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Toxicological Assessment of Rubber Industry Effluent on Rat Cellular System

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Abstract:- This study assessed the toxicological effect of rubber industry effluent on rat's cellular system, such as the biochemical responses, physiology and histopathology of selected tissues. Twenty six (26) rats was divided into five (5) groups and was exposed for a period of twenty eight (28) days. Standard enzyme assays were conducted for alanine transaminase (ALT), catalase (CAT), and superoxide dismutase (SOD), and antioxidant such malondialdehyde (MDA) and ascorbic acid (ASB) of selected tissues of rat's cellular systems such as serum, heart, liver, stomach, kidney and colon exposed to contaminated rubber effluent for the period of 4 weeks. In general, exposure to sub lethal concentrations of rubber effluent showed a significant decrease in the activity of serum of the enzymes of rat's cellular system when compare to control ($p < 0.05$). In specific, the activity of alanine transaminase of serum exposed to contaminated rubber effluent was found to be 0.09 ± 0.00 in group B when compared to 0.06 ± 0.00 of Group A (control). The activity of liver in the same group was found to be 0.11 ± 0.00 when compare to 0.05±0.00 of control. The activity of MDA of serum exposed to contaminated rubber effluent was found to be 0.13 ± 0.00 when compare 0.09±0.00 of control. There is a similar increase in groups exposed to contaminated rubber effluent when compare to control. The results shows that lipid peroxidation was high in rats exposed to rubber effluent. It was also noticed, there was a gradual increase in liver and selected tissues of ALT, MDA, CAT, ASB and SOD in all experimental groups. This study predicts that rubber effluents could induce oxidative stress, thereby affecting general physiology. Thus, the serum biochemical response of rats is affected resulting in oxidative stress. These findings can be used as valuable biomarkers for the assessment of toxicity and also monitoring of water quality. The present study further reveals that Rattus novergicus exposed to rubber effluent may have altered protein metabolism, among others, at the subcellular level and this may be indicative of impairment of the function of the tissues.

Keywords:- *Rubber Effluent; Rat's Cellular System; Lipid Peroxidation; Industry; Toxicological.*

I. INTRODUCTION

Rubber processing industries have become a very important industry in our society today. They discharge effluent into the environment. The rubber waste is also known as rubber effluent. Rubber is found in plants as milky white latex but the chemical composition however, varies from species to species. Chemically, rubber is a polyterpene consisting of a long chain (500 – 5000) of isoprene units joined together end to end to form giant molecules called polymers which are coiled up like tiny springs [1].

The rubber processing sector produces huge amounts of wastewater with high levels of organic matter, suspended particles, and nitrogen [2]. Rubber manufacturing wastewater has large quantities of gaseous and liquid effluent that, if not adequately treated and disposed of, can cause serious environmental harm. The term "Effluent" refers to wastewater that runs out of a treatment plant, sewer, or industrial outfall, whether treated or untreated. It relates to pollutants that are released into surface waterways in general" [3]. Water pollution refers to any alteration in its natural properties caused by the addition of anthropogenic pollutants to the point that it can no longer function as a source of drinking water for people or maintain biotic organisms [4]. Because of their close association with the external environment, toxicants entering water bodies can affect water quality parameters, which can pose threat to public health [5].

- *Rubber Effluent*

According to recent studies and investigations, trash and runoff that flows directly into the environment, particularly the aquatic environment, has a major acute and chronic impact on the ecosystem, which can cause severe bio-diversity loss and drive a significant reduction in biodiversity [6]. These chemicals used in making rubber products and in combination with other ingredients are considered to cause toxicity in the environment [7]. Basic Polymer and ingredients used for making rubber products, either individually or after combinations make some carcinogenic liquids, Also, chemical dust comes in contact with the liquids, some gets contaminated in water and thus, directly or indirectly, affects living things especially human beings [7].

