found in rats' weights after 1.5 and 3 months (table 5). In group 4, the weights of rats significantly decreased in response to the different treatments in 1.5 and 3 months while no significant difference was found in rats' weights after 1.5 and 3 months.

During the experimental period, the rats showed signs of debility; group (3) rats fed on bread made from irradiated flour/70 adults' infestation showed a loss of body and arms hair. For group (4) fed on bread made from irradiated flour/100 adults infection, loss of hair on the whole body was observed (figure 1).

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Table 6 showed an increase in MDA level in rats' group G3 and G4 compared to control group G1. On the other hand, G2 has significantly decreased compared to control. The data revealed a significant decrease in GSH content in G3 and G4 groups compared with control group G1 after 1.5 and 3 months of treatment. However, in G2 a significant increase in GSH was noticed.

Data in table 6 revealed a significant reduction of GST activity and GGT content after 3 months as compared to 1.5 months. However, the GST activity has significantly declined in G2, G3 and G4 when comparing with control group G1 after 1.5 or 3 months. Moreover, GGT content was significantly increased in G2, G3 and G4 comparing with control group G1 after 1.5 or 3 months. Data further revealed a significant increase in AST and ALT activities in all groups as compared to the control group (G1) after 1.5 and 3 months. However, there was no significant difference between 3 months and 1.5 months (table 7).

Data in table 8 showed a significant increase in hematological parameters, WBCs count and lymphocyte count in rats' groups G2, G3 and G4 compared with control group (G1). Meanwhile, RBCs count and Hb significantly declined in all rats' group except G2 in RBCs under investigation compared with control group G1 after 1.5 and 3 months.

Tables 5. Change in weights (g) in different rats groups at time interval (6 and 12 weeks) after secession of treatments.

Weight / Groups	Initial	After 6 weeks	After 12 weeks
Group 1	52.5±0.76^{A}	88±1.77^{A}	128.67±2.49^{A}
Group 2	52.67±0.84^{A}	69.66±1.58^{B}	82±1.91^{B}
Group 3	51.66±1.44^{A}	55.83±1.07^{C}	57.33±1.42^{C}
Group 4	53.167±0.83^{A}	48.83±1.44^{D}	48.16±1.35^{D}

Group (1): rats fed on bread made from 1kg un irradiated flour/ no adults infection. ;Group (2): rats fed on bread made form 1kg irradiated flour (3kGy)/ no adults infection ;Group (3): rats fed on bread made 1kg irradiated flour/ 70adults infection ;Group (4): rats fed on bread made form 1kg irradiated flour/ 100 adults infection. Means with different letters are significantly different at p<0.05 between groups (one way ANOVA followed by Tukey pairwise comparison test).



Figure 1. Loss of hair in rats fed with different diets. **G1:** rats fed on bread made from 1kg un irradiated flour/ no adults infection; **G2:** rats fed on bread made form 1kg irradiated flour (3kGy)/ no adults infection; **G3:** rats fed on bread made 1kg irradiated flour/ 70adults infection; **G4:** rats fed on bread made form 1kg irradiated flour/ 100 adults infection.

Table 6. MDA level, GSH, content, GST activity and GGT content in different rats' groups at time interval 6 and 12 weeks after secession of treatments.

Parameters / Groups	MDA level (nmol/ g $_{tissue}$)		GSH content (mg/g $_{tissue}$)	
	6 weeks	12 weeks	6 weeks	12 weeks
Group 1	141.89±1.2^{C}		44.37±1.07^{B}	
Group 2	118.60± 1.47^{D}	71.54± 2.61$^{D@}$	58.70± 0.92^{A}	84.05± 0.93$^{A@}$
Group 3	161.09± 2.02^{B}	213.23± 1.4$^{B@}$	17.50± 0.79^{C}	15.99± 0.7^{C}
Group 4	193.93± 2.02^{A}	279.2± 1.19$^{A@}$	10.97± 0.75^{D}	12.57± 0.45^{D}
Parameters / Groups	**GST activity (U/g $_{tissue}$)**		**GGT content (U/L)**	
	6 weeks	**12 weeks**	**6 weeks**	**12 weeks**
Group 1	6.21±0.21^{A}		3.67±0.46^{D}	
Group 2	4.76±0.17^{B}	5.54± 0.13$^{A@}$	9.84± 0.57^{C}	15.44± 0.57$^{C@}$
Group 3	3.68±0.17^{C}	4.53± 0.28$^{B@}$	16.21± 0.66^{B}	34.93± 0.55$^{B@}$
Group 4	2.62±0.13^{D}	3.14± 0.14$^{C@}$	32.23± 0.91^{A}	55.20± 1.14$^{A@}$

Group (1): rats fed on bread made from 1kg un irradiated flour/ no adults infection. ;Group (2): rats fed on bread made form 1kg irradiated flour (3kGy)/ no adults infection ;Group (3): rats fed on bread made 1kg irradiated flour/ 70adults infection ;Group (4): rats fed on bread made form 1kg irradiated flour/ 100 adults infection. Means with different letters are significantly different at p<0.05 between groups (one way ANOVA followed by Tukey pairwise comparison test). @ Statistically significant at p<0.05 between 6 and 12 weeks (2 sample *T-test*).

