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Investigations on Hematotoxic and Nephrotoxic Analysis in Aniline Induced Wistar Rats.

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Abstract-

The Nephrotoxic and Hematotoxic risks of aniline have been identified and evaluated in the current investigation using male Wistar rats. Aniline was administered orally to Group A rats for 30 days using gavage at a dose of 20 mg/kg body weight per day, while Group B rats received standard saline water as treatment. After the completion of treatment, the male Wistar rats were sacrificed for specialized tests. Serum biomarkers of kidney injury, creatinine and blood urea increased their concentration. Sodium and potassium concentrations decreased and histological changes such as random apical sloughing of the brush border of proximal tubular cells occurred. Proximal and distal tubular cells exhibited marked degenerative changes.

Key words: - Aniline; Nephrotoxicity; Rats; Biomarker; Histology.

INTRODUCTION

Today, one of the top priorities of environmental impact assessment studies is the requirement for safety evaluation of everyday chemicals and their widespread exposure at every level of organization in the ecosystem. Scientific, regulating authorities, and public sectors have become concerned about the health impacts of many of the chemicals used in personal care products and domestic utilities (Daughton & Terms, 1999). International efforts are being made to investigate these chemicals' environmental occurrences, fates, impacts, and ecological and human health hazards. The Scientific Committee on Cosmetic Products and Other Consumer Products (SCCNFP, 2002). The term toxicology describes how chemicals affect living things negatively. Animal studies that are extrapolated to human populations are typically used to determine a compound's toxicity (Cota et al., 2000). The method of exposure plays a role in a substance's toxicity. The main methods of exposure to toxic compounds include cutaneous, inhalation, and ingestion. Determining exposure and establishing the contribution of interacting elements that can change toxicity are crucial components of toxicological evaluation. For a full assessment of toxicology and its prognosis, quantitative expressions of toxicity and exposure are crucial. A large number of biochemical analyses of blood have been used in in vivo studies using laboratory animals to evaluate the toxic potential of chemical compounds. In recent decades, efforts have been made to validate clinical chemistry tests as organ-specific biomarkers (Travlos, 1996). The majority of industrialized countries manufacture aniline and its monochlorophenyl derivatives. Aniline was manufactured in excess of 400 million pounds in 1975 just in the US. The aromatic amine aniline is used as an intermediate product in the manufacture of herbicides, azo dyes, drugs, paint, and toothpaste. These substances are also used in the synthesis of several organic compounds, including those that are used in medicines, dyes, antioxidants, chemicals

for use in agriculture, and many other industrial applications. The vast majority of human exposure to aniline and its derivatives takes place in industrial settings, and the main source of exposure to aniline compounds in people is industrial use. (Rankin et al,1985). The methemoglobinemia that is produced as a result of acute aniline chemical exposure results in tissue hypoxia. even if many aniline derivatives in humans result in symptoms or renal damage. (Kiese, 1974). Aniline is a potential environmental pollutant. Due to its toxicity to both humans and animals, aniline is on the European Union's priority list of chemicals. This study looked into the possibility of acute nephrotoxicity and hemototoxicity caused by aniline. Methemoglobinemia is one of the main symptoms of aniline poisoning (Anon, 2001). The rise in the erythrocytes' ability to produce methemoglobin (MetHb) heme iron transitioning from its ferrous to its ferric state (McLean et al., 1969) Despite the fact that Met Hb formation is reversible It is not a direct means of increasing red blood cell lysis, but rather red blood cell oxidative damage. Physically, Met Hb cannot bind oxygen reversibly and is inactive. The acute effects of many of these chemicals on renal function have not been thoroughly studied, despite the fact that many aniline derivatives cause symptoms or renal injury in humans In addition, ring halogenated derivatives of aniline may also be nephrotoxic given that several aliphatic and aromatic halogenated hydrocarbons have been demonstrated to cause nephrotoxicity in humans and animals (Munday et al., 1991).

Further research should be done to more precisely define the possible harm to humans from exposure to these chemicals, as there aren't many studies that go into great detail about the nephrotoxic potential of these commonly used halogenated aniline derivatives. Purpose of this study was to examine the acute nephrotoxic potential of aniline. In this work, male wistar rats were used as test subjects to determine the acute nephrotoxic potential of aniline because this rat strain is often more sensitive to kidney toxins than female.