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In rubber processing industry, to increase dry rubber content (DRC) of rubber latex, water and other impurities are being removed. Rubber end products are also cleaned with water in the industry. Contaminated water is discharged into the open land or aquatic bodies on a regular basis, causing toxicity. Ammonia solution (NH3), formic acid (CH_20_2), Tetra methyl Thiuram Disulfide, Zinc Oxide, and diamonium phosphate (NH4)2HPO4 are commonly found in rubber wastewater. Before releasing waste water (effluent) from the factory into the environment, a strategy and treatment procedures must be implemented [7].

Rubber processing generates materials that are utilized in the production of rubber industrial goods. A large quantity of water is utilized, and a large amount of effluent is produced, which is then released into rivers or the environment, creating pollution that is harmful to human health [8]. Heavy metals are major environmental contaminants in rubber toxicity, and their toxicity is becoming more of an issue for biological, genetic, and environmental considerations [9].

The effects of rubber industry effluents on the cellular system of rats were studied using a toxicological evaluation.

II. MATERIALS AND METHODS

The effluent samples for this research study were collected from the rubber factory division of Rubber Research Institute of Nigeria. 20 litres of rubber effluents were collected from the factory flow out unit, six (6) drops of nitrous oxide (N_2O) (Ahmed, 2019) was added to the effluent collected to keep the originality and state of the effluent. Physicochemical analysis was carried out on the samples using the method described by Apha (1992).

Albino wistar rats *(Rattus novergicus)* of both sexes, weighing 150-200mg/kg were acclimatized for one (1) week before commencement of exposure to rubber effluent. The experimental rats were divided into five (5) groups (compartments) of 5 rats each in four groups and 6 rats in one group and each group were assigned to a concentration of the toxicant; with distilled water as control. They were treated in laboratory conditions of 24-hour in a day, the rats were exposed to temperature of 26 ± 2°C and relative humidity of 70 ± 20°C. The animals were fed with standard rodent chow (Ladokun feed Nigeria®) *ad libitum* and water. The rats in each groups were treated with 1mL of 5, 10, 15 and 20 % (rubber effluent diluted with distilled water, v/v) and treated for 28 days. Similar treatment was also given to the group (control) receiving distilled water. The experimented animals were classified into 5 groups as follows; each group was labeled and treated with the diluted effluent as stated in the table below.

GROUPS	NO OF RATS	TREATMENT	PERIOD OF TREATMENT
A	**5**	Control - Distilled water only (1000 mL)	4 weeks
B	**6**	5% - 50 mL Rubber effluent / 950mL Distilled water	4 weeks
C	**5**	10% - 100 mL Rubber effluent / 900mL Distilled water	4 weeks
D	**5**	15% - 150 mL Rubber effluent / 850mL Distilled water	4 weeks
E	**5**	20% - 200 mL Rubber effluent / 800mL Distilled water	4 weeks

Table 1:- Animal Groups and Treatment

The feeding and exposure period lasted for of 28 days. The animals were kept and treated in a plastic cage and fed ad libitum with commercial rat chow throughout the period of administration after 7 days of acclimatization.

Note: The toxicant was administered to the rats morning and evening
Doses of Toxicant: 1000 µl
Body Weight of rats: 150 - 200 kg
Routes of Administration: Oral administration
Source of Toxicant: Rubber Research Institute of Nigeria, Benin-City.

Before being sacrificed by jugular puncture, the rats were anesthetized by putting them in a container containing cotton wool saturated in chloroform and were promptly dissected, with the various organs such as serum, heart, liver, stomach, kidney and colon properly harvested and excised. Fat removed, wiped with clean tissue paper, and weighed into an ice cold 0.25M normal saline solution beaker.

Weights of the harvested tissues were recorded, then a part of each was chopped, diced into very small bits, and homogenized in a basin of ice cubes using a pre-cooled pestle and mortar. Tissue homogenates obtained were diluted to 1 in 30 using a 0.25M normal saline solution. For biochemical investigations and enzyme assays, a portion of each organ was homogenized. The homogenates were diluted and kept at -8°C refrigerator until they were needed for analysis.