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Table 7. AST (U/l) and ALT (U/l) activities in different rats groups at time interval 6 and 12 weeks after secession of treatments.

Parameters / Groups	AST activity (U/l)		ALT activity (U/l)	
	6 weeks	12 weeks	6 weeks	12 weeks
Group 1	52.13±0.81^{D}		12.30±1.13^{D}	
Group 2	66.55±1.3^{C}	73.68±0.34$^{C@}$	22.55±0.57^{C}	32.74±1.12$^{C@}$
Group 3	95.24±0.68^{B}	101.9±0.72$^{B@}$	39.03±1.54^{B}	52.80±1.28$^{B@}$
Group 4	104.85±0.65^{A}	120.82±0.99$^{A@}$	79.59±3.03^{A}	91.23±2.11$^{A@}$

Group (1): rats fed on bread made from 1kg un irradiated flour/ no adults infection. ;Group (2): rats fed on bread made form 1kg irradiated flour (3kGy)/ no adults infection ;Group (3): rats fed on bread made 1kg irradiated flour/ 70adults infection ;Group (4): rats fed on bread made form 1kg irradiated flour/ 100 adults infection. Means with different letters are significantly different at p<0.05 between groups (one way ANOVA followed by Tukey pairwise comparison test). @ Statistically significant at p<0.05 between 6 and 12 weeks (2 sample *T-test*).

Table 8. Counts of Lymphocyte (10xg/L) and WBCs (10xg/L) RBCs (10x12/L) and Hb% (g/dL) in different rats groups at time interval 6 and 12 weeks after secession of treatments.

Parameters / Groups	WBCs count (10^{x}g/L)		Lymphocytes count (10^{x}g/L)	
	6 weeks	12 weeks	6 weeks	12 weeks
Group 1	3.033±0.18^{D}		57.25±0.86^{D}	
Group 2	4.51±0.13^{C}	61.96±0.98^{C}	61.96±0.98^{C}	65.31±0.97$^{C@}$
Group 3	5.13±0.09^{B}	66.65±0.7^{B}	66.65±0.7^{B}	72.86±0.84$^{B@}$
Group 4	6.28±0.13^{A}	71±0.33^{A}	71±0.33^{A}	78.16±0.61$^{A@}$
Parameters / Groups	RBCs count(10^{x}12/L)		Hb%(g/dL)	
	6 weeks	12 weeks	6 weeks	12 weeks
Group 1	6.5±0.11^{B}		12.2±0.14^{A}	
Group 2	8.27±0.13^{A}	6.76±0.07$^{A@}$	10.88±0.13^{B}	9.91±0.10$^{B@}$
Group 3	5.89±0.1^{C}	4.49±0.11$^{B@}$	10.23±0.09^{C}	9.33±0.12$^{C@}$
Group 4	5.26±0.15^{D}	2.68±0.1$^{C@}$	9.8±0.1^{C}	8.93±0.09$^{C@}$

Group (1): rats fed on bread made from 1kg un irradiated flour/ no adults infection. ;Group (2): rats fed on bread made form 1kg irradiated flour (3kGy)/ no adults infection ;Group (3): rats fed on bread made 1kg irradiated flour/ 70adults infection ;Group (4): rats fed on bread made form 1kg irradiated flour/ 100 adults infection. Means with different letters are significantly different at p<0.05 between groups (one way ANOVA followed by Tukey pairwise comparison test). @ Statistically significant at p<0.05 between 6 and 12 weeks (2 sample *T-test*).

Histopathological Determinations

Figure 2A presented the normal histological structure of control rats' liver (fed on bread made from 1kg of un-irradiated, uninfected flour); the hepatic lobule appeared with normal size. Furthermore, rats feeding on irradiated flour for 1.5 and 3 months showed unremarkable changes in hepatocyte respectively (figure 2 B-C).

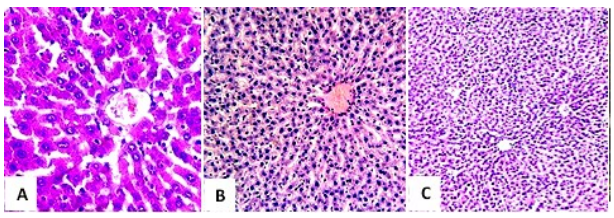


Figure 2. Photomicrographs in liver section of rats. **A:** normal rats liver tissue (H&E ×400). **B:** liver of rats receiving radiated bread for 1.5 months showing unremarkable changes. (H&E ×200). **C:** liver of rat receiving radiated bread for 3 months showing unremarkable changes. (H&E ×100).

