ISSN No:-2456-2165

Effect of Activated Charcoal on Sperm and Haematological Parameters of Wistar Rats Induced with Snake Venom

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Abstract:- Activated charcoal is a form of carbon processed to have small and low-volume pores that increases the surface area available for adsorption or chemical reactions. The adsorbent nature of the activated carbon can draw toxins from the body. It is cheap, available, affordable, and accessible. In Nigeria, where snake bites are rampant and the cost of chelation therapy is high, it becomes imperative to explore readily available and natural antidotes in the management of snake poisoning. The present study investigates the effect of prepared activated charcoal on sperm and hematological parameters of wistar rats induced with snake venom. The studied rats were grouped into four, four rats per group based on the treatment: group I: Control, Group II; Activated charcoal only, group III: activated charcoal and venom and Group group IV: venom only. Semen samples were thereafter collected from the cauda epididymis and blood was collected through ocular puncture. Sperm analysis and haematological parameters were determined using standard procedures. The sperm cell count, motility, live/dead and volume of cells were used to study the effect of activated charcoal on testicle of Wistar rats induced with snake venom. The result of sperm analysis showed sperm motility for group I (75±2.40), group II (40±0.40), group III (50±0.51), group IV (57.50±0.75); Live/dead ratio: group I (96.5±0.76), group II (60±0.52), group III (84.50± 2.19), group IV (93.25±2.24); sperm volume group I (5.18±0.01), group II (5.18±0.01), group III (5.18±0.01), group IV (5.18±0.01); while for the sperm count group I (140±0.54), group II (77.25±1.89), group III (75.5±1.91), group IV (79±1.99). The result of haematological parameters indicated packed cell volume for group I (47.00±1.92) group II (40.67±0.52), group III (44.00±1.93), group IV (41.33±1.03) and the rest followed the same trends. The result showed that activated charcoal has beneficial haematological and reproductive effects in Wistar rats. The sperm count was observed to have increased significantly (p>0.05) which is an indication that activated charcoal reduced spermatoxicity.

Keywords:- *Activated Charcoal, Wistar Rats, Snake Venom, Spermatozoa, Haematological parameters.*

I. INTRODUCTION

Activated carbon which is also known as activated charcoal, is a form of carbon processed to have small and low-volume pores that increases the surface area available for adsorption or chemical reactions. its degree of micro porosity is high, hence 1 gm of activated carbon has a surface area in excess of 3,000 m^2 (32,000 sq ft), as determined by gas adsorption. An activation level sufficient for useful application may be obtained solely from high surface area, further chemical treatment often enhances adsorption properties (Dillon *et al.,* 1999).

Activated carbon is usually derived from charcoal and is sometimes used as bioscher. When derived from coal it is referred to as activated charcoal. Activated coke is derived from coke. Activated carbon is carbonaceous, highly porous adsorptive medium that has a complex structure compose primarily of carbon atoms. The network of pores is activated carbons are channels created within a rigid skeleton of disordered layers of carbon atoms, linked together by chemical bonds, sacked unevenly, creating a highly porous structure of nooks, crannies, cracks and crevices between the carbon layers (Elliott *et al.,* 1999). Activated carbons are manufacture from coconut shell, peat, hard and soft wood, lignite coal, bituminous coal, olive pits and various carbonaceous specialty materials. Chemical activation or high temperature steam activation mechanisms are used in the production of activated carbons from these raw materials (Bourke, 1999). The intrinsic pore network in the lattice structure of activated carbons which is caused by exposing the activated carbon to heat at high temperature will cause little pockets or holes to form this makes it highly absorbent. The adsorbent nature of the activated carbon can draw toxins from the body. Hence it is used to remove poison from the skin when insects bite.

Activated carbon has become a popular ingredient in beauty and skin care but there is no much research to this effect. The adsorption of metals to activated charcoal is poor and consequently it is seldom used in management of metal poisoning though it is associated with treatment of poisoning substances. It can also be used to treat chronic kidney disease

which enhances the outcome of renal dialysis (Alkhatib and Al Zailaey, 2015).This is the key the performance of activated carbon can be derived from many different sources and produced in varying production process. The raw materials used. Activation process and process parameters determine the physical properties and performance characteristics of the resulting carbon. Snakebites envenoming comprise of a major public health problem among communities from the Savanna region of West Africa notably in Benin, Burkina – Faso, Cameroon, Ghana, Nigeria and Togo. Snakebite envenoming with an evidence of about 100,000 persons per year (Kasturiratne *et al*., 2008) comprises public health which is major problem among region of west Africa, notably in Benin, Burkina-faso, Cameroon, Ghana, Nigeria and Togo. Victims died and amputations occured from snake bites. Several studies showed that activated carbons have been found useful in the treatment of poisons but little or no work has been reported on the use of activated charcoal to treat snake poison and its usefulness cannot be over emphasized. Hence, this research work was designed to determine the effect of activated charcoal on the sperm, kidney and liver of wistar rat induced with snake venom

