Morphological and Biochemical Changes in Male Rats Fed on Genetically Modified Corn (Ajeeb YG)

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Abstract : This study was designed to evaluate the safety of genetically modified (GM) corn (Ajeeb YG). Corn grains from Ajeeb YG or its control (Ajeeb) were incorporated into rodent diets at 30% concentrations administered to rats (n= 10/group) for 45 and 91 days. An additional negative control group of rats (n= 10/group) was fed AIN93G diets. General conditions were observed daily, total body weights were recorded weekly. At the termination of the study periods, some visceral organs (heart, liver, kidneys, testes and spleen) and serum biochemistry were measured. The data showed several statistically significant differences in organs/body weight and serum biochemistry between the rats fed on GM and/or Non-GM corn and the rats fed on AIN93G diets. In general, GM corn sample caused several changes by increase or decrease organs/body weight or serum biochemistry values. This indicates potential adverse health/toxic effects of GM corn and further investigations still needed. Journal of [Gab-Alla, A. A., El-Shamei, Z. S., Shatta, A. A., Moussa, E. A., and Rayan, A. M. **Morphological and Biochemical Changes in Male Rats Fed on Genetically Modified Corn (Ajeeb YG)**. *J Am Sci* 2012;8(9):1117-1123]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 152

Key words: organs/weight; GM corn; serum biochemistry; rats

1. Introduction

Ajeeb YG (YieldGard corn, event MON-00810-6) is a genetically modified insect resistant corn produced by incorporated the MON 810 (Monsanto) borer resistance trait in the best corn germplasm "Ajeeb" (a trade mark of Dekalb). For the last decades there has been a growing interest from the food crop industry to construct and produce genetically modified (GM) crops with the primary goal to significantly increase the yield and avoid the use of pesticides. At present, GM crops are grown and consumed by humans in many countries, for example corn expressing the insecticidal genes (*cry* genes) from *Bacillus thuringiensis* (Bt) (Eizaguirre et al., 2006).

An important requirement in toxicological experiments is the ability to assess the effects of xenobiotics (a chemical which is found in an organism but which is not normally produced or expected to be present in it) on specific organs. For many organs, this is done through macroscopic examination of the organs, measuring organ weight, and histopathological examination of the tissue. Organ weight can be the most sensitive indicator of an effect of an experimental compound, as significant differences in organ weight between treated and untreated (control) animals may occur in the absence of any morphological changes (Bailey et al., 2004). Biochemical analyses are useful in chronic toxicity studies because in-vivo effects of clinical treatments are reported. When certain types of cells are damaged, they may leak enzymes into the blood, where they can be measured as indicators of cell damage (Rochling, 2001). Dietary changes may lead to series of reactions which can cause disruption of normal physiological activity bringing changes in biochemical constituents of the body fluid of test animals (Schilter et al., 2003). Blood biochemical screening is a useful indicator for nutritional research. It is supportive for more accurate and reliable diagnosis of various physiological disorders and can be comprehensively interpreted by correlating with other nutritional parameters (Singh et al., 2002). Clinical pathological evaluation is being used as one of the safety assessment tools when some novel food sources are exploited for their appraisal as safe human food ingredient (Malley et al., 2007). Liver and kidney function test along with serum contents were used to evaluate safety of novel foods (Farag et al., 2006). Serum protein and albumin are often used for the 86 evaluation of the protein (Jung et al., 2003). Physiological responses of growing rats can be useful tool to indicate food safety and quality of test diet (Olivera et al., 2003).

Some studies did report some serum chemistry changes due to GM foods utilization these includes (Hammond et al., 2004) they observed a few statistically significant differences between rats fed on GM corn (MON 810) and its control. The authors decided that these differences not considered being test article related as they were of small magnitude and fell within ±2SD of the mean of the reference groups. The slight reduction in A/G (albumin/globulin) ratio for high dose MON 810 males was attributed to the slightly lower albumin and slightly higher globulin levels for MON 810 males, neither of which were individually statistically different from controls. The A/G ratio for MON 810 males also fell within ±2SD of

