



(RESEARCH ARTICLE)



Reproductive indices and haematological response of male albino rats (*Rattus norvegicus*) exposed to Carburetor cleaner

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Abstract

Albino rats were exposed to carburetor cleaners with varying routes of exposure using five treatments viz control group 1, & groups 2, 3, 4 & 5 representing (inhalatory, feed incorporated, oral and dermal routes) respectively. Haematological parameters as; PCV, HB, RBC, McV, Mch, and Mchc were evaluated, while the semen analysis such as; Motility quantitative (M1), Debris quantitative (D1), sperm count (C), morphology primordial (Mp), and morphology structure (Ms) were determined. The results revealed the following mean value of PCV for groups 1, 2, 3, 4, and 5 as 47.5, 40.0, 35.0, 20.5, and 23.25 respectively with significant differences ($P < 0.05$) across the group, for HB 15.38, 14.25, 12.10, 8.30 and 8.78 for group 1, 2, 3, 4 and 5 respectively with significant difference ($P < 0.05$) across the group. RBC and McV had a mean value of 5.27 and 9.08, 4.15 and 9.59, 3.57 and 10.01, 2.33 and 8.77, 1.85 and 14.66 for groups 1, 2, 3, 4, and 5 respectively with significant differences ($P < 0.05$) across the group. While Mch and Mchc had a mean value of 2.94 and 0.32, 3.42 and 0.36, 3.45 and 0.35, 3.59 and 0.41, 5.51 and 0.39 for groups 1, 2, 3, 4, and 5 respectively with significant difference ($P < 0.05$) across the group. For semen analysis, M1 was 66.25, 52.5, 43.75, 31.25 and 23.75 for group 1, 2, 3, 4 and 5 respectively, Debris D1 was 6.25, 7.5, 8.75, 28.75 and 7.5 for group 1, 2, 3, 4 and 5 respectively, C and Mp were 53.25 and 6.25, 47.5 and 6.25, 42.0 and 17.75, 36.25 and 27.50, 37.25 and 30.0 for group 1, 2, 3, 4 and 5 respectively also. Finally, Ms was 1.0, 1.0, 1.25, 1.50 and 1.75 for group 1, 2, 3, 4 and 5 respectively. The results generally indicate that carburetor cleaner exerts a negative effect on the body irrespective of the route of exposure and extreme care should be taken when handling and utilization.

Keywords: Carburetor; Male albino rats; Reproductive indices; Haemoglobin; Anaemia; Occupational health

1. Introduction

A carburetor is a device that mixes fuel and air together and pulling the mixture into the cylinders for combustion in an internal combustion engine [1]. Carburetors are vulnerable to accumulation of residues of petrol, and this affects its functionality. These residues require a strong solvent before it can be wiped clean [2]. Carburetor cleaners are chemical sprays which are used to remove residues that build-up and deposit on carburetors, it is designed to safely clean without damaging the gaskets, rubber and plastic parts [2]. They are either single can aerosols or come in gallon-sized portions; the toxic nature of the main ingredient makes it hazardous and hence requires a level of education and careful handling [3]. The carburetor cleaner is made up of oil and organic solvents, some of the organic solvents used include; Acetone, Xylene, Toluene, methyl ethyl ketone and ethyl benzene [2]. There have been many studies by different authors on the individual chemicals, but few on its possible synergetic effect considering that Carburetor cleaners contain more than one of these solvents. Automobile mechanics are the set of persons predisposed to the exposure of Carburetor cleaners, especially in developing countries where the level of education of the mechanics is usually low and adequate safety

