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# The Pathophysiological Effects of Acrylamide in *Albino Wister* Rats

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**ABSTRACT**

*Studies of the pathophysiological effects of suspected compounds are conducted in rodent species, especially rats and mice, to determine the potential toxic effects of a particular compound. In the assessment of acrylamide (ACR) which is available as a dietary compound in daily food stuffs, the potential toxicity was determined following the method described earlier. In this study, Albino Wister rats were used and were observed for clinical abnormalities, changes in food consumption, and symptoms of toxicity over a period of two months following the oral administration of ACR. Among the parameters used to assess the effect of ACR were include ovarian histopathology, blood sugar, haemogram and lipid profile. The most notable clinical abnormalities observed in a few rats were a rough coat and decreased activity. None of the rats died or showedbehavioural change resulting from treatment with ACR. The concentration of serum biochemical parameters and haemogram showed significant differences between normal and treated rats. Histological examination of the ovaries of the treated rats showed great abnormalities as well. In fact, oral ACR doses are practically toxic with regard to rats after exposure for two months at a dose rate of 30 mg/kg, suggesting the compound is quite non-innocuous.*

**Key Words:** Acrylamide, lipid profile, ovarian histopathology, haemogram, pathophysiology study

## INTRODUCTION

Determination of ovarian toxicity is very important for the evaluation of the safety of drugs and chemicals, because any abnormities in the ovaries indicates an impairment of the female reproductive capacity. Results from morphological analysis of the ovaries, reported that any deviation in the morphology of the follicles and corpus luteum resulting from a synchronized combination might represent a first indicator of ovarian toxicity [1].

Acrylamide (ACR) is a very small, hydrophilic molecule. This means that it is easily transported throughout the body by passive diffusion [2]. Therefore, all tissues and organs are theoretically a target for ACR, including ovaries.

Until 2002 it was believed that ACR exposure was only occupational and, to some extent, due to cigarette smoking, water and cosmetics. In 2002 however, the Swedish National Food Agency reported the presence of ACR in widely consumed food items such as biscuits, cereals, bread, potato chips, and any foodstuffs involving Maillared reaction asparagines and sugar heated to more than 200ºC [3].

The cellular toxicity of ACR is initiated when it is bio-transformed into a more potent and highly reactive molecule. Furthermore, the oxidative biotransformation of ACR by cytochrome P450 2E1 (CYP2E1) is the most important pathogenic pathway, resulting in glycidamide metabolite which is more reactive towards proteins, including hemoglobin and DNA, than ACR itself [4, 5].

Studies have shown positive dose-response relationships between acrylamide exposure and cancer in several hormone-sensitive organs such as mammary glands and the uterus [6-9].Some data suggests that the exposure of females may contribute to reproductive toxicity, decreases in fertility, as well as changes in pre- and post-implantation losses. This has been recorded in both mice and rats, at doses 15 mg/kg/day or greater [10, 11].

Since ACR is present in a wide range of food items, the general population is at a high risk of exposure through their diet. Usually, toxicity tests are first done on animal models, typically rodents. These tests provide information with regard to the onset of toxic signs and the recovery of the survivors. This information is extremely critical because of the potential of humans and animals to be exposed to compounds claiming to be therapeutic, but in fact which have inherent toxic effects [12, 13].

Many studies have been conducted on the effect ACR on the male reproductive system, but little is known about their effect on females. Accordingly, this study has been performed to evaluate the effect of various doses of ACR on the ovary histopathology in female albino rats, in addition to testing some of the hematological parameters and lipid profiles of the experimental groups.

## MATERIALS AND METHODS

**Animals:** The study was performed in the Faculty of Education, School of Science, Biology Department animal house under standard laboratory conditions, 12:12 light/dark photoperiod at 23 ± 2ºC. Twenty four Albino Wister rats weighing 280-300 g and aged6-8 weeks were kept in polystyrene plastic cages. They were acclimated for about one week prior to the start of the experiment. The animals had ad libitum access to water and diet through the study.

**Experimental Design:** ACR was purchased from Sigma Aldrich and prepared in $dH_2O$, pH 7.0, at room temperature.

The animals were randomly divided into four groups, each with 6 rats. These were group 1: control group, rats were given distilled water only; group 2: low dose ACR, rats were given 2 mg/kg body weight; group 3: mild dose ACR, rats were given 10 mg/kg body weight; group 4: high dose ACR, rats were given 30 mg/kg of body weight. The applied dose was selected according to the earlier study performed by Tyl and Friedman[14].

