Vitamin E, Liver And Kidney Functions Of Rabbit During Hypothermia

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Abstract: Effects of oral vitamin E supplementation on the health statuses of liver and kidney of rabbits during hypothermia were studied. 24 male New Zealand White rabbits of 8 – 10 weeks old and weighed about 800 – 1000g were used in the study. The rabbits were randomly assigned to three treatment groups as: T_1 (control), T_2 (hypothermia) and T_3 (hypothermia + vitamin E). Experimental duration was 6 weeks. Animals were fed similar diets throughout the experimental period except that T_3 animals received oral vitamin E daily at 460mg/kg body weight in the last 4 days of study. 24 hours after the last administration of vitamin E T_1 rabbits were dipped into water of body temperature (37^0C) whereas T_2 and T_3 rabbits were dipped into water of 10 - 12^0C with their heads up for 5-minutes after which their blood samples were immediately collected and snap frozen. Body temperatures of the animals after dipping were T_1 (37.9 ± 0.5), T_2 (34 ± 1.0) and T_3 (34.2 ± 0.7), respectively. Alanine amino transferase (ALT) serum concentrations were similar ($P > 0.05$) for T_1 and T_3 but T_2 had significant higher ($P < 0.05$) ALT. Aspartic amino transferase (AST) was significantly higher ($P < 0.05$) in T_2 compared with T_1 and T_3 that had similar ($P > 0.0$) AST values. Alkaline phosphatase (ALP) serum concentration was significantly different ($P < 0.05$) for T_1, T_2 and T_3 with T_2 showing the highest concentration. Blood urea nitrogen (BUN) levels were similar ($P > 0.05$) in T_1 and T_3 while T_2 had a significant higher ($P < 0.05$) BUN value. Creatinine levels were similar ($P > 0.05$) in T_1 and T_3 while T_2 had a significant ($P < 0.05$) higher value. It was concluded that vitamin E supplementation can up-regulate liver and kidney functions in rabbits during hypothermic condition.

Key words: Vitamin E, liver and kidney functions, hypothermia and rabbit

1 INTRODUCTION

In the rabbit hypothermia is defined as the fall in body temperature to less than 35^0C. When this occurs it can lead to the production of reactive oxygen species (ROS) leading to oxidative stress in the animals. During this period, the animal well-being can be compromised, especially in the absence of antioxidants to detoxify the products of ROS produced as a result of the oxidative stress [1]. Vitamin E is a well-known antioxidant vitamin for reducing oxidative stress in animals. In reducing the effect of oxidants, Vitamin E donates hydrogen atom from its hydroxyl group which reacts with hydroxyl radicals (oxidants) thereby reducing them to water and renders ROS harmless to the cells and tissues of the animals [2]. One of the factors militating against rabbit production is climatic condition as it relates to ambient temperature where they are produced [3]. To this point, rabbits often suffer from hypothermia, especially during the peak of prolonged rainy and harmattan seasons mimicking the winter period in the temperate region. At such periods efficiency of production is usually compromised as the animal is no more within its comfort zone [3]. Also, in deep hypothermic conditions ROS and hydroxyl radicals are produced leading to cells damages that can result in programmed cell death if ROS are not detoxified or removed from the cells of the animal [1]. The enzyme ALT is mostly abundant in liver of animals [4]. A high level of ALT in blood serum has been shown to be a dependable biomarker of oxidative damage in the cells of the liver of rabbits [5]. [6] has shown that the activity of ALT in the liver is 3000 times its activity in the blood serum and any damage in the cells of the liver will result to the releasing of high amount of ALT in the blood of the organism. [7] also showed that Vitamin E had a protective effect on the liver in the presence of oxidative stress evidenced by normal levels of ALT in Wister rats exposed to organophosphate pesticides compared to the control. AST is an enzyme that is found in tissues of organs such as the muscle, liver, kidney and heart and particularly in the liver [4; 7]. [5] demonstrated that ROS produced by some xenobiotics are capable of damaging some tissues of the liver, thereby releasing AST into the blood. ALP is another enzyme that has been demonstrated to be a good biomarker of oxidative stress in animals [4]. It is mainly found in the liver and bones but particularly in the liver. Just like other liver enzymes, they are released into the blood stream when there is an oxidative damage in the liver tissues, including the bile duct [8]. Blood creatinine is also used to determine the level of oxidative damage in kidney of rabbits. The study of [9] has shown that blood creatinine increased when rabbits were treated with cisplatin (chemotherapeutic agent). This suggests that creatinine is a good biomarker of oxidative stress. High concentrations of blood creatinine are indications of kidney damage or malfunction. [4 and 9] demonstrated that high level of BUN is associated with damage or malfunctioning of the kidney in animals under oxidative stress. This therefore also, suggests that BUN test is one of best practices to check the level of damage in the kidney when animals are subjected to oxidative stress, such as the one induced by cold stress. Therefore, the objectives of the study are to determine the effect of oral vitamin E supplementation on ALT, AST and ALP levels in serum to assess the health status and functioning of the liver of rabbits exposed to hypothermia as well as to determine the effect of oral vitamin E supplementation on serum creatinine and urea levels in rabbits exposed to short-term hypothermia to assess the health status and functioning of the kidney, respectively.