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precautions is usually not adopted in handling the chemical (Carburetor Cleaners). The routes of exposure are usually oral, dermal and inhalatory routes. Acetone also known as propanone is a very common industrial solvent, it is colorless and flammable at room temperature [4]. An oral exposure to acetone can damage the mucosa of the mouth while when ingested in large amounts can lead to unconsciousness and death, and a dermal exposure can cause irritations and damage the skin [5]. Acetone is also known to target the central nervous system and may also cause drowsiness, confusion and lack of coordination [6]. It has also been linked to hypotension and digestive disorders [6]. Long term exposure to acetone according to animal studies include; kidney and liver damage, nerve damage, increased birth defect and a reduction in reproductive ability [5]. Exposure to the eye is can also cause irritation and permanent eye damage [5; 6]. A study conducted on a group of workers exposed to acetone indicated evidence of neurotoxicity [7]. Xylene is another component of carburetor cleaners, and it's also used as an industrial solvent (In the manufacturing of paints, varnishes and shellacs) and also in the medical profession. It is clear and has a sweet odour [8]. Exposure to Xylene causes a range of effects depending on the duration with <14 days (acute) and >365 days (chronic), also the amount of Xylene being exposed to determines its adverse effect [8]. Inhaling Xylene can lead to depression of the central nervous system which can cause headaches, dizziness, nausea and vomiting. This effect on the central nervous system is due to the liposolubility of Xylene in the neuronal membrane [9; 8; 10]. Long term exposure can cause insomnia, agitation, impaired concentration and short-term memory; it can also cause irritation of the nose and throat [11]. Exposure to Xylene at levels up to 200ppm or more can irritate the lung, because chest pain, shortness of breath and extreme exposure can lead to pulmonary edema [11]. Xylene has also been linked to possible teratogenic effect based on animal information, it can also lead to death when ingested [12]. Toluene is another component of carburetor cleaner and found also in gasoline, shoe polish etc. [13], Toluene have been reported to damage the central nervous system, it is also known to cause irritation of the eyes, dizziness, muscle spasticity, loss of muscle strength etc. the long-term effect varies depending on age, duration of exposure and frequency with general cognitive impairment being associated with heavy long-term exposure [14; 15]. Methyl ethyl ketone (MEK) is another component of Carburetor Cleaners and it's quickly absorbed by ingestion, inhalation and dermal exposure, acute exposure to MEK causes irritation of the eyes and nose, lack of coordination, abnormality in brain, liver, lungs and kidney while chronic exposure leads to decrease in brain weight and slight anemia [16]. Ethyl benzene has been classified as potential carcinogen by IARC, and some experiments conducted using rats and mice showed increased incidence of kidney and testicular tumors in male and female rats, lung tumors and liver tumors in male and female mice respectively [17]. The aim of this study is therefore to determine the effects of exposure to Carburetor cleaning agents on reproductive and haematological parameters in albino rats and relating the effect to humans considering that humans share similar physiology with albino rats.

2. Material and methods

2.1. Experimental design

Twenty-five (25) male eight (8) weeks old albino rats weighing 240 – 250 g were used for this experiment, and were allowed to acclimatize to laboratory condition (25°C). They were housed in wire mesh cages during the duration of study. Complete Randomized design was used and the animals were divided into five different groups and carburetor cleaner solvent was used as the treatment; Group 1(control, no chemical), Group 2 (1 ml/kg, inhalatory), Group 3 (1 ml/kg, Incorporated in feed), Group 4 (0.01 ml, orally) and Group 5 (a drop on the skin, Dermal route).

2.2. Biochemical Analysis

Standard procedures were ensured during the collection of the blood, and sperm samples prior to biochemical analysis. Sperm fluid/semen was collected from the sperm duct by maceration on the glass slide and the analysis procedure was done according to [18], Sperm motility, viability and abnormalities were determined using one step eosin method [19] and the epididymal sperm count was done with Neubauer haemocytometer (Deep 1/10 mm, LABART, Munich, Germany) and light microscope at 40× magnifications.

2.3. Method of Data Analysis

Data were analyzed using Tukey test at a level of 5% probability, using Assitat Software Version 7.7 en (2017).