Biochemical analysis: tissues (serum, heart, liver, stomach, kidney and colon) homogenate were evaluated for Alanine aminotransferase (ALT) by the method introduced by Reitman and Frankel (1957) and modified by Schmidt and Schmidt (1963). Lipid peroxidation product by malondialdehyde (MDA) and Ascorbic acid (ASB) determination of tissue homogenate was determined using the methods of Varshney and Kale (1990); and Roe and Kurethor (1943) respectively. Enzymic antioxidant activities; superoxide dismutase (SOD) was assayed by the method of Misra and Fridovich (1972), while catalase (CAT) was done following the method of Sinha (1971). Statistical tools such as Duncan Multiple Range Test were

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applied to calculate the significance of the differences between control and experimental means. P values of 0.05 or less were considered statistically significant [10].

III. RESULTS

Table 2 present Alanine transaminase (ALT) activity of *Rattus novergicus* cellular system exposed to oral administration of rubber effluent. Activity of serum enzyme in the test groups (groups B to E) was found to be significantly higher ($p<0.05$) when compared to Group A (control). ALT activity in the selected tissues did not show any definite pattern, for instance, it was not found to be effluent concentration dependent. However, in most cases rats treated with 5% effluent (Group B) showed significantly higher ($p<0.005$) enzyme activity relative to any of the other test groups (Groups C-E) except in serum where ALT activity of Group B rats was significantly lower than that of Group C rats. Except in serum, ALT activity of selected tissues of rats in Groups D and E was not significantly different ($p>0.05$).

GROUPS	SERUM	HEART	LIVER	STOMACH	KIDNEY	COLON
MA	0.06 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.06 ± 0.00^{a}	0.06 ± 0.00^{a}
MB	0.09 ± 0.00^{b}	0.11 ± 0.00^{b}	0.11 ± 0.00^{b}	0.11 ± 0.00^{b}	0.13 ± 0.00^{b}	0.11 ± 0.00^{b}
MC	0.10 ± 0.00^{c}	0.09 ± 0.00^{c}	0.06 ± 0.00^{c}	0.06 ± 0.00^{c}	0.06 ± 0.00^{c}	0.09 ± 0.00^{c}
MD	0.09 ± 0.00^{b}	0.08 ± 0.00^{d}	0.08 ± 0.00^{d}	0.09 ± 0.00^{d}	0.08 ± 0.00^{d}	0.10 ± 0.00^{d}
ME	0.08 ± 0.00^{e}	0.08 ± 0.00^{d}	0.08 ± 0.00^{d}	0.09 ± 0.00^{d}	0.08 ± 0.00^{d}	0.07 ± 0.00^{e}

Table 2:- Specific Activities (nmol/min/mg protein) of Enzyme Alanine Transaminase (ALT) of Elected Tissues of Rats Given Oral Administration of Rubber Effluent.

The results above are means of six determinations (mean ± SEM). Group A: The rats exposed to 100% distilled water (control). Group B: The rats exposed to rubber effluent (EF) (5%). Group C: The rats exposed to rubber effluent (10%). Group D: The rats exposed to rubber effluent (15%). Group E: The rats exposed to rubber effluent (20%).

The values on the same column carrying different superscripts are significantly different ($p < 0.05$).

Table 3 presents Malondialdehyde (MDA) concentration of *Rattus novergicus* cellular system exposed to oral administration of rubber effluent toxicant. Specifically, Group B showed a significantly ($p < 0.05$) lower activity in serum and selected tissues when compared to Group A (control). In Group C, there was found to be a significantly ($p < 0.05$) lower activity in stomach but significantly ($p < 0.05$) higher activity in serum and other tissues (liver, heart, stomach and colon) when compared to group A (control). In Group D, there was a significantly ($p < 0.05$) lower activity in serum and other tissues (heart, liver, stomach and colon) except kidney in group D which showed no significant difference when compared to Group A (control). Group E showed a significantly difference ($p < 0.05$) lower activity in serum and stomach but significantly ($p < 0.05$) higher activity in heart, kidney, liver and colon when compared to group A (control).