2 MATERIALS AND METHOD

Animals and Management

The experiment was carried out at the rabbitry section of the Teaching and Research Farm of the Rivers State University, Port Harcourt, Nigeria. Before the commencement of the study, the hutches, feeding and

water troughs, including the floor of the rabbitry were thoroughly washed with detergent, water and hypochlorite and allowed to dry for one week to ensure that the site is 'pathogenic-free' before the rabbits were introduced into the hutches. 24 male New Zealand White rabbits of 8-10 weeks of age and weighing between 800-1000g were used in the study.

Experimental Procedures-Animals

The 24 rabbits on arrival at the rabbitry wing of the Rivers State Teaching and Research farm of the Rivers State University were immediately weighed to obtain their initial body weights and randomly assigned to their hutches in three (3) treatment groups of eight (8) animals each as: T_1 (control), no vitamin E, no hypothermia; T_2, no vitamin E + hypothermia and T_3, vitamin E supplementation + hypothermia, respectively.

Experimental Diets

The rabbits in the three treatment groups were similarly fed for five weeks during which they became fully acclimatization to the study environment. They were given growers diet that were in pellet forms ad libitum twice per day and supplemented with Centrosema pubecen forage as to enable them practice refection. The experiment lasted for six weeks.

Administration of Vitamin E, Hypothermia and Blood Sample Collection

Rabbits in T_3 group received oral supplementation of vitamin E (dl- Alpha Tocopheryl Acetate) via drinking water at the level of 460mg/kg of body weight for 4 days in the sixth week of the experiment. Twenty-four (24) hours after the last vitamin E supplementation, hypothermia was introduced as: T_1 (Control) rabbits were immersed into water of body temperature (37^0C) up to the neck for 5 minutes without any anesthetic or tranquilizer. T_2 and T_3 rabbits were cooled by immersion into cold water (10-12^0C) also for 5 minutes. Blood samples were collected from individual rabbits immediately after dipping and snap frozen in all the treatment groups using a 2ml syringe via their ear vein and placed in well labeled ethylene diamine tetracetic acid treated vial tubes for the respective treatments for later analyses for liver enzymes and for kidney function biomarkers, respectively. Additionally, as the animals were dipped rectal temperatures were taken and recorded for all animals in each group.

Blood Sample Analysis - Liver Biomarkers

Alanine amino transferase and aspartic amino transferase were analysed according to the method of [10]. ALT catalyses the reversible transfer of an amino group from alanine to α-ketogluterate forming glutamate and pyruvate. The pyruvate produced is reduced to lactate by lactate dehydrogenase (LDH) and NADH. The spectrophotometer was set at 240nm and adjusted to zero with distilled water during the reading of ALT concentration. AST, the principle is based on formation of the chromogenic di-nitrophenylhydrazine of pyruvate. Di-nitrophenylhydazine reacts with α- ketogluterate as well as pyruvate. The absorbance value is usually high. The amino group is enzymatically transferred by AST present in the sample from aspartate to the carbon atom in α-oxaloacetate and L- glutamate. The intensity of the colour produced is directly proportional to the enzyme activity. AST was determined by measuring the increase in absorbance at 530- 550nm. Alkaline phosphatase was done according to the method of [11]. ALP of an alkaline pH hydrolyses p-Nitrophenylphosphate to form p-Nitrophenol and phosphate. The rate of formation of p-Nitrophenol is measured as an increase in absorbance which is proportional to the ALP activity in the sample. Absorbance was taken at 405nm.

Blood Sample Analysis - Kidney Biomarkers

Urea concentration was determined according to the method of [12]. Urea in acidic medium condenses with diacetylmonoxime at 100^0C to form a red coloured complex. Intensity of the colour formed was directly proportional to the amount of urea present in the sample. Creatinine was measured with alkaline picrate according to the method of [13].

Statistical Analysis

Data obtained were analysed using Analysis of Variance (ANOVA) of general linear model (GLM) procedure of SAS. Treatment means were compared using Tukey's test. Completely randomized design was used as the experimental design, and therefore the model was $Y_{ij} = u + X_i + E_{ij}$.

Where; Y_{ij}= individual observation of treatment, u = population mean, X_i= treatment effect and E_{ij}= the error term. A α-level of 0.05 was used for all statistical comparisms to represent significance.