Material and Method:-

Animal

Male Wistar rats were obtained from the Datta Meghe, College of Pharmacy, Wardha. Adult albino rats of the Male Wistar strain that were healthy and pathogen-free were chosen for the experiment in the current investigation. Only male rats of a similar age (17 weeks) and weight (about 125-150 gram) were utilised in the study since males of some species have faster biotransformation rates than females (Omkar, 1994). Stock of experimental animals and raised there under typical laboratory conditions. Before the scheduled trial, the animals were acclimated to the laboratory environment for 7–10 days. The animals were housed in polypropylene cages in a room under controlled environmental condition of light (12 hr. light / dark cycles), temperature (25±2°C) and relative humidity (52±5%). Water was available at all times during the experiment. The NIH criteria for the care and use of laboratory animals were followed by all the animals (NIH, 1985).

Chemical:

The formula for the organic molecule aniline is C6H5NH2. The simplest aromatic amine is aniline, which consists of an amino group joined to a phenyl group. It is an important commodity chemical for industry and a flexible starting point for the synthesis of fine chemicals. It is commonly known as: aminobenzene. It has a 93.13 molecular weight for the

molar mass of aniline. ID: Phenylamine in IUPAC. 184.1 °C is the boiling point of aniline and 1.02 g/cm3 is the density. It has a weak base with an aromatic or fleshy odor.

Experimental design.

The male Wistar rats were divided into two groups, and each group had nine animals. 20 mg/kg body weight of aniline was administered through gavage for 30 days. The aniline is less miscible with water, so boil the water for mixing the aniline, then cool it for dosing. Animals in the control group received access to regular drinking water. We continued the exposure paradigm for 30 days. The methods utilized in this investigation adhered to the requirements of OECD Testing Guideline No. 452. (1981). Observations were made once every day during the exposure period, observations were made. Body weights were recorded two times per week, body weights were recorded.

Observation and Examination

The overall rat behavior was observed throughout the inquiry for any signs of toxicity, both before and after the daily treatment. Toxicity was evaluated using key toxicity indicators such as mortality, and hydration food and water consumption each day.Throughout the thirty days of the research, no mortality was noted. After post-exposure rat sacrifices by the use of chloroform, remove kidney tissue and blood from the heart by using syringe. The abnormalities noticed included decreased haemoglobin, salt, and potassium levels, and an increase in serum creatinine and blood urea, alteration of kidney histology.

Haematologicaland Biochemical studies :

After being treated with chloroform, the animals were immobilized on the dissecting tray in order to collect blood samples. Blood samples were drawn from the heart using the cardiac puncture procedure and a sterile needle in order to evaluate both haematological and biochemical characteristics. Each animal had blood drawn in around 2 ml portions, and EDTA was used to keep the blood from clotting. Table 1.1 contains a list of the haematological and biochemical variables analyzed.

Table- 1.1 Haematological and Biochemical Parameters.

Parameter	Unit	Method
Creatinine	mg%	Jaffes reaction for serum creatinine
Urea	mg%	Urease test for urea
Sodium	mg%	Flamephotometric estimation
Potassium	mg%	Flamephotometric estimation
Haemoglobin	gm/dl	Sahli's method (Kolmer et al, 1969; Medwey, 1973) using sahlishaemometer.

Histological studies:-

At the 30th day of the exposure period, tissues from the kidneys of the control and test animals were obtained for histological and scientific analyses. The tissues were cut into small pieces and fixed for 24 hours in freshly made Bouins fixative. The tissues were then routinely dehydrated, fully rinsed with tap water, rehydrated, cleaned in xylene, and embedded in molten paraffin. Sections were cut at a thickness of 3–4 microns, and the standard hematoxylin–eosin (H&E) staining procedure was used (Luna, 1968).

Results:-

Behavioural pattern and mortality rate-

Throughout the time that they were given aniline treatment, all animals survived. The chosen dose concentrations were applied over the course of a 30-day exposure, and neither the treated group nor the control group experienced any deaths. Thus, the chosen dose, exposure time, and exposure route during the current study may be regarded as non-lethal for the animals.