GROUPS	SERUM	HEART	LIVER	STOMACH	KIDNEY	COLON
MA	0.09 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}
MB	0.13 ± 0.00^{b}	0.03 ± 0.00^{b}	0.03 ± 0.00^{b}	0.06 ± 0.00^{b}	0.03 ± 0.00^{b}	0.03 ± 0.00^{b}
MC	0.13 ± 0.00^{b}	0.03 ± 0.00^{b}	0.03 ± 0.00^{b}	0.03 ± 0.00^{c}	0.03 ± 0.00^{b}	0.03 ± 0.00^{b}
MD	0.13 ± 0.00^{b}	0.03 ± 0.00^{b}	0.03 ± 0.00^{b}	0.03 ± 0.00^{c}	0.02 ± 0.00^{a}	0.03 ± 0.00^{b}
ME	0.20 ± 0.00^{c}	0.03 ± 0.00^{b}	0.03 ± 0.00^{b}	0.03 ± 0.00^{c}	0.03 ± 0.00^{b}	0.03 ± 0.00^{b}

Table 3:- Specific Activities (nmol/min/mg protein) Malondialdehyde (MDA) Of Selected Tissues Of Rats Given Oral Administration Of Rubber Effluent.

The results above are means of six determinations (mean ± SEM). Group A: The rats exposed to 100% distilled water (control). Group B: The rats exposed to rubber effluent (EF) (5%). Group C: The rats exposed to rubber effluent (10%). Group D: The rats exposed to rubber effluent (15%). Group E: The rats exposed to rubber effluent (20%).

The values on the same column carrying different superscripts are significantly different ($p < 0.05$).

Table 4 presents Catalase (CAT) activity of *Rattus novergicus* cellular system exposed to oral administration of rubber effluent toxicant. Activity of serum and kidney from the test groups (B to E) were found to be significantly ($p < 0.05$) reduced activity in serum, heart, kidney and colon when compared to group A (control) but significantly ($p < 0.05$) higher activity in liver and stomach when compared to group A (control). In Group C, there was a similar pattern to that of Group B, in which a significantly ($p < 0.05$) reduced activity was found in serum, heart, kidney and colon but significantly ($p < 0.05$) higher activity in liver and stomach when compared to group A (control). Group D showed a significantly lower activity in serum and kidney but significantly ($p < 0.05$) higher activity in heart, liver stomach and colon as the level of toxicant increased when compared to group A (control). Group E also followed a similar trend to Group D, in which there was a significantly ($p < 0.05$) lower activity in serum and kidney but significantly higher activity in heart, liver, stomach and colon when compared to group A (control).

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GROUPS	SERUM	HEART	LIVER	STOMACH	KIDNEY	COLON
MA	0.20±0.00^{a}	0.15±0.00^{a}	0.15±0.00^{a}	0.15±0.00^{a}	0.17±0.00^{a}	0.15±5.00^{a}
MB	0.25±0.00^{b}	0.16±0.00^{b}	0.15±0.00^{a}	0.15±0.00^{a}	0.15±0.00^{b}	0.17±0.00^{b}
MC	0.24±0.00^{c}	0.22±0.00^{c}	0.21±0.00^{b}	0.20±0.00^{b}	0.25±0.00^{c}	0.16±0.00^{c}
MD	0.30±0.00^{d}	0.16±0.00^{b}	0.16±0.00^{c}	0.19±0.00^{c}	0.18±0.00^{d}	0.17±0.00^{b}
ME	0.35±0.01^{e}	0.21±0.00^{d}	0.20±0.00^{d}	0.24±0.00^{d}	0.22±0.00^{e}	0.20±0.00^{d}

Table 4:- Specific Activities (nmol/min/mg protein) Catalase (CAT) of Selected Tissues of Rats Given Oral Administration of Rubber Effluent.

The results above are means of six determinations (mean ± SEM). Group A: The rats exposed to 100% distilled water (control). Group B: The rats exposed to rubber effluent (EF) (5%). Group C: The rats exposed to rubber effluent (10%). Group D: The rats exposed to rubber effluent (15%). Group E: The rats exposed to rubber effluent (20%).

The values on the same column carrying different superscripts are significantly different ($p < 0.05$).