II. MATERIALS AND METHODS

- *Collection, preparation of sample and activation of the sample*

African star apple fruits were purchased from the market and the seeds were removed. The seeds will be broken to separate the shell from the dicotyledon after which the shells will be sundried for two weeks and some days. The dried shells will be grinded into powder using a mechanical grinder. The powdered shell will be stored in a cool and dry place for further analysis.

Approximately 15.1 g of the raw sample was mixed with 15 cm^3 of 1M H_3PO_4, $ZnCl_2$ and KOH separately. The sample mixture was kept for 24 hours after which they were put into a furnace at 800 ^oC for 5 mins. The sample was removed and cooled in ice water bath; excess water was drained and allowed to stand at room temperature (Gimba et *al.,2004]*. The activated carbon generated was washed using 0.1M HCl to remove surface ash followed by rinsing with distilled water to remove residual acid. The sample was then dried in an oven at 110 ^0C for 1 hour. Washing was completed when a pH of 6-8 was ascertained (Ahmendna et *al.,* 2000).

III. CHARACTERISATION OF THE ACTIVATED SAMPLE

- *Determination of Bulk Density (P_b)*

Laska method was used to determine the values of bulk density P_b. To determine P_b. the glass beaker was emptied, desiccated and tarred, then it was filled to overflowing with each absorbent. The sides of the beaker was tapped lightly ten times with glass rod, then leveled by rolling the rod across the edge of the beaker six times. Then the beaker was reweighed and the medium density was calculated according to (Laska *et al.,*2005).

$P_b = \underline{M_t}\ V_t$

Where P_b = Bulk Density
M_t = Mass
V_t = Volume

- *Determination of pH Measurement*

Approximately 1.0 g of each activated carbon samples was put inside a 25 ml Erlenmeyer flask separately. Then 100 ml of distilled water was be poured inside the Erlenmeyer flask individually. The solution was heated separately for 5 minutes in gentle boiling condition. Later, the solutions was cooled at room temperature and distilled water was added to 100 ml of the solution and was stirred well, the pH of the solutions was determined separately using a calibrated pH meter (Ahmedna *et al*., 1997).

- *Ash Content Determination*

Approximately 1.0 g of each activated carbon samples was weighed into a crucible dish and the dried samples were transferred into a crucible. The samples ignited until it was completely burned using muffle furnace at 500 ^oC for 4hours and then it was cooled in desiccators. The weight loss is obtained and the ash content is calculated using the equation below (Bansode *et al.,* 2003).

$$\text{Ash content} = \frac{\text{weight of sample after ash process}}{\text{Weight of sample before ash process}} \times 100\ \%$$

Moisture Content Determination

According to Standardization and Industrial Research Malaysia (SIRIM) method approximately, 1.0 g of each activated carbon samples was weighed in a crucible dish and was placed in a drying oven for 2 hours at 110 ^oC. The samples were then cooled in a desiccators and weighed immediately to prevent moisture absorption. The moisture content was then determined using the equation below (Yoshiyuki and Yutaka, 2003).

$$\text{Moisture content} = \frac{\text{loss of weight}}{\text{Weight of the sample after drying}} \times 100\ \%$$

- *Determination of Percentage Yield*

The percent yield for each activated carbonwas obtained by taking initial mass of sample at the end of the activation process. The percentage yield was then determined using the equation below (Yoshiyuki and Yutaka, 2003).

ISSN No:-2456-2165

$$\% \text{ yield} = \frac{W_f}{W_i} \times 100\,\%$$

Where W_f = the initial mass of the dry impregnated samples
W_i = the final mass of the sample at the end of the activation process.

IV. PREPARATION OF ACTIVATED CARBON MASK

- 2 ½ teaspoons of the unflavored gelatin with 2 tablespoons of water in a microwave-safe bowl was properly mixed.
- A teaspoon of activated carbon was added, Mixed thoroughly but gently until the charcoal was thoroughly mixed with the gelatin to avoid any clumps.
- The mixture was placed in the microwave for 10 seconds. This allowed the mixture to thicken. it was stirred for a few more times to make sure no clumps after the mixture was removed from the microwave,.
- The mixture was placed in the freezer for 3 minutes to cool it down.