the mean (1.79 ± 0.34) of the reference groups even though it was slightly, but statistically significantly lower that both the control and reference groups. There were no differences in A/G ratio for females. The authors stated that this finding was not considered to be test article related. The values of AST (aspartate aminotransferase) and Glu (glucose) in rat male group treated with transgenic *Bacillus thuringiensis* (1000 mg/kg body weight) were significantly lower than control group, while, in (5000 mg/kg body weight) female group, the ALP (alkaline phosphatase) was significant higher (Peng et al., 2007) . Also, the author declared that AST and ALT (Alanine aminotransferase) are considered to be sensitive indicators of hepatocellular damage and within limits can provide a quantitative assessment of the degree of damage sustained by the liver. Except for AST, there were no significant alterations in other liver function parameters including total protein, bilirubin, and other liver enzymes including alkaline phosphatase and ALT. Some parameters of serum biochemeistry of rats such as albumin and total protein increased while other parameters like AST, ALT, ALP, urea, urea nitrogen and uric level of female serum samples increased compared to the control group and male groups and the authors attributed that to individual alterations and diet formulations (Kilic and Akay, 2008). Several statistically significant differences in serum chemistry values were between rats consuming AIN93G diets and those formulated with 59122 and 091 control maize grain (He et al., 2008). However, the author attributed these differences to the formulation of diets containing higher concentration of maize grain than the AIN93G diet rather than being attributable to consumption of 59122 maize grains. Moreover, Appenzeller et al. (2009) found statistically significant differences for two clinical chemistry response variables between female rats consuming the 1507×59122 diet compared with female rats consuming the 091 control diet. Mean serum sodium concentration (NA) was lower ($p < 0.05$) in female rats consuming the 1507×59122 diet compared with the 091 control diet. However, this statistical difference was not considered biologically significant or related to exposure to the test substance.

Consumer concerns regarding GM products relate mostly to unanticipated health effects that may arise from direct consumption of GM products or products from animals fed GM ingredients (Malarkey, 2003; Dona and Arvanitoyannis, 2009 and Martinez-Povida et al., 2009). Therefore, this study is aimed to measure some vital organs/body weight and serum biochemistry of rats as parameters to evaluate the safety of genetically modified corn (Ajeeb YG).

2. Materials and Methods

2.1. Plant materials

Transgenic corn sample (Ajeeb YG) and its near-isogenic line (Ajeeb) were obtained from the agricultural administration, Hehia, Sharkia governorate, Egypt. The Cairo based company Fine Seed International is partnering with Monsanto to distribute the variety in Egypt.

2.2. Diet formulation

Flours from Ajeeb YG and Ajeeb corn grains were formulated into rodent diets at concentration of 30%. These diets were produced in accordance with AIN93G guidelines (Reeves et al., 1993). An additional AIN93G grain-based diet was included as negative control. Mixing ratios of standard and experimental diets are given in Table (1).

Table 1. Ratios of standard and experimental diets (%)

Groups	Diets (%)		
	Ajeeb YG(GM)	Ajeeb (CO)	AIN93 (ST)
Group I	0	0	100
Group II	0	30	70
Group III	30	0	70

2.3. Animals and housing

Thirty male apparently healthy rats, approximately three-weeks of age with an average body weight of 45 ±5 g were obtained from the Research Institute of Ophthalmology (Giza– Egypt). All animals were housed individually with ad libitum access to water and commercially obtained AIN93G feed. Animal rooms were maintained at a temperature 22 ± 2 ºC and relative humidity of 40–70%, with a 12 h light/dark cycle. Rats were acclimatized for 5 days with AIN93G control diet and then divided into treatment groups randomly as 10 rats/group with mean body weights across each group not varying more than 10%. Experimental groups were fed three different diets, first group of rats were fed AIN93G diet as an additional negative control; referred as standard group (ST group). The second group of rats fed diet formulated with 30% (wt/wt) Ajeeb corn grains; referred as control group (CO group). The third group of rats fed diet formulated with 30% (wt/wt) Ajeeb YG; referred as genetically modified diet (GM group).

2.4. Body and organ weights

Body weight was recorded weekly. Organs weight was measured at two study intervals, after 45 and 91 days.

2.5. Biochemical analyses

Blood samples were collected under light ether anesthesia from the medial canthus of the eye of five rats out of each group on days 45 and 91. The blood samples were allowed to coagulate and were centrifuged at 3000 rpm for 5 min. Sera obtained following centrifugation were assayed for aspartate

aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, uric acid, total lipid (TL), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), creatinine (CREA), total protein (TP), albumin (ALB) and globulin (GLOB). All these determinations were carried out using laboratory kit reagent (Bio-diagnostic, Giza, Egypt) as described by Ode et al. (2011). The reaction products were read using spectrophotometer (model 6505 UV/Vis, JENWAY, UK) and from the obtained data, some other parameters were calculated as follows:

Globulin = Total protein – Albumin,

VLDL (mg / dl) = (Triglycerides / 5),

LDL (mg / dl) = (Total cholesterol - HDL - VLDL)

2.7. Statistical Analysis

Statistical analysis was carried out on the entire data collected using one-way ANOVA and Duncan New Multiple Range post hoc test from SPSS 16.0 package to compare the mean values of the test groups with the controls.