Table 5 presents Ascorbic Acid (ASB) activity of *Rattus novergicus* cellular system exposed to oral administration of rubber effluent toxicant. Activity of serum and other tissues from group B to E were significantly lower ($p < 0.05$) when compare to group Group A (control) like that of CAT. Specifically, in Group B, the administration of rubber effluent toxicant caused a significantly lower activity in liver, kidney, stomach and colon when compared to Group A (control). In Group C, there was a significantly ($p < 0.05$) higher activity in serum but a significantly lower activity in liver, stomach and colon when compared to Group A (control). Group D followed similar trend as Group C, in which there was a significantly higher activity in serum but a significantly ($p < 0.05$) lower activity in liver, stomach and colon when compared to Group A (control). Group E showed a significantly lower activity in heart, liver, kidney and colon when compared to Group A (control).

GROUPS	SERUM	HEART	LIVER	STOMACH	KIDNEY	COLON
MA	1.39±0.00^{a}	1.14±0.01^{a}	1.95±0.02^{a}	1.32±0.01^{a}	2.26±0.01^{a}	1.71±0.03^{a}
MB	0.85±0.00^{b}	1.44±0.01^{b}	1.45±0.01^{b}	1.72±0.00^{b}	1.42±0.00^{b}	1.37±0.00^{b}
MC	1.10±0.01^{c}	1.46±0.01^{c}	1.45±0.01^{b}	1.40±0.00^{c}	1.58±0.01^{c}	1.57±0.00^{c}
MD	1.08±0.01^{d}	1.37±0.01^{d}	0.96±0.01^{c}	0.92±0.00^{d}	1.19±0.01^{d}	0.85±0.00^{d}
ME	0.80±0.00^{e}	0.92±0.00^{e}	1.12±0.01^{d}	1.06±0.01^{e}	0.93±0.00^{e}	0.95±0.00^{e}

Table 5:- Specific Activities (nmol/min/mg protein) Ascorbic Acid (ASB) of Selected Tissues of Rats Given Oral Administration of Rubber Effluent.

The results above are means of six determinations (mean ± SEM). Group A: The rats exposed to 100% distilled water (control). Group B: The rats exposed to rubber effluent (EF) (5%). Group C: The rats exposed to rubber effluent (10%). Group D: The rats exposed to rubber effluent (15%). Group E: The rats exposed to rubber effluent (20%).

The values on the same column carrying different superscripts are significantly different ($p < 0.05$).

Table 6 presents Superoxide Dismutase (SOD) activity of *Rattus novergicus* cellular system exposed to oral administration of rubber effluent toxicant. Like CAT and ASB, activity of serum and other tissues from group B to E were found significantly ($p < 0.05$) lower activity in serum, heart, stomach and kidney when compare to group A (control) s while it showed a significantly higher activity in liver and colon when compared to Group A (control). In Group C, there was a significantly ($p < 0.05$) lower activity in serum and all tissues except colon which showed a significantly higher activity when compared to Group A (control). Group D showed a significantly lower activity in serum, heart and liver while there was a significantly ($p < 0.05$) higher activity in stomach, kidney and colon when compared to Group A (control). In Group E, there was a significantly ($p < 0.05$) lower activity in heart, stomach and kidney while there was a significantly ($p < 0.05$) higher activity in serum, liver and colon when compared to Group A (control).

GROUPS	SERUM	HEART	LIVER	STOMACH	KIDNEY	COLON
MA	211±2.76^{a}	246±2.01^{a}	190±0.40^{a}	140±1.09^{a}	149±1.59^{a}	309±0.59^{a}
MB	150±2.06^{b}	165±1.07^{b}	189±4.38^{b}	158±0.2^{0b}	145±0.75	146±1.41^{b}
MC	160±0.48^{c}	175±0.45^{c}	170±0.24^{c}	162±0.15^{c}	154±0.14^{c}	350±3.94^{c}
MD	147±0.30^{d}	138±0.15^{d}	147±0.25^{d}	138±1.74^{d}	191±5.59^{d}	379±7.29^{d}
ME	171±3.07^{e}	132±1.44^{e}	180±1.84^{e}	152±1.01^{e}	153±0.33^{e}	249±2.54^{e}

Table 6:- Specific Activities (nmol/min/mg protein) of Enzyme Antioxidant Superoxide Dismutase (SOD) of Selected Tissues of Rats Given Oral Administration of Rubber Effluent.