Body Weights-

The mean body weight of all experimental animals was within the range of gm on the day of the initiation of treatment. The body weight of the control group was found to increase at a steady rate during the period of study and had attained, after a 30 day period, 139.0 ± 10.11 gm. On the other hand, the animals treated with aniline exhibited less weight gain in comparison to their control group. On the day that treatment began, the mean body weight of all experimental animals ranged from 141 ± 5.89 to 148.33 ± 4.11 The body weight of the control group was observed to increase steadily throughout the course of the trial and reached 198.44 ± 1.54 gm from 141 ± 5.89 after 30 days. However, compared to the rats in the control group, the aniline-treated rats showed a reduced weight gain.

Table 1.2:- Body weight of the experimental animals during 30 days oral dose of aniline, values are Mean ± SEM, n=9 for each group, denote statistically significant differences to the respective controls $p<0.0001$.

Days	**Body Weight in gm**	
	Control	**Treated**
On initiation of experiment/ 0day	141 ± 5.89	148.33 ± 4.11
At the end of experiment/30th day	198.44 ± 1.54	147.78 ± 1.73

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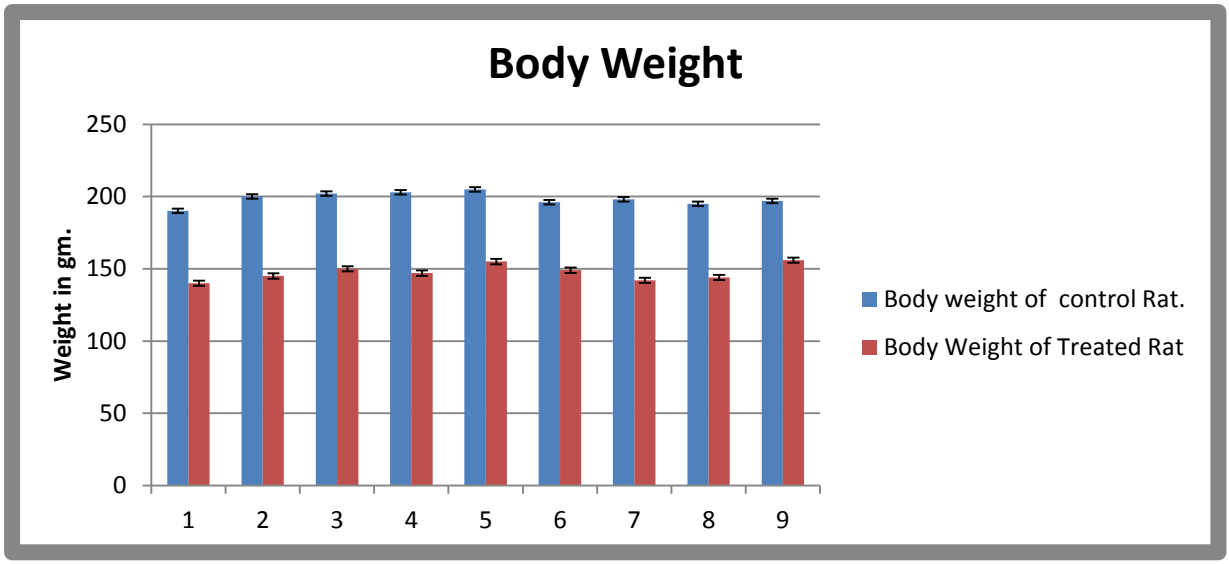


Fig 1.1: Changes of body weight in experimental male rats exposed by Aniline oral route for 30 days. after an interval of 30 days and weights were recorded. Data presented as mean ±SEM, n=9 in each group. Statistically significant differences to the respective controls:$p<0.001$

Kidney Weight :

The control group's kidney weight was found to be 1.15 ± 0.02 and the treated groups' kidney weights were noted 1.04 ± 0.01. These alterations in the relative weight of the kidney changes were statistically significant.