3. Results and Discussion

3.1. General signs in the rat

No rat mortality among ST and CO groups during the experiment durations. In GM group, only one rat was died at the seventh week.

3.2. Body Weights

The body weight is a parameter considered as a very good predictor of side effects in various organs (Séralini et al., 2011). Figure (1) demonstrates the weights of rats fed on standard diet (ST group), control diet (CO group) and genetically modified diet (GM group). The data showed that there were no significant differences ($P<0.05$) between the examined three groups from the beginning of the experiment until the seventh week. After that the weights of rats from GM group showed decreases comparing with the ST and CO groups. The reduction in weight gain compared with control may not be due to an adverse effect per se, but due to poor dietary palatability or a nutritionally poorly balanced diet due to incretions incorporation of the test material in the animal feed (Abdullah, 2008). In the same field, some GMOs (Roundup tolerant and MON863) affect the body weight increase at least in one sex (Séralini et al., 2007 and Zhu et al., 2004). In another study**,** Séralini et al. (2007) found that the weights of rats fed with MON 863 varied significantly when compared to the control groups. Female weight increased by 3.7%, while male weight decreased by 3.3%. These weight changes could be indicative of organ dysfunction.

3.3. Organs weights

Relative organ weights of male rats after 45 and 91 days of feeding are given in Table 2 and 3, respectively. After 45 days of feeding, no differences were seen in the relative weight of heart for CO and GM groups (Table, 2) whereas, there were significant differences found between the same two groups after 91 days of feeding (Table, 3).

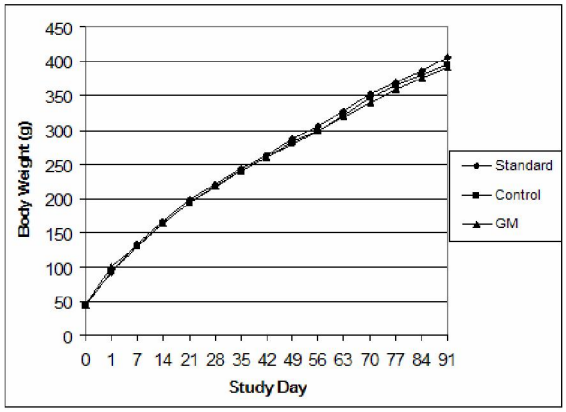

Fig (1): Mean weekly body weights of male rats. Rats were fed on experimental rodent diets containing one of three corn grain sources for at least 91 consecutive days. Non-GM commercial reference corn (standard diet, ST group). Ajeeb, near-isogenic (control diet, CO group) and Ajeeb YG, transgenic corn (genetically modified, GM diet).

Otherwise, kidney weights differed in both period of study as this of GM group was higher than those of ST and CO groups (Table 2, 3). The liver weight of the three groups after 45 days of feeding were not differed significantly (Table, 2) but after 91days of study, liver of GM group was significantly higher than ST and CO groups (Table, 3). With respect to spleen weights, values of GM group showed significant differences than those of ST and CO groups after 45 and 91 days of feeding (Table 2, 3). Finally, testes from GM group after 45 days feeding showed lower weight values than those of ST and CO groups (Table, 2). However, the data in Table (3) showed that no significant differences were found in testes weights after 91 days feeding between ST, CO and GM groups.

In the same aspect, the data obtained from 19 animal studies by Séralini et al. (2011), showed that consuming GM corn or soybeans leads to significant organ disruptions in rats and mice, particularly in livers and kidneys. In addition they found other organs may be affected too, such as heart and spleen, or blood cells. The kidneys of males fared the worst, with 43.5% of all the changes, the liver of females followed with 30.8%. For instance, they reported that individual kidney weights of male rats fed with the 33% MON863 diet were statistically significantly lower compared to those of animals on control diets. The authors pointed out that livers and kidneys "are the major reactive organs" in cases of chronic food toxicity.

3.4. Serum biochemistry

A number of statistically significant differences in serum chemistry values were observed between rats consuming standard diet and those formulated with GM corn and control corn grains after 45 and 91 days of feeding (Table 4, 5).