3. Results

3.1. Effects of Carburetor cleaner on Semen analysis of Albino Rats

The results for semen analysis is outlined in Table 1, The control group (group 1) had a mean value of 66.25 for M1 (Motility quantitative), while the group exposure to the treatment through the inhalatory route (group 2) had a mean

value of 52.50, the group that had the treatment incorporated in their feed (group 3) had a mean value of 43.75, those through the oral route (group 4) had a mean value of 31.25 and those exposed through the dermal route (group 5) had a mean value of 23.75. Statistically, there were significant differences among the groups when comparing group 2, 3, 4 and 5 with the control group (Group 1). Group 1, Group 2 and Group 3 had a mean value of 2.0 respectively for M2 (motility quantitative 2), while Group 4 and 5 had a mean value of 1.0 respectively. For D1 (Debris quantitative), group 1, group 2 and group 3 had a mean value of 6.25, 7.50 and 8.75 respectively while group 4 and group 5 had a mean value of 28.75 and 7.50 respectively with no significant difference ($P>0.05$) recorded when comparing group 2 and group 3 with the control while group 4 and group 5 had a significant difference ($P<0.05$) when compared to the control. The sperm count carried out (C) had a mean value of 53.25, 47.50 and 42.50 in group 1, group 2 and group 3 respectively, while it had a mean value of 36.25 and 37.25 in group 4 and group 5. Group 2 was the only group that had no significant difference ($P>0.05$) when compared to the control. The Morphological primordial (Mp) had a mean value of 6.25 respectively in group 1 and group 2 while group 3, group 4 and group 5 had a mean value of 17.75, 27.50 and 30.0 respectively. There was a significant difference ($P<0.05$) for group 3, 4 and 5 when compared to the control. Group 1, 2 and 3 had a mean value of 1.0, 1.0 and 1.25 respectively while group 4 and group 5 had a mean value of 1.5 and 1.75 respectively for Ms (Morphological structure) with a significant difference only in group 3, 4 and 5.

3.2. Effects of Carburetor cleaner on Haematological Parameters of Albino Rats

The results for the effect on carburetor cleaner on Haematological parameters of Albino rats are shown in Table 2. Group 1, 2 and 3 had a mean value of 47.5, 40.0 and 35.0 respectively for Pack cell volume (PCV) and group 4 and group 5 had a mean value of 20.5 and 23.25 respectively for PCV also with significant difference ($P<0.05$) in group 3, 4 and 5 when compared to the control group and only group 2 had no significant difference ($P>0.05$).

Table 1 Semen Analysis

	M1	D1	C	Mp	Ms
Group 1 (Control)	66.25 ± 2.39 ^d	6.25 ± 1.25 ^a	53.25 ± 2.69 ^b	6.25 ± 1.25 ^a	1.00 ± 0.00 ^a
Group 2 (Inhalatory Route)	52.50 ± 3.23 ^c	7.50 ± 1.44 ^a	47.50 ± 4.80 ^b	6.25 ± 1.25 ^a	1.00 ± 0.00 ^a
Group 3 (Feed Incorporated)	43.75 ± 2.39 ^b	8.75 ± 1.25 ^a	42.00 ± 1.08 ^a	17.75 ± 5.17 ^b	1.25 ± 0.25 ^b
Group 4 (Oral Route)	31.25 ± 3.39 ^b	28.75 ± 3.15 ^b	36.25 ± 5.14 ^a	27.50 ± 4.33 ^c	1.50 ± 0.29 ^b
Group 5 (Dermal route)	23.75 ± 3.39 ^a	7.50 ± 1.44 ^b	37.25 ± 2.25 ^a	30.0 ± 2.04 ^c	1.75 ± 0.25 ^b

Key: Motility quantitative (M1), Debris quantitative (D1), sperm count (C), morphology primordial (Mp) and morphology structure (Ms); ^{a-b} Different letters in the same column indicate significant difference ($P<0.05$) within the group; ^{A-B} Different letters in the same column indicate significant difference ($P<0.05$) across the group