V. COLLECTION OF WISTER RATS

36 Male rats between 6 – 8 weeks old were purchased from animal house, University of Ibadan, Ibadan, Oyo state. Their weights were between 95 – 123 g, the rats were acclimatized for 21 days before snake poison was introduced. The rats had free access to rat pellets and water throughout the experimental period, the snake venom was induced orally by means of cannula.

The studied rats were grouped into four based on the treatment:
Group 1: Control
Group II: Activated Charcoal only
Group III: Activated charcoal + Venom
Group IV: Venom Only

➢ *Semen sample collection*

The rats were sacrificed after cervical dislocation, Orchidectomy was performed by open castration method, a pre-scrotal incision was made and the testicles were milked out of the incision site, the spermatic cord was exposed, litigated and incised. Semen samples were thereafter collected from the cauda epididymis(oyeyemi and Ubiogoro,2005).

➢ *Sperm Volume.*

The volume was determined by using a calibrated measuring cylinder.

➢ *Sperm Count.*

The spermatozoa was counted by hemocytometer using the improved Neubauer (deep 1/10 mm, labart, Germany) Chamber as described by Pant and Srivastava ,(2003). Sperms counted from each rat ranged between 400 and 410, these were used for morphological studies.

➢ *Morphological abnormalities and percentage viable cells assay*

They were determined from total 400 spermatozoa in smears obtained with Wells and Awa stains (0.2 g of Eosin and 0.6 g of Fast green dissolved in distilled water and Ethanol in ratio 2:1). Live/ Dead ratio was determined using 1%Eosin and 5 % nigrosinin, 3 % Sodium Citrate dehydrate solution (Saba *et al*., 2009)**.**

➢ *Collection of blood samples and Analysis*

Wistar rats were anaesthetized with diethyl ether after which blood samples were obtained from the tails of the rats into heparinized bottles collected blood samples were immediately centrifuged to separate the red blood cells from serum (Okolo *et al*., 2016). The packed cells volume(PCV) were determined by using haematocrit centrifuge at 300rpm for 5 minutes. Haemoglobin cells, red blood cells (erythrocyte indices) were determined by using Heamacytometer (Deice and Levis, 1991).

➢ *Statistical Analysis*

Student t-test was used to analyze the data, the difference of the means were considered significant at $p< 0.05$

VI. RESULT AND DISCUSSION

The sperm cell count, motility, live/dead and volume of cells were used to study the effect of activated charcoal on testicle of Wistar rat induced with snake venom. This evaluation was done to determine the fertility of the male rat. Alteration of the sperm cells in this study can be grouped into primary and secondary abnormalities according to the classification by Noarkes *et al*., (2004), abnormalities in the studied rats. Sperm abnormalility tail cell. Rudimentary tail abnormality was the only primary sperm abnormality,while bent mid-piece, curved mid-piece, bent tail,curved tail, normal tail, without head, normal head without tail and looped tail were the secondary abnormalities observed. The abnormality observed rudimentary tail sperm was slightly higher in group II than that of the control. Sperm cells with rudimentary tails are usually immotile and are unable to fertilize mature ovum. Bent mid-piece and bent tails were higher followed by curved-mid piece. The other secondary sperm abnormalities had no significant difference with the values observed in control. Secondary abnormalities arise as a result of a fundamental problem with the process of maturation where abnormal sperm cells are matured from damaged seminiferous tubules (Thomas and Thomas, 2001). The sperm motility of the studied rats were significantly lower than that of the control, sperm ratio for live/dead were lower in group II while groups III and IV were in the same range with the control . This indicated that the venom (group IV) and activated charcoal (group III) did not affect the liveability of spermatozoa but the mixture of both (group II) caused deformation of the cells and rendered them less motile or immotile. These observations are similar to those of Adebowale *et al*.,(2009) but contrary to the report Sof Farombi *et al*.,2007 and Ishihara *et al*.,2000 who

discovered that *Curcuma longa Garcia* kola prevented peroxidative changes in sperm and testicular. The sperm count was observed to have reduced significantly ($p>0.05$)which is an indication that activated charcoal reduced spermatogenesis, this is similar to the observation of Adebowale *et al*.,(2009) and Parveen *et al*.,(2003) but it is contrary to the observation of Abel-magnied *et al*.,(2001). They reported increase in sperm count when extracts of root and leaf of *Leganaria breviflora* were induced in Wistar rats.