The mean serum total protein, albumin and globulin concentrations were significantly differed, especially between CO and GM groups after 45 days of feeding (Table, 4). After 91 days of feeding on different experimental diets, there were no significant differences observed for total protein and globulin values between the groups. However, from Table (5) there were significant differences have been found in albumin values between CO and GM groups.

Table 2. Organ/body weight ratios of rats after 45 days of feeding (mean*100 ± SD).

	Groups		
Organ	**ST**	**CO**	**GM**
Heart	0.2836 ±0.002[b]	0.3547±0.008[a]	0.3546±0.004[a]
Kidney	0.7093 ±0.005[b]	0.7049±0.009[b]	0.8040±0.047[a]
Liver	3.512 ±0.364[a]	3.413±0.061[a]	3.474±0.109[a]
Spleen	0.2152 ±0.004[b]	0.2158±0.002[b]	0.3075±0.043[a]
Testes	1.185 ±0.037[a]	1.115±0.117[a]	0.8278±0.152[b]

*The same letter in the same row is not significant different (P<0.05).

Table 3. Organ/body weight ratios of rats after 91 days of feeding (mean*100 ± SD).

	Groups		
Organ	**ST**	**CO**	**GM**
Heart	0.3935 ±0.017[b]	0.3643±0.003[b]	0.4442±0.038[a]
Kidney	0.8521 ±0.103[b]	0.8521±0.042[b]	1.488 ±0.064[a]
Liver	2.745 ±0.140[b]	2.630 ±0.137[b]	3.332 ±0.027[a]
Spleen	0.2367 ±0.013[a]	0.2277±0.010[a]	0.1992±0.002[b]
Testes	1.2079 ±0.311[a]	1.238 ±0.115[a]	1.491 ±0.070[a]

*The same letter in the same row is not significant different (P<0.05).

In general, total protein and albumin concentrations of rats from GM group were lower than that of ST and CO groups. Dhanotiya (2004) observed liver damage, hepatitis and cirrhosis of the liver, reduced albumin synthesis and overall decreased plasma protein. Decreased plasma protein was also found in nephrosis, and massive albuminuria in which protein was lost in the kidneys.

Serum AST concentrations of rats from GM group was higher than those for ST and CO groups, but AST of ST group displayed significantly different than CO and GM group (Table, 4). On the other hand, after 91 days of feeding there were no significant differences were observed between the groups (Table, 5). The amount of AST in the blood is directly related to the extent of tissue damage (Rochling, 2001).

Concentrations of ALT and ALP in rats consuming diet formulated with GM corn were higher than the values observed in ST and CO groups (Table, 4). The ALT concentrations of rats from GM group were significantly lower than those from ST and CO groups (Table, 5). Mohamadnia et al. (2010) reported that the increase in the levels of ALT or AST or both may occur in situations of common bile duct stone e.g. transient biliary obstruction; medications e.g. acetaminophen; common liver disease causes e.g. alcohol abuse, cirrhosis, hepatotoxins, viral hepatitis, steatohepatitis (fatty liver); uncommon liver diseases e.g. autoimmune hepatitis, hemochromatosis, alpha-1-antitrypsin deficiency and Wilson's disease.

Additionally, compared to the ST and CO groups, mean serum total lipid, triglyceride and cholesterol concentrations were high in rats from GM group. The values were statistically different between the three groups, but the differences are clearly noticed between CO and GM groups. Similarly, mean serum total lipid and cholesterol concentrations were high in rats from GM group, while triglyceride concentration of GM group was lower than those for ST and CO groups (Table, 5). Rai et al. (2009) attributed the elevation in total serum cholesterol level to blockage of liver bile ducts causing reduction or cessation of its secretion to the duodenum subsequently causing cholestasis.

Significant differences were found in HDL values between the examined groups after 45 days of study, but the rats from GM group showed lower value than those for ST and CO groups (Table, 4). After 91 days of feeding on different experimental diets, there were significant differences for HDL concentration between the examined groups. In general the value of GM group was the lowest (Table, 5).

Table 4. Serum chemistry values of rats after 45 days of feeding (mean ± SD).