Table 2 Haematological Analysis

	PCV	Hb	RBC	McV	McH	McHc
Group 1 (Control)	47.50 ± 3.23 ^c	15.38 ± 0.44 ^c	5.27 ± 0.28 ^c	9.08 ± 0.76 ^a	2.94 ± 0.21 ^a	0.32 ± 0.01 ^a
Group 2 (Inhalatory Route)	40.00 ± 3.44 ^c	14.25 ± 1.16 ^c	4.15 ± 0.20 ^b	9.59 ± 0.45 ^a	3.42 ± 0.15 ^a	0.36 ± 0.00 ^b
Group 3 (Feed Incorporated)	35.00 ± 2.04 ^b	12.10 ± 0.75 ^b	3.57 ± 0.37 ^b	10.01 ± 0.86 ^a	3.45 ± 0.22 ^a	0.35 ± 0.00 ^b
Group 4 (Oral Route)	20.50 ± 2.26 ^a	8.30 ± 0.43 ^a	2.33 ± 0.17 ^a	8.77 ± 0.60 ^a	3.59 ± 0.25 ^a	0.41 ± 0.24 ^b
Group 5 (Dermal route)	23.25 ± 2.49 ^a	8.78 ± 0.22 ^a	1.85 ± 0.45 ^a	14.66 ± 3.22 ^b	5.51 ± 1.09 ^b	0.39 ± 0.03 ^a

Key: Pack cell volume (PCV), Haemoglobin (Hb), Red blood cell (RBC), Mean corpuscular Volume (McV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHc); ^{a-b} Different letters in the same column indicate significant difference ($P<0.05$) within the group; ^{A-B} Different letters in the same column indicate significant difference ($P<0.05$) across the group

When group 4 and group 5 were compared together, there was no significant difference ($P>0.05$). Haemoglobin (Hb) had a mean value of 15.38, 14.25, 12.1, 8.3 and 8.78 for group 1, group 2, group 3, group 4 and group 5 respectively with a significant difference ($P<0.05$) recorded in group 3, 4 and 5 when compared to the control. Group 1, 2, 3, 4 and 5 had a mean value of 5.27, 4.15, 3.57, 2.33 and 1.85 respectively for red blood cell (RBC) with significant difference ($P<0.05$) in group 3, 4 and 5. Mean corpuscular Volume (McV) had mean value of 9.08, 9.59, 10.01, 8.77 and 14.66 for group 1, group 2, group 3, group 4 and group 5 respectively with no significant difference ($P>0.05$) in group 2, 3 and 4 when compared to the control group. Group 1, 2, 3, 4 and 5 had a mean value of 2.94, 3.42, 3.45, 3.59 and 5.51 respectively for

McH (Mean Corpuscular Haemoglobin). Statistically, there was also no significant difference ($P>0.05$) in group 2, 3 and 4 when compared to the control group. For McHc (Mean Corpuscular Haemoglobin Concentration), group 1 had a mean value of 0.32, group 2 had 0.36, group 3 had 0.35, group 4 had 0.41 and group 5 had 0.39 with no significance difference ($P>0.05$) recorded for only group 2, 3 and 4.