ACR and distilled water were given orally once a day using a force-feeding needle for exactly eight weeks. After that period, the animals were anesthetized and blood was collected. Serum from non-heparinized blood was used for blood sugar and lipid profile tests (cholesterol, triglyceride, HDL and LDL) using standard kit and measured using an auto-analyzer (LISA 200, France). On the other hand, heparinized blood was prepared in order to measure some hematological parameters such as RBC, MCV, HCT%, HGB, WBC, PLT, MCH, MCHC using an automated hematocoulter (Beckman Coulter, Germany).

In addition, the ovaries were collected, fixed in 10% formalin; routinely processed and sectioned at 4-5 mm thickness. The obtained tissue sections were mounted on glass slides, deparaffinized and stained with Hematoxylin and Eosin stain(Thermo Fisher Scientific, USA)following the standard method. The sections were then examined and observed under a light microscope at 100X and 400X magnifications (Leica, Germany).

**Statistical Analysis**

Statistical analysis of the obtained data was performed using SPSS (Version 16) (SPSS Inc., Chicago, USA). The results were expressed as mean ± SD using an independent paired t-test. The probability values of less than alpha 0.05 ($P<0.05$) were considered to be statistically significant.

## RESULTS

**Hematological Analysis:** Results from the RBC count showed no significant differences between the control and tested groups. Similarly, there were no significant differences between the control and low dose group in MCV, whereas, MCV in the mild dose group was significantly lower than in the control. Likely, no significant differences were recorded between the control and low dose groups in terms of HCT%, but the mild dose group was significantly lower than the control (Table 1).

**Table 1:Mean values and standard errors for some hematological parameters in control, low and mild dose groups with significant differences*p⧏0.05**

| Parameters | Control | Low dose (2mg) | Mild dose (10mg) |
|---|---|---|---|
| RBC*$10^{12}$/l | 6.1886±0.09041 | 6.0429±0.12478 | 6.3886±0. 07392 |
| MCV*fl | 61.2143 ±2.99559 | 55.0571 ±0.37152 | 53.9429 ±1.41284 *↓ |
| HCT % | 39.7286±0.61556 | 40.4000±0.95668 | 37.8571±0.47352 *↓ |
| HGB g/dl | 13.750±0.27295 | 14.3500±0.14083 | 13.5667±0.13824 |
| WBC*$10^{9}$/l | 7.4000 ±0.20702 | 11.0000 ±0.36187 *↑ | 8.8429±0.16454 *↑ |
| PLT*$10^{9}$/l | 5.3814 ±2.00510 | 4.3829 ±2.35750 *↓ | 5.2414 ±1.33503 *↓ |
| MCHpg | 20.2429±0.38101 | 19.7000±0.14639 | 19.1286±0.24173 *↓ |
| MCHC g/dl | 34.7714±0.22008 | 34.8714±0.23575 | 36.1429±0.21791 |

There was no significant difference in terms of HGB levels when comparing the control with the low and mild dose groups. On the other hand, the WBC count was significantly higher in both low and mild dose groups compared with the control group. Different from WBC, the PLT counts in both low and mild groups were significantly lower than in the control group. However, there wasn't any significant difference between the control and low dose groups in regarding MCH, but there were significant decreases in the mild dose group compared with the control group. No significant differences were recorded in MCHC when comparing the control group with both the low and mild dose groups.

**Blood Sugar and Lipid Profiling:** With regard to blood sugar levels, there were significant increases in the low, mild and high dose groups compared with the control group. Similarly, cholesterol levels were significantly higher in both the low and mild groups compared with the control group, but there were significant decreases in cholesterol levels in the high dose group compared with the control group. In spite of significant increases in the triglyceride level of the low dose group compared to the control group, there were significant decreases in both the mild and high dose groups compared with the control group. On the other hand, HDL levels in each of the low, mild and high dose groups were significantly lower as compared with the control group. However, LDL levels in both the low and mild dose groups were recorded as showing significant increases when compared with the control group and there were no significant differences between the control and the high dose group in LDL levels (Table 2).

**Table 2:Mean values and standard errors for blood sugar and some lipid profile in control, low and mild dose groups with significant differences*p⧏0.05**

| Parameters | Control | Low dose (2 mg) | Mild dose (10mg) | High dose (30mg) |
|---|---|---|---|---|
| Blood sugar | 157.67±4.51418 | 300.67±4.68093 *↑ | 200.67 ±1.40633*↑ | 212.00 ±2.63312*↑ |
| Cholesterol mg/dl | 81.6667 ±1.40633 | 121.33 ±1.05409*↑ | 104.00±3.46410*↑ | 61.6667±1.05409 *↓ |
| Triglyceride | 74.8333±1.57938 | 92.1667±1.83333*↑ | 40.5000±2.26201 *↓ | 45.6667±0.21082 *↓ |
| HDL | 52.1667±0.83333 | 44.5000±1.05672 *↓ | 46.1667±0.47726 *↓ | 34.6667±0.55777 *↓ |
| LDL | 19.1667±1.35195 | 36.0000±0.77460*↑ | 43.3333±1.28236*↑ | 19.3333±1.11555 |