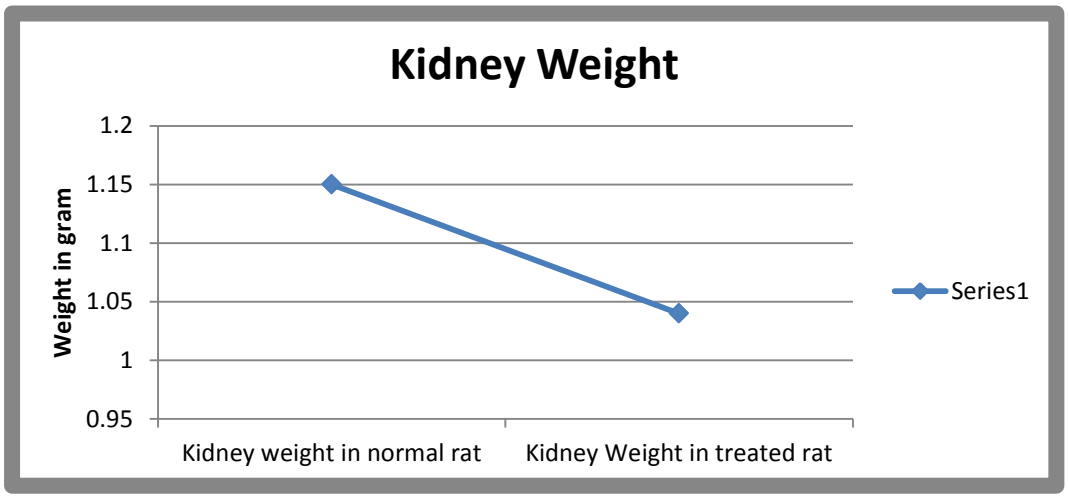
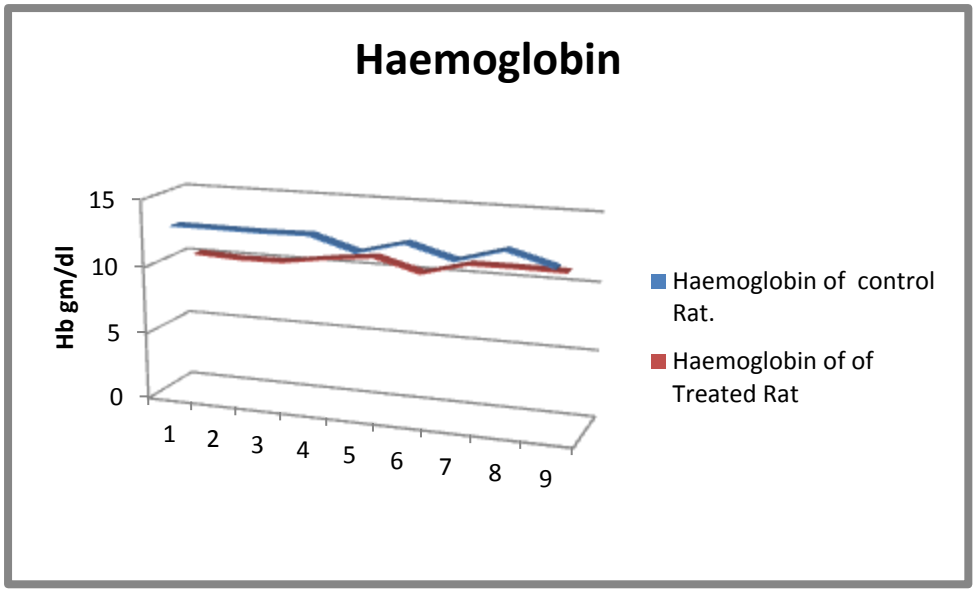


Fig 1.2 Absolute weight of Kidney during 30 days Oraltraeatment of aniline on male rat.

Haemaglobin:

In the control group, the haemoglobin concentration was recorded at (12.79 ± 0.15 (g/dl). But in the treated groups, a gradual decline in the level of haemoglobin has been noted, and after 30 days, the lowest level of haemoglobin was recorded in all animals of the treated groups. The overall reduction in Hb concentration showed a significant difference ($p<0.001$) from control vehicle treated animals.



Fig; 1.3 Haemoglobin (g/dl) of peripheral blood in the treated and control rat of the Values are Mean ± SEM of five (09) nos. of rats, and p<0.001.