4. Discussion

The experiment carried out generally had a negative impact on the albino rat as indicated by the abnormally high and low haematological parameters in the treated group compared to the control group this implies that carburetor cleaners contain chemicals that generally affects the body negatively. Looking at the different routes of exposure, there were some routes of exposure that had a more adverse effect than the other. This implies that the route of exposure plays a significant role in the toxicity of chemicals. This is because some routes may have more resistant mechanisms, while some routes may be more vulnerable to toxic effects. The pack cell volume (PCV) was observed to be generally lower in the treated group than in the control group, Pack cell volume measures the amount or percentage of blood that is composed of red blood cells [20], factors that raise or increase the amount of other blood constituent generally decrease PCV [21]. One of the factors identified is an abnormal increase in white blood cells due to infection and diseases [22]. Damages to the kidney can also lead to lower value of PCV because the kidney also plays a pivotal role in regulating the PCV [23]. Damages done to the kidney (leading to kidney diseases) also affects the red blood cell negatively by reducing the amount of red blood cell in circulation and this might be why the red blood cell analyzed was also low, the kidney is known to play a vital role in red blood cell production via erythropoietin hormone secreted by the peritubular capillary lining cells of the kidney [24; 25; 26]. Chemicals present in carburetor cleaners that is known to cause damages and tumors to the liver and kidney include; Acetone, MEK and Ethyl benzene [5; 16], This shows that carburetor cleaner in the cause of damaging the liver and kidney indirectly reduce the amount of red blood cells, PCV and Hb thereby inducing anemia at the end. Ethyl benzene which is derived from benzene is also linked to the cause of a type of anemia called Aplastic anemia [27]. McV was also observed to be high in the treated groups except in group 4 when compared to the control group. High McV indicates macrocytic anaemia [28], this might be because the general low amount of red blood cells must have caused the few red blood cells produced to be larger in size in a bid to meet up with the demands of oxygen by the body. This is based on the biological phenomenon called physiological hypertrophy, although this is usually common among muscle cells [29]. McH was generally high in the treated groups compared to the control group and according to [30] a high value of McH is a sign of macrocytic anemia. This means that carburetor cleaners have the ability to induce anemia in exposed animals. High level of McH is known to arise due to liver damage, and studies have shown that acetone which is one of the components of carburetor cleaners can cause liver damage [5]. Toluene is also established to cause damages to the liver especially when ingested [31], Hence it can also be said that Carburetor cleaners can damage the liver when one is exposed. McHc which measures the amount of haemoglobin in each red blood cell was also observed to be generally higher in the treated groups compared to the control group even though the variations were affected by the routes of exposure. High McHc is known to indicate anemia [32], All this effects on the haematology are due to the adverse effect of Carburetor cleaners. The overall result was affected by the routes of exposure, hence even though the treated groups showed a negative result; some groups with certain routes of exposure showed a more adverse effect pointing out that the toxicity of the treatment were affected by the routes of exposure. For PCV, HB and McV, group 4 had the least value of 20.50, 8.30 and 8.77 respectively; showing that exposure through the oral route had the most adverse effect on PCV, HB and McV this might be because in the oral route, the treatment is rapidly absorbed and transported to the liver for detoxification and in the course of that over labors the liver and damages it faster in the process compared to other routes of exposure. In RBC, the least value was recorded in the group exposed through the dermal route (group 5) while the same group had the highest value for McH when compared to the control group with a significant difference ($P<0.05$). Group 4 (oral route) had the highest value of McHc 0.41 compared to 0.32 in the control group. In the semen analysis, the treated group showed low sperm motility when compared to the control group with a significant difference across the group ($P<0.05$). The debris quantitative was higher in the treated groups compared to the control group, In the Mp and Ms, the very high values recorded in group 3, 4 and 5 indicated that the mode of exposure increases the reproductive toxicity of the solvent (carburetor cleaner), the sperm count (C) was also generally low in the treated groups compared to the control group. This result is in agreement with [33] and [34] that reported a negative effect of organic solvents such as acetone on sperm quality and might be due the disruption of testicular physiology by solvents like acetone and ethyl benzene [17].

5. Conclusion

The results clearly points out that exposure to carburetor cleaners have serious adverse effect on mammals due to the constituting organic solvents, since man shares similar physiology and anatomy with the mammalian rat, it is advisable for exposure prone individuals such as auto-mechanics to be properly enlightened on the risk associated with the use

of the solvent and also on the protective measures to adopt to minimize contact with the solvent and prevent the possible detrimental health effects.

Compliance with ethical standards

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All individuals who have contributed to this work have been listed as authors.

Disclosure of conflict of interest

No potential conflict of interest reported by the authors.

Statement of ethical approval

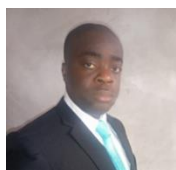
University standard written ethical permission was sought for, granted and has been preserved by the author(s).

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