Response variable	Group		
	ST	CO	GM
TP (g/dl)	5.395 ±0.218[b]	6.489±0.297 [a]	5.372 ±0.259[b]
ALB (g/dl)	3.323 ±0.017[a]	2.978±0.092[a]	2.689 ±0.106[b]
GLOB (g/dl)	2.072 ±0.442[b]	3.511±0.380[a]	2.682 ±0.215[b]
AST (U/ml)	29 ±1[b]	40.5 ±0.5[a]	43 ±2[a]
ALT (U/ml)	9.5±0.5[b]	10±0[ab]	10.5±0.5[a]
ALP (U/l)	167.23 ±14[b]	116.76±0.83[c]	219.39±14.32[a]
TL (mg/dl)	204.54 ±26.22[a]	143.19±27.97[b]	227.62±11.54[a]
Triglyceride (mg/dl)	18.08 ±0.953[c]	20.66 ±1.37[b]	24.79±0.551[a]
Cholesterol (mg/dl)	49.65 ±3.7[b]	52.69±1.64[b]	79.629±2.34[a]
HDL (mg/dl)	19.41 ±0.485[a]	19.272±0.875[a]	17.61 ±1.29[a]
VLDL (mg/dl)	3.616 ±0.190[c]	4.131±0.275[b]	4.958±0.110[a]
LDL (mg/dl)	26.619 ±3.226[b]	29.285±2.168[b]	57.059±1.216[a]
Uric acid (mg/dl)	0.789 ±0.041[b]	0.955±0.152[b]	2.767±0.089[a]
Urea (mg/dl)	39.012 ±8.714[b]	41.403±4.269[b]	57.546±16.004[a]
Creatinine (mg/dl)	0.449 ±0.016[b]	0.474±0.008[b]	0.549±0.016[a]

*The same letter in the same row is not significant different (P<0.05).

Table 5. Serum chemistry values of rats after 91 days of feeding (mean ± SD).

Response variable	Group		
	ST	CO	GM
TP (g/dl)	6.371±0.218[a]	6.129±0.622[a]	5.906 ±0.235[a]
ALB (g/dl)	2.886±0.085[a]	2.929±0.146[a]	2.561 ±0.263[b]
GLOB (g/dl)	3.484±0.236[a]	3.200±0.714[a]	3.346 ±0.267[a]
AST (U/ml)	40.666 ±0.577[a]	41.333±0.577[a]	42±1.000[a]
ALT (U/ml)	9±1.00[c]	12±0.00[b]	14±0.333[a]
ALP (U/l)	110.33±15.55[b]	75.942±4.713[c]	134.849 ±13.261[a]
TL (mg/dl)	169.58 ±15.73[b]	229.02±8.74[a]	253.496±33.217[a]
Triglyceride (mg/dl)	20.386±0.549[c]	26.967±1.457[b]	34.159 ±2.75[a]
Cholesterol (mg/dl)	86.650 ±1.87[b]	118.03±3.278[a]	122.88±2.646[a]
HDL (mg/dl)	22.832±0.898[a]	19.527±0.115[b]	18.608±0.607[b]
VLDL (mg/dl)	4.077±0.110[c]	5.399±0.291[b]	6.832 ±0.551[a]
LDL (mg/dl)	59.741 ±2.068[c]	93.105±3.178[b]	97.440 ±2.670[a]
Uric acid (mg/dl)	1.423 ±0.139[b]	1.425±0.108[b]	1.631±0.110[a]
Urea (mg/dl)	36.840 ±8.714[b]	38.47±2.263[b]	52.026±4.575[a]
Creatinine (mg/dl)	0.402±0.007[b]	0.474±0.008[b]	0.549±0.016[a]

*The same letter in the same row is not significant different (P<0.05).

From Table (4) it can be seen that after 45 days of feeding, the concentrations of VLDL and LDL for CO groups were lower (P<0.05) than that of GM group. Similarly, there were significant differences between all groups for values of VLDL and LDL after 91 days of study (Table, 5). Adamu et al. (2008) stated that any significant alteration of lipids in their plasma levels could lead to a variety of clinical disorders in the affected animals.

As uric acid and urea and creatinine reflecting renal function, statistical differences were observed between mean uric acid, urea and creatinine values of ST and CO rat groups compared to rats consuming the GM diet after 45 days of study (Table, 4). In the same manner, after 91 days of feeding, there were statistically differences were observed between mean uric acid, urea and creatinine values of rat from ST and CO groups compared to rat from GM group (Table, 5). Our results were in accordance with those obtained by de Vendômois et al. (2009) who observed that the deficiency in kidney function in male rats is different between animals fed NK 603 and MON 863 genetically modified corn. This is characterized by an increase in plasma creatinine levels and retention of ions, which were associated with a chronic interstitial nephropathy, as originally admitted in the Monsanto MON 863 (Hammond et al., 2006).

Conclusion

The results of this study showed several changes in organs/body weight and serum biochemistry in the rats fed on GM corn. These findings indicate potential adverse health/toxic effects of GM corn and further investigations still needed.

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