Urea:

All experimental animals and the control group's urea concentrations were measured. After 30 days, the urea content in the control group was reported to be (31.74 ±1.70). The blood urea content in the aniline-treated animals was only slightly higher than that of the animals in the control group (42.77± 0.17). The statistical significance of this difference is p0.001.

Table 1.3:- Kidney weight after 30 days oral dose of aniline, values are Mean ± SEM, n=9 for each group, denote statistically significant differences to the respective controls p<0.0001.

Days	**Amount of Urea and in gm**	
	Control	**Treated**
At the end of experiment/30th day	31.74 ±1.70	42.77 ± 0.17

Effect of aniline on kidney histology:

On either side of the spinal column, in the retroperitoneal tissue of the posterior abdominal cavity, are the kidneys, two sizable, reddish, bean-shaped organs. They have complicated tubular glands and are extensively vascularized. Approximately 25% of the cardiac output is directed toward them. The urine is originally an ultrafiltrate of the blood that the kidneys produce and then modify through selective resorption and specialised secretion by the kidney cells. The ureters transport the finished urine to the urinary bladder, where it is kept until it is expelled through the urethra. The organ is quickly impacted by harmful compounds that enter

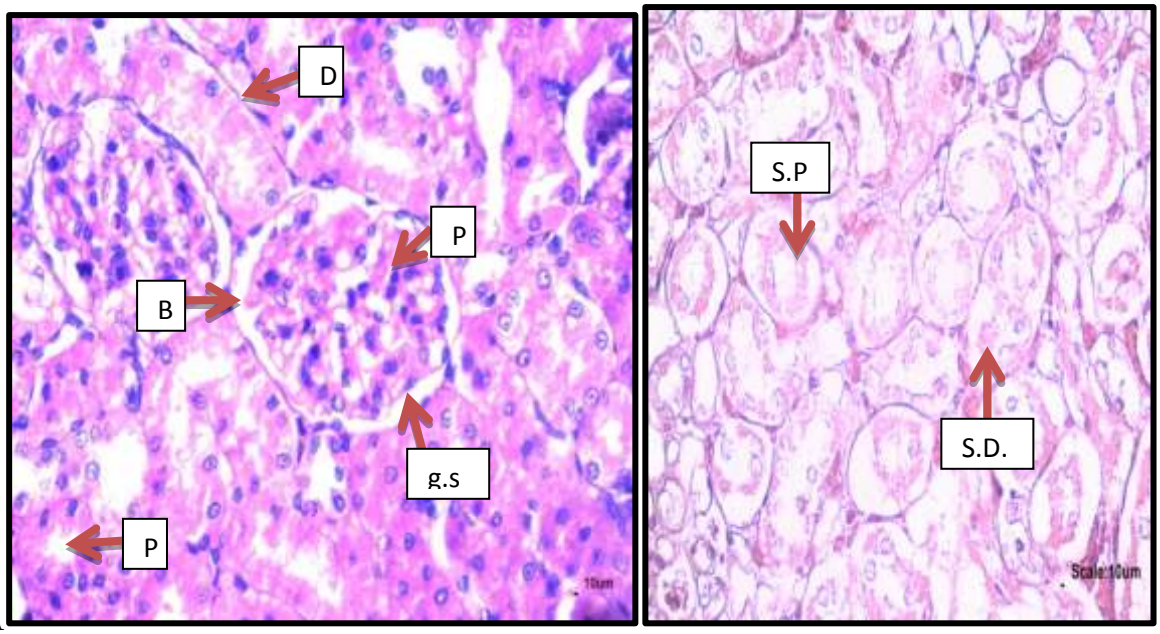
the body through the bloodstream or that are produced during the metabolic process since it functions as an effective filter to eliminate waste products formed during metabolism.

Control group:

The kidney's outer cortex and inner medulla are two distinct regions. Renal corpuscles and straight and convoluted nephron tubules make up the cortex. The distinctive characteristics of the medulla are unique capillary networks, collecting ducts, and straight tubules. The uriniferous tubules that make up the kidney parenchyma are sandwiched between blood vesicles and a small amount of interstitial connective tissue. The terminal tubule is a component of the nephron, a structural and functional unit. Each nephron begins as a Bowmen's capsule, a double-walled cup-shaped expansion that houses the glomerulus, a tuft of capillaries. The Bowmen's capsule and glomerulus are the components of the renal corpuscles. Glomeruli with squamous epithelial cells and podocytes.