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The results above are means of six determinations (mean ± SEM). Group A: The rats exposed to 100% distilled water (control). Group B: The rats exposed to rubber effluent (EF) (5%). Group C: The rats exposed to rubber effluent (10%). Group D: The rats exposed to rubber effluent (15%). Group E: The rats exposed to rubber effluent (20%).

The values on the same column carrying different superscripts are significantly different ($p < 0.05$).

IV. DISCUSSION

From previous literature review, no research work has been recorded on toxic effect of rubber effluent on biochemical responses on rat's cellular system.

This present study looked at the toxicological assessment of rubber industry effluent on rat's cellular system that is the effect of waste water effluent on the biochemical responses and physiology and histopathology of serum, heart, liver, stomach, kidney and colon and enzyme antioxidants when exposed for four weeks by oral administration of effluent. At the end of the research, the following were observed by examining the specific activity of ALT, SOD and CAT as well as MDA and ASB.

Alanine aminotransferase (ALT) is an enzyme found in the liver which is used as a liver function test to ascertain damage caused to the liver. The function of alanine aminotransferase (ALT) is to catalyze the transfer of an amino group from alanine to alpha-ketoglutarate in the alanine cycle to generate glutamate and pyruvate. ALT is usually utilized as a diagnostic tool for evaluating hepatocellular injury, for determining liver health. In this study, Alanine transaminase (ALT) activity of *rat's novergicus* cellular system exposed to oral administration of rubber effluent as presented in Table 2. This observation is in accordance with the findings [6], which showed the effect of water contaminated with phthalate, benzene and cyclohexane on Clarias gariepinus' cellular system. Abnormal values of serum ALT have been observed for different diseased conditions, but most especially in hepatic diseases. The data presented in the experimental Table 2 revealed that *Rattus novergicus* exposed to rubber effluent must have altered protein metabolism, among others, at the subcellular level and this may be indicative of impairment of the function of the tissues. When there is a damage to smooth; liver or heart musculature, serum level of some enzymes such as, alanine transaminase (ALT) is reduced. These diagnostic enzymes are valuable tools used in early detection of muscle wastage as a result of ischemia, injury or inflammation [6].

Previous research found similar results [11]. The activity levels of ALT are the hepatic indicators that are examined to check for the effects of polycyclic aromatic hydrocarbons (PAH). These are hepatocyte organelle indicators that leak into the tissues as a result of damage induced by reactive metabolites produced by xenobiotic metabolism in the liver. The results reveal that the content of ALT increased where the effluent water samples were given to the rats. The results demonstrate a steady rise, with a significant increase at the 0.05 level ($P < 0.05$). This indicates that the PAH had the greatest impact on the liver, resulting in significant liver damage and the induction of inflammatory cells in the liver. Furthermore, the effluent was found to have a negative impact on hepatic cells. The presence of PAH and other compounds identified in the effluent water sample may be linked to the chemicals employed in the treating rubber latex, causing these effects [11].

The specific concentration of MDA of *Rattus novergicus* exposed to oral administration of rubber effluent as shown in Table 3. This study is in agreement with an earlier report by an increase was determined in hydrogen peroxide and superoxide anion levels after cyanide treatment [12, 13]. It was also reported that cyanide given to rats in toxic dosage inhibits antioxidant enzymes like GP-x and depletes non-enzymatic antioxidants like intracellular GSH [14]. It was also reported that cyanide causes an increase in lipid peroxidation and a decrease in GSH levels (blood, brain and liver) and GP-x (liver and brain), SOD (brain and liver) a CAT (blood and brain) enzyme activities [15]. While the rats in group A (control) kidney have similar response with rats in group D but significantly different for rats in group B, C and E ($p<0.05$). This result is in corroboration with another study which was also observed [16]. It was accepted that protection against lipid peroxidation differs from tissue to tissue [17]. It was also reported that cyanide leads to neurotoxicity in neuronal membranes due to oxidative damage because of lipid peroxidation [18, 19]. Different experimental studies revealed that acute and chronic cyanide poisoning leads to oxidative stress [14, 20, 21], as confirmed by the current studies.