Figure 3 presented a liver section of rats fed on bread made from irradiated flour/ infected with 70 adults *T. confusum* at time intervals of 1.5 and 3 months. After 1.5 months, the results showed mild degenerative changes associated with spotty necrosis and inflammatory aggregate (A), mild degenerative changes associated with focal portal inflammatory reaction (↓)(B). While at time interval 3 months, there were some degenerative changes (DC) associated with dilated portal blood vessels (PV) and portal inflammation (↓) and fibrosis (C) while moderates degenerative changes as well as portal fibrosis and inflammation (↓) were associated with cholestasis (stagnation of bile inside bile ductile (bd) and ducts (D).

Data revealed that liver section of rats fed on bread made from 1kg irradiated flour/ 100 *T. confusum* adults infection for 5.9 months showed moderate degenerative changes

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associated moderates portal inflammation (↓) (A) while in (B) showed moderate degenerative changes associated with spotty necrosis and inflammatory aggregate (↓). However, at 3 months period there were marked degenerative changes, dilated congested portal blood vessels (PV) and perivascular inflammatory infiltrates (c) marked degenerative changes and early cirrhotic changes (↔) showing marked degenerative changes and early cirrhotic changes (↔) (D). Early dysplastic changes (▼) (note the anisonucleosis, irregular chromatin pattern and prominent nucleoli (E).

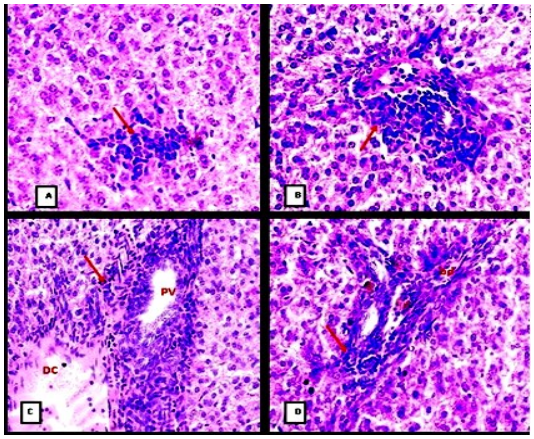


Figure 3. Photomicrographs in liver section of rats fed on bread made from irradiated flour (3KGy)/70 T. confusum adults' infection. For 1.5 month feeding **(A)** showing mild degenerative changes associated with spotty necrosis and inflammatory aggregate (↓); **(B):** mild degenerative changes associated with focal portal inflammatory reaction (↓); **(C):** A liver of rats receiving. While at 3 months showing some degenerative changes (DC) associated with dilated portal blood vessels (PV) and portal inflammation (↓); **(D):** showing moderate degenerative changes as well as portal fibrosis & inflammation (↓) associated with cholestasis (stagnation of bile inside bile ductile (bd) and ducts) (H&E ×400).

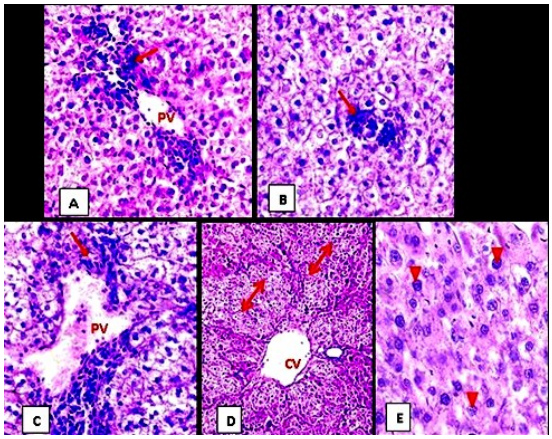


Figure 4. Photomicrographs in liver section of rats fed on bread made from irradiated flour (3KGy)/100 T. confusum adults' infection. For 1.5 month feeding **(A)** showing moderate degenerative changes associated moderates portal inflammation (↓); **(B):** moderate degenerative changes associated with spotty necrosis and inflammatory aggregate (↓). **(C):** liver section of rats feeding on bread made form 1kg irradiated flour/ 100 adults infection at time interval 3 months showing marked degenerative changes, dilated congested portal blood vessels (PV) and perivascular inflammatory infiltrates(↓). **(D):** marked degenerative changes and early cirrhotic changes (↔). (E): early dysplastic changes (▼) (note the anisonucleosis, irregular chromatin pattern and prominent nucleoli). (H&E × 400).

DISCUSSION

Environment pollution is the important problems which were faced by the living organism; therefore, the current study was targeted to reveal the impact of stored flour infestation on the health of beetle and rats. The obtained results indicated that the number of alive adult and the number of larvae was less in irradiated flour than in unirradiated ones and number of dead insects was more in irradiated flour than in unirradiated ones.