Aniline Treated Kidney:

Histopathological alterations in the kidney's architecture were discovered after 30 days of exposure. Massive red blood cell deposition throughout the kidneys is a sign of haemorrhagic regions that are large in size. Tubular epithelium is being lost. The proximal tubular epithelial cells displayed degenerative changes, and the lumen of the proximal tubules occasionally seemed to be filled with dead epithelial cells. There was also the accumulation of fibrinous material within the Bowmen's capsule, sloughing of tubular epithelial cells, and glomerular atrophy.



A B

Fig- 1.4 A. Showing Control group kidney after thirty day normal Glomeruli size with Bowman capsule(B), glomerular space(g.s.), squamous epithelium cell, podocyte and P.C.T.(P) and D.C.T.(D) and **Fig- 1.4 B** Showing Treated group kidney after thirty days Sloughing of brush border of P.C.T.(S.P.) and Sloughing of brush border of D.C.T.(S.D.).

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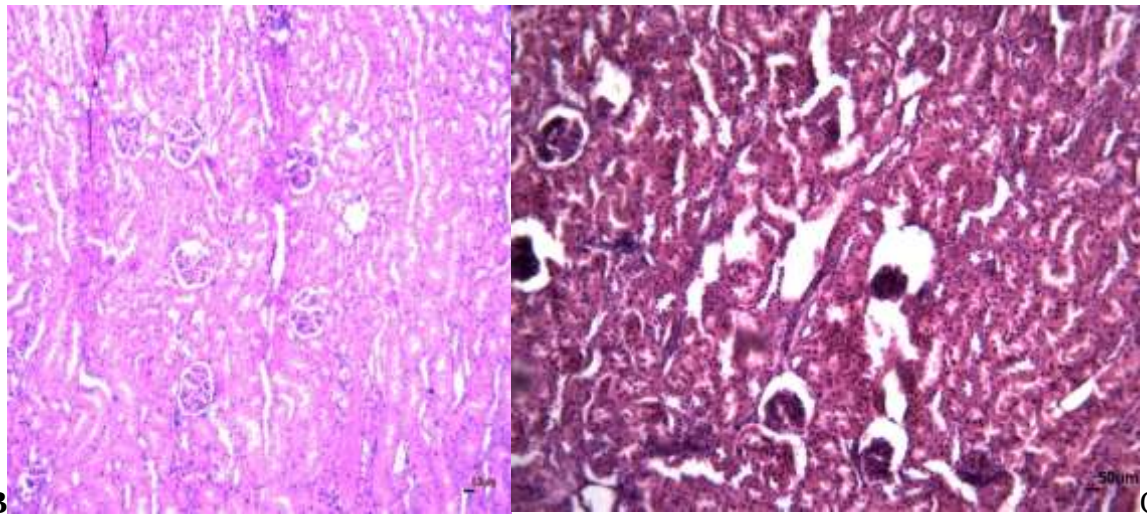

Fig- 1.4 B. Showing Control group kidney after thirty days normal Glomeruli size and 1.4C Showing aniline treated group kidney Glomeruli Atrophy.

Analytical statistics

To analyse the data, descriptive statistics were employed. To compare the means of untreated and treated male wistar rats, a paired t test was used. The data were presented as Mean and SER for the biochemical parameters. The cutoff for statistical significance was P 0.001.

Discussion:

The results of the current study demonstrated that the chemical aniline is nephrotoxic. It is clear from detailed investigations that extended topical exposure to aniline causes a variety of clinical problems in the experimental animals' kidneys. The potential for aniline deposition increases when the kidneys reabsorb more water from the filtrate, resulting in a higher concentration of the chemical that causes histopathological alterations, as was discovered in the current study. Although results from experiments cannot always be generalized to humans, using less aniline-containing toothpaste, paint, and hair dye is always advised for improved human health safety.

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