Catalase (CAT) is an important antioxidant that helps to reduce oxidative stress by eliminating cellular hydrogen peroxide and producing water and oxygen. Reactive species generated in the cell during normal cellular metabolism can react chemically with cellular macromolecules such as nucleic acids, proteins, and lipids, producing oxidative changes and possible harm to their biological functions. In this study, Table 4 showed the specific activity of CAT in *rattus novergicus* exposed to rubber effluent and the resultant effect on cellular activities. The specific activity of CAT was significantly lower in serum and kidney of all contaminated groups (B-E). This alteration in activity agrees with [22], which observes that any disruption in the equilibrium of antioxidants and reactive species leads to a physiological state known as "oxidative stress," according to research. Many age-related degenerative illnesses, such as diabetes mellitus, hypertension, anemia, vitiligo, Alzheimer's disease, Parkinson's disease, bipolar disorder, cancer, and schizophrenia, are thought to be linked to catalase deficiency or dysfunction. It was also reported according to [23], the effect of benzene on the enzymes of carbohydrate metabolism, brush border membrane (BBM) and oxidative stress in kidney and other rat tissues. The activity of gluconeogenic enzymes G6Pase and FBPase declined when expose to benzene. In similar development, the activities of catalase significantly reduced when associated with the increase in lipid peroxidation. The result indicated that benzene brought about nephrotoxicity and lowered the

enzymes of metabolism of carbohydrate and BBM. This is most likely to cause oxidative stress.

Ascorbic acid (ASB) or Vitamin C, was discovered to be a crucial molecule in the prevention of scurvy and has since grown in popularity due to its antioxidant qualities. The constant activation of inflammation is widely recognized to be the cause of many illnesses. To produce protective benefits, ASB might lower oxidative damage and inflammation. Ascorbic acid has been linked to a variety of metabolic effects, including antioxidant and reducing properties. In this study, the specific concentrations of Ascorbic Acid (ASB) in Table 5 was in agreement with [24] which states that the exposure of *rattus novergicus* to polychlorinated biphenyls (PCB) will lead to an increase in the activity of liver microsomal that is mixed with the function oxidase system, plasma cholesterol and urinary ascorbic acid.

SOD is an antioxidant that effectively removes the superoxide anion by converting free radicals to oxygen and hydrogen peroxide, avoiding the formation of peroxynitrite and further damage. Many pathogenic processes in the body are closely linked to free radicals. SOD has received a lot of attention for its capacity to scavenge because of this scavenging capabilities [25]. In this investigation, as shown in Table 6. It is a good biomarker in the estimation of malathion-induced oxidative stress affecting blood and liver. Also in a similar work [26], it was observed that there was significantly high decline in the activities of CAT and SOD as well as elevation in MDA and was observed in the group receiving NPs at the rate of 150 mg/kg BW. It's also noted that TiO2 can caused histological alterations in the liver. This finding showed that very high dose of TiO2 NPs can significantly cause harmful effects on liver and blood when compared to lower doses.

V. CONCLUSION

From the results obtained from this present study, consumption of rubber waste water (effluent) may increase oxidative stress as seen in test rats. Ingestion of rubber waste water (effluent) may also alter the specific activity of enzyme ALT and antioxidant enzymes as evidenced by the reduced activity of enzyme ALT in Groups B-D and increased activity of the same enzyme in serum, heart, liver, stomach, kidney and colon in Group E. Also there was reduced activity in antioxidants CAT and ASB. In SOD there was increased activity of the same antioxidant enzyme in selected tissues. MDA showed alteration in cellular activity, as there was reduced activity in some selected tissues in Groups E, and increased activity of the same antioxidant in other experimental groups. Consumption or injection of rubber waste (effluent) may lead to tissue damage as evidenced in the test rats.These findings can be used as valuable biomarkers for the assessment of toxicity and also monitoring of water quality.

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