The results of the effect of activated charcoal demonstrated the ability to reduce packed cells volume (PCV) by 13. 46 %. Activated charcoal group (II) and venom (group IV) reduced the White blood cells count (WBC) when induced only but when the mixture of both (group III) was administered the activated charcoal was able to reduce the effect of the venom on the blood parameters this is in accordance with the report of Nwafor,(2003) and (Sandanghmatirah,(2017), they observed activated charcoal reduction in PCV, WBC, Hb and erythrocytes parameters in ration administered with anti-malaria drug (Quinine). Activated charcoal counteracted with the inhibitory compounds in the venom which perhaps is capable of affecting the heamatological profiles this may result from the fact that the molecules of activated charcoal compete with that of venom. It was observed that activated charcoal doesn't enhance the elimination of substituted which have already been absorbed into the system circulation but constitute a useful method for the removal of the compounds remaining in the gastro intestinal track (Tominaru, 1996). The venom reduced the heamatological parameters, activated charcoal had a beneficial effect in reducing the toxilogical but the result shows Effects of poisons on blood profile when given as early as possible.

In the study, treatments of rats with activated charcoal and mixtutre of activated charcoal and venom caused a slight increase in the activity of serum total protein, albumin and ALP while decrease was observed in ALT and AST.this is contrary to the report of Azoz and Rafat,2012, Ibrahim *et al*.,2012 and Azah, 2014. They observed an increase in activity of serum, Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline phosphatase (ALP) with increase in total protein and albumin. Samuel *et al.*,2017 observed a reversed when treated with activated charcoal, this is in accordance with the observation of this study. Increase in ALT and AST levels in rats show damaged to the structural integrityof the liver. This is due to the leakage of these enzymes from liver cytosol into the blood stream(Concept et al., 1993).the high AST and ALT activities are accompanied by high liver microsomal membrane fluidity, free radical generation and alteration in the liver tissue (Ibrahim et al,2012). Increase level of ALP shows damage or an obstruction of the bilary treewhich distrupt flow of blood to the liver (Farida *et al*,2012).. The decrease in serum levels of these enzymes may be due to the prevention of their leakage from the liver cytosolby activated charcoal probably due to reduction in blood level by venom.

The serum urea was deceased significantly with the administration of activated charcoal in this study but showed only a slight increase in creatinine, decrease in serum urea in similar to the observation of Cheong and Roh,2006, they observed a slight decrease in serum urea but also observed a decrease in creatine level this is not in accordance to this study because a slight increase was observed in the creatinine serum level. this indicate activated charcoal protect the snake venom induced toxicity on kidney. Activated charcoal with low protein diets have been reported to control effectively some uremic symptomsin patients with different stagesof renal disease,this is achieved through the binding of urea and otherurinary toxins to charcoal and its excretion with feaces this creates a concentration gradient for continued diffusion of these toxins.Ash,2009 slight increase in serum protein showed showed no damage to both hepatic and renal damage, it also indicates that no binding veom to plasma proteins which can cause the alteration in a high number of enzymes and can also prevent protein synthesisin hepatocytes Goering,1993. Increase in serum protein ccontents may be attributed to an increase in hepatic Deoxyribonucleic acid (DNA) and Ribonucleic Acid (RNA).

Table 1 : Characteristics of Studied Wistar rats Spermatozoa

Treatment	**Motility**	**Live/Dead**	**Volume**	**Count**
Group 1				
1	70±2.50	98±0.77	5.2±0.01	145±2.38
2	80±2.44	95±0.75	5.1±0.01	133±1.87
3	70±2.50	98±0.77	5.2±0.01	139±1.94
4	80±2.44	95±0.75	5.2±0.01	144±2.35
Mean+SD	75± 2.40	96.5±0.76	5.18±0.01	140±0.54
Group II				
1	10±0.05	40±0.34	5.2±0.01	80±2.12
2	40±0.32	60±0.56	5.2±0.01	69±1.66
3	50±0.55	60±0.49	5.1±0.01	81±2.34
4	60±0.67	80± 0.68	5.2±0.01	79±1.44
Mean+SD	40±0.40	60±0.52	5.18±0.01	77.25±1.89