**Histopathology:** The histopathological section of ovaries from the control negative group showed a normal texture that contained follicles at different stages of development. Ovaries treated with low doses of ACR showed the apparent regression of follicles with fluid filled cavities (black arrows), whereas ovaries treated with mild doses of ACR showed an obvious reduction in the number of follicles (yellow arrow). On the other hand, ovaries treated with

high doses of ACR displayed a marked reduction and the regression of ovarian follicles, together with the atrophy of the ovary and atretic follicle (red arrow) (Figure 1).

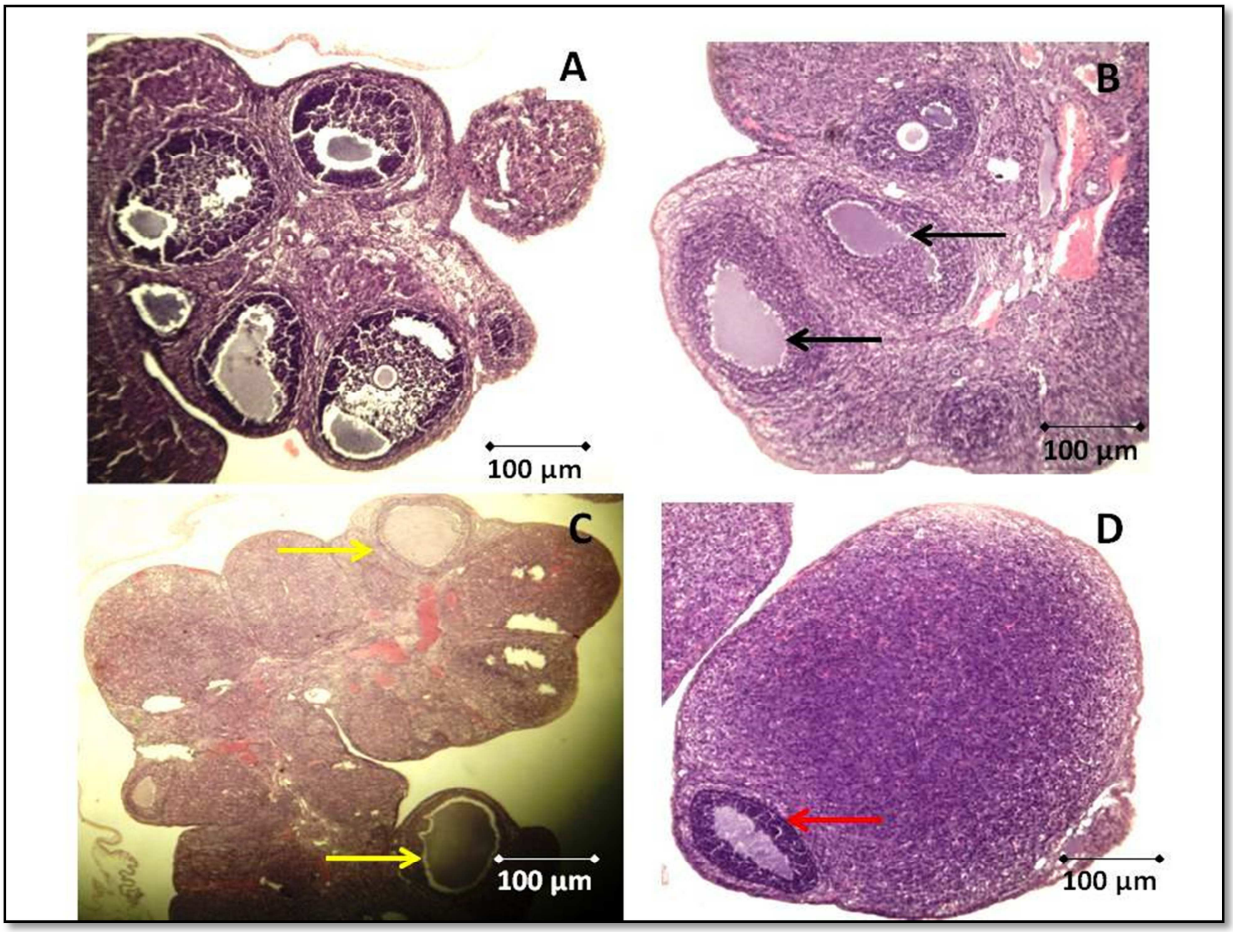


**Figure 1: Histopathological section of ovaries from Albino *Wister* rat stained with H & E illustrate: (A) Control negative showing normal texture that contains follicles at different stages of development, (B) Ovaries treated with low doses of ACR, showing apparent regression of follicles with fluid filled cavities (black arrows), (C) Ovaries treated with mild doses of ACR showing obvious reduction in the number of follicles (yellow arrow), and (D) Ovaries treated with high doses of ACR displaying a marked reduction and the regression of ovarian follicles, together with the atrophy of the ovary, in the given section showing only one atretic follicle (red arrow). Scale bars; 100 µm**