In addition, GR and ECI decreased when the insect feed on gamma irradiated flour. Also, the percent weight loss of the flour was more after 12 weeks than 6 weeks and more in non-irradiated flour than in irradiated ones and the antifeedant was increased when the number of insect increased from 70 to 100 adults. These agreed with that obtained by Ahmadi *et al.* [21] who found that gamma radiation significantly affect the nutritional indices of *T. castaneum*. Moreover, Gabarty and Abou El Nour [22] studied the effect of artificially infestation of 3KGy irradiated flour with *Corcyra cephalonica*, *Ephestia kuehniella*, *T. confusum*, they declared that there were major alterations in the insects population and high weight loss and lowering of the nutritional composition of the flour.

The obtained data indicated that the activities of AST and ALT were high in *T. confusum* fed on irradiated flour. This increase may be due to the change in the flour properties after irradiation. Since, Khattak and Klopfenstein

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[23] reported changes in the amino acid profiles of gamma irradiated wheat seeds. Additionally, Haiba and Abd-El Aziz [24] recorded a significant decrease in protein and carbohydrate contents of gamma irradiated potato tubers.

When un-irradiated and irradiated flour were infected with 70 or 100 *T. confusum* adults /25mg and left for 6 and 12 weeks the number of insects changed over this period of time, the number of adults' insects increased and the progeny of larvae appeared which lead to more secretion of BQ. This agrees with that obtained by El-Desouky *et al.* [5] who found that the highest concentration of the benzoquines BQ was secreted by *T*. *castaneum* in the presence of the maximum adult number at 4 months storage time. Also Markarian *et al.* [25] discovered 37% methoxybenzoquinone and 63% ethyl-benzoquinone from *T. castaneum* secretion. Different BQ from *Tribolium* spp. have been previously recognized by Hodges *et al.* [26]; Pappas and Wardrop [27]; Villaverde *et al.* [28].

Our results indicated that weights of rats were significantly decreased of rats fed on flour infected with 100 insects after 1.5 and 3 months and rats lost their hair, this may be due to nutritional insufficiency that influenced both hair structure and hair growth. Impacts on hair growth comprise acute telogen effluvium (TE), a recognized result of surprising weight lack or decline in protein consumption [29].

The oxidative damage in a cell or tissue occurs when the concentration of ROS generated exceeds the antioxidant capability of the cell, this agrees with the result of the present study. Thus, it could be regarded to statistically lower in the antioxidant levels.

GSH subiquitoustri peptides widely distributed in animal tissues which exist in two inter convertible forms either thiol reduced form (GSH) or disulfide oxidized form (GSSG) [30]. Hepatic damages which were found in liver tissue as result of increase in GST activity, it initiates extracellular break down of GSH, provides cell with cysteine supply to increase of GGT, which is associated with increase of free radical production and GSH depletion. Consistent with our result, Salama and Montaser [31] confirmed reaching the same result. The increase in the activity of liver enzymes AST and ALT may be attributed to the damage of liver parenchymal cells and extra hepatic tissues were followed by a release of intracellular enzymes into circulation. The excessive production of free radicals and lipid peroxide might have caused elevation of hepatic enzymes [32]. Additionally, Halder *et al.* [33] showed that leakage of hepatic enzymes AST and ALT and GGT used as biochemical markers for hepato cellular damage and hepatic dysfunction. The hematopoietic tissue is the most toxic-sensitive tissues in the body. Disturbance of the hematological parameters was clear in the data of our study, which may be regarded to oxidative damage of DNA and production of DNA adducts [34, 35]. Also, Abdel-Mageid and Ahmed [36] reported that, the reduction in RBCs could be attributed to diminished ability of blood forming organ to produce their cells or increase in lipid peroxidation of RBCs cell membranes which is in line with our results. Elhassaneen and Abd El-Moaty [37] reported that feeding on infested flour resulted in decline of the protective characteristics of antioxidants accompanied by great plasma oxidants and ROS concentrations.

CONCLUSION

From previously mentioned data, we conclude that rats fed on bread produced from stored flour infested with Tribolium confusum suffered from decreased weight, and loss of antioxidant activity, Hb and RBC. Biochemically, it increased lipid peroxidation, GGT and liver enzymes of rats accompanied by hair loss and histological alteration of the liver. Therefore, we must protect flour away from pest infestation.

Authors' contributions

All authors designed the study. SAR, RMS, RSA and TSE performed the experimental works of the insect and infestation of the flour. SII achieved the experiments on the rat and wrote its part in the manuscript. RMS analyzed the data. SAR wrote the part of the insect. All authors revised and approved the manuscript.

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***Conflicts of interest*:** *Declared none.*

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