Group III				
1	60±0.86	98±3.12	5.1± 0.01	88±2.45
2	20±0.06	60±1.76	5.2±0.01	66±1.34
3	70±0.89	85±0.98	5.2±0.01	79±1.98
4	50±0.22	95±2.88	5.2±0.01	69±1.76
Mean +SD	50±0.51	84.50± 2.19	5.18±0.01	75.5±1.91
Group IV				
1	60±0.88	98±3.11	5.2±0.01	88±2.43
2	50±0.56	95±2.77	5.1±0.01	78±1.86
3	60±0.77	85±0.97	5.2±0.01	69±1.66
4	60±0.77	95±2.11	5.2±0.01	81±1.99
Mean+SD	57.50±0.75	93.25±2.24	5.18±0.01	79±1.99

Table 2 : Deformed sperm

Mean sperm deformed	**Group I**	**Group II**	**Group III**	**Group IV**
Tail-less head	4.50±0.02	4.50±0.33	5.00±0.04*	4.25±0.07
Headless tail	4.50±0.01	5.00±0.03	4.75±0.02	4.0±0.05
Bent tails	8.00±0.44	11.25±1.07*	11.5±0.06*	10.25±0.85*
Curved Tails	8.75±0.37	10.75±1.16*	11.50±0.06*	11.25±0.91*
Bent Mid-piece	8.75±0.05	11.5±0.08*	11.50±0.36*	10.25±0.90*
Looped tail	2.00±0.01	3.0±0.02*	3.0±0.02*	2,25±0.02
Curved mid- piece	7.25±0.05	9.75±0.67*	11±0.04*	10.5±0.87*
Rudimentary tails	2.00±0.02	2.25±0.06	1.75±0.02	1.75±0.02
Total mean deformed sperm	37.00±0.97	58.00±3.42	60.00±0.62	54.50±3.69
Total cells	406.25±4.25	405±4.70	405±4.70	403.75±4.50

Data was expressed as mean ± SD, * significantly different from control ($p<0.05$)

Table3: Effect Of Activated Charcoal on Heamatological parameters

Heamtological parameters	**GroupI**	**Group II**	**GroupIII**	**Group IV**
Packed Cell Volume (PCV)	47.00±1.92	40.67±0.52	44.00±1.93	41.33±1.03
White Blood Cell Count (WBC/L)	9.62×10^9 ±0.39	8.9×10^9 ±0.85	8.9×10^9 ±0.58	7.70×10^9 ±1.5
Neutrophil (N)	36.67 ±0.64	33.33 ±1.28	33.67 ±1.03	14.00 ±0.39
Lymphocytes (L)	65.67 ±4.75	62.00 ±1.54	60.00 ±0.00	59.67 ±0.26
Monocytes (M)	16.33 ±3.23	3.33 ±0.27	04.00±0.39	3.33±0.18
Eosinophil (E)	0.33±0.13	2.00 ±0.77	2.33 ±0.37	2.00 ±0.48
Basophil (B)	0.00±0.00	0.33 ±0.43	1.00 ±0.09	0.33 ±0.19
Platelet Count (PLT)	544×10^9 ±104.70	744.33×10^9 ±109.07	642.33×10^9 ±26.04	574.33×10^9 ±43.61
Mean Cell Volume (MCV/Red Cell)	64.30 ±0.65	63.20 ±0.42	59.73 ±1.64	62.20 ±0.11
Mean Cell Haemoglobin (MCH)	21.69 ±0.38	20.93 ±0.07	19.50 ±0.08	20.90 ±0.06
Mean Corpuscular Haemoglobin Concentration (MCHC)	33.93 ±0.40	32.77 ±0.11	31.80 ±0.96	32.77 ±0.09

Table :4 Effect of activated charcoal on Serum levels of AST,ALP,ALT, Protein and Albumin

Treatment	Control	AC Only	AC + Venom	Venom Only
Total Protein g/dl	3.10±0.04	3.37±0.03	3.10±0.04	3.17±0.06
Albumin g/dl	1.20±0.04	1.37±0.03	1.47±0.03	1.40±0.08
ALP U/L	15.33±2.18	15.67±2.18	23.00±11.93	15.33±0.26
AST U/L	437.00±87.38	339.00±92.76	228.33±32.97	339.00±50.98
ALT U/L	121.00±43.49	91.00±4.23	85.33±6.41	81.33±11.80

ALT: Alanine Transaminase , AST: Aspartate Transaminase and ALP: Alkaline phosphatase

VII. CONCLUSION

It can be concluded that the snake venom increase sperm cell abnormalities but introduction of activated charcoal reduced the abnormalities. Activated charcoal seems to be protective against hepato-renal damage induced the snake venom

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