## DISCUSSION

The experimental screening method is essential in order to establish the assessment toxicity and efficacy of ACR. In the current study, ACR causes significant increases in the levels of total cholesterol, LDL in both low and mild doses, and similar effects on the triglyceride level in low doses of ACR as compared with the control group. Since lipid metabolism, such as production of triglyceride and cholesterol synthesis, takes place partially in the liver and it has been documented that ACR causes liver damage [15, 16], this leads to an increase in the synthesis of plasma lipoproteins and high mobilization of lipids from the liver. Consequently, the results can be explained by the ACR involvement in lipid peroxidation [17]. Low triglyceride at mild and high ACR doses seems to be due to the inhibition of the production of cAMP, resulting from low levels of insulin caused by ACR intake, followed by the inhibition of both cAMP dependent protein kinase activity and triglycerol decomposition [18]. Similar results have been recorded by Hammad [15] using 10mg and 30mg ACR for 6weeks and El –Shafey [19]using 50mg/kg for 5 days. Data from a number of investigators agree with the results of the present work [15, 20, 21]. Moreover, Teodor with co-workers[17] reported similar results for total cholesterol, LDL and triglyceride using 50 mg/kg once a day for 10 days.

Most of the hematological parameters showed no significant changes in the groups treated with ACR compared with the control group. However, at mild doses of ACR, the levels of MCH, MCV, platelets, and HCT were significantly lower than that found in the control group. This could be described as microcytic hypochromic anemia [22]. Anemia is mediated by cytokines produced by inflammatory cells, which lead to decreases in iron availability. Microcytic hypochromic anemia mainly results from iron deficiency [23]and, to some degree, results from chronic disease[24]. Microcytic hypochromic anemia results from a depressed response to erythropoietin. However, this mechanism may be important in chronic renal failure, which might happen as a consequence of ACR administration[16]. On the other hand, inflammation causes a change in the dynamics of iron recirculation: iron is retained in the reticuloendothelial system (the so-called reticuloendothelial block) rather than being released to developing red cells in the marrow [24]. Tarskikh (2006) reported that ACR intake leads to decreases in erythrocyte membrane acid resistance and activate lipid peroxidation[25]. Consistent with the current results, Ali*et al.* (2014) and Osman *et al*. (2016) reported significant decreases in HCT [20, 26].

Total WBC were significantly higher than in the control group, indicating immune system activation, reflecting the incidence of inflammation. Similar results have been reported by Ghorbel *et al*.[27], and there have been statistically non-significant increases in WBC count recorded by Jin with co-workers[28].

Totani*et al*. (2007) and Gabr*et al*. (2010) reported low serum insulin levels in rats which had taken ACR, which could be the reason for the high blood sugar levels in the low, mild and high ACR dose groups of the current study[29, 30].

Histopathological study of ovaries from Albino *Wister* rats stained with H & E illustrated that there are no obvious changes in the normal texture of the control negative group and ovaries containing follicles at different stages of development. At the same time, in the mild ACR dose group, the ovaries showed an obvious reduction in the number of follicles. The high ACR dose group revealed a marked reduction and regression of ovarian follicles, together with the atrophy of the ovary and atretic follicles. On the other hand, ovaries treated with low doses of ACR showed an apparent regression of follicles with evidence of fluid-filled cavities.

Recently Saleh et al. [31]revealed that the testes of acrylamide-fed rats showed decreased number of seminiferous tubules containing mature sperms and degenerative changes in sperm germ cell layers. Some sperms of epidermal cauda showed head deformity. Additionally, same researchers demonstrated that in female, acrylamide induced cystic ovarian changes, degenerative changes of zona pelluida, granulosa cells and oocytes. Post implantation loss and decrease in the number of full term fetuses also detected. They also found that resorption sites showed necrotic fetal tissue with vacuolation of amniotic cells.

## CONCLUSION

In conclusion, the overall results of this study clearly demonstrate that the oral administration of ACR of various and chronic doses (over 2 months' duration) induced some behavioral alterations, changes in lipid profile, blood sugar and haemogram, with the regression of active ovaries and a significant adverse effect on the experimental animals, indicating that ACR offers a potential toxicity profile and health threatening features that indicate that ACR should be inhibited for consumption in humans.

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