3 RESULTS

Rabbits in all treatment groups were seen to be in good health throughout the period of the experiment as they were observed and checked daily. Mean body temperature of control group was significantly higher (37.9 ± 0.5) than those of hypothermic group without vitamin E (34 ± 1.0) and hypothermia group with vitamin E supplementation (34.2 ± 0.7), respectively. This was indication that hypothermia was experienced with animals in treatments T_2 and T_3, respectively. The results of ALT, AST and ALP serum concentrations are shown in Table 1

Table 1: *Serum Levels of Liver Biomarkers with or without Hypothermia*

	Treatments				
Items	T_1 n = 8	T_2 n = 8	T_3 n = 8	SEM	P-Value
ALT (iu/l)	104.88^a	110.25^b	106.50^a	0.9	0.028
AST (iu/l)	45.37^a	50.50^b	44.88^a	0.7	0.001
ALP (iu/l)	42.63^a	46.88^b	44.50^c	0.5	0.012

Means with different superscripts within the same row are significantly ($P < 0.05$) different; SEM = standard error of the mean. Legends: ALT = alanine amino transferase, AST = aspartic amino transferase and ALP = alkaline phosphatase

Hypothermia and vitamin E had effect on all the liver enzymes studied. ALT and AST levels were significantly

higher ($P < 0.05$) in T_2 rabbits compared with T_1 and T_3 groups that had similar levels ($P > 0.05$) of ALT and AST, respectively. Hypothermia had effect on ALP as ALP was significantly higher ($P < 0.05$) in T_2 rabbits compared with T_1 and T_3 groups. However, ALP level was significantly lower ($P < 0.05$) in T_1 group compared with T_3 group that also had a significant lower ($P < 0.05$) ALP level compared with T_2 group. The results of BUN and creatinine serum levels are shown in Table 2.

***Table 2**: Serum Levels of Kidney Biomarkers with or without Hypothermia*

	Treatments				
Items	T_1 n=8	T_2 n=8	T_3 n=8	SEM	P-Value
BUN(mmol/l)	4.64[a]	5.25[b]	4.66[a]	0.1	0.045
Creatinine (mmol/)	81.63[a]	96.00[b]	85.25[a]	1.3	0.001

Means with same superscripts within the same row are not significantly different ($P > 0.05$); SEM= standard error of mean. Legend: BUN = blood urea nitrogen.

BUN level was significantly ($P < 0.05$) higher in T_2 rabbits compared with T_1 and T_3 rabbits with similar concentration ($P > 0.05$) of BUN. Similarly, creatinine level was significantly higher ($P< 0.05$) in T_2 rabbits compared with T_1 and T_3 groups that had similar concentrations ($P > 0.05$) of creatinine.

4 DISCUSSION

ALT is an enzyme mainly found in the liver cells. The enzyme aids in the conversion of amino groups to pyruvate and glutamate [7]. Thus, ALT is involved in the metabolism of protein. However, abnormal high levels of ALT are often an indication of ALT released into the blood by the liver. High levels of ALT is usually associated with liver damaged or malfunctioning leading to liver leakage resulting in the high levels of ALT. In this study, hypothermia significantly increased ALT in T_2 group compared with T_1 and T_3 groups with similar levels of ALT. This is an indication that hypothermia could damage the liver. However, in the presence of vitamin E the adverse effect of hypothermia can be reduced or reversed. This finding agrees with that of [2]. These workers showed that vitamin E supplementation reduced ALT levels in the serum of animals and restored normal liver function during oxidative stress. AST is another liver enzyme that is used to determine the health status of the liver. Although AST is not as specific as ALT for liver test due to its presence in the heart and muscles, it is usually measured together with ALT to better diagnose for liver problems. During liver damage AST is released into the blood stream leading to high levels of AST in the blood indicating a problem with the liver or muscles. AST aids in catalyzing the conversion of α-ketogluterate and aspartic acid to glutamate and oxaloacetate [5]. In this study, hypothermia and vitamin E had effects on AST as AST was significantly higher in T_2 rabbits compared with T_1 and T_3 groups that had similar AST level. This suggests that vitamin E is capable of reversing damage done to the liver during hypothermia in rabbits by restoring AST level in the serum to normal. This is further confirmed in this study because the effects of hypothermia and vitamin E activities similarly affected ALT and AST in a similar fashion. This finding agrees with those of [2] and [4] who demonstrated that vitamin E supplementation reduced AST level in the serums of laying birds, mice and ruminants and also improved their liver function during oxidative stress induced by heat stress, obesity, and clinical mastitis according to their independent studies. Alkaline phosphatase is another enzyme found in bones, bile ducts and in the liver. Therefore, high level in the blood stream may indicate liver inflammation, blockage of bile ducts or a bone disease. It also plays a major role in protein synthesis, DNA, RNA and removal of phosphate groups in alkaloids [14]. In this study, hypothermia and vitamin E had effects on ALP as ALP was significantly higher in T_2 rabbits compared to T_1 and T_3 groups, respectively. However, ALP level was significantly lowered in T_3 rabbits compared with the T_2 group. This shows that vitamin E improved rabbit liver function during hypothermic condition. This finding also agrees with the finding of [5] and [8] who demonstrated decrease in ALP level when rabbits and gold fishes on oxidative stress induced by contraceptive pills and xenobiotics were treated with vitamin E, respectively.Blood urea nitrogen is a component of urea and the method which the body reduces excess of it through the kidney is by filtration. Blood urea nitrogen is by-product of protein metabolism and in these process amino acids gives rise to ammonia and later converted to urea which is transported by blood into the kidneys where they are finally eliminated. In this process, the kidneys filter the blood from urea and finally release urea into urine for elimination from the body [9]. From the finding of this study, BUN was significantly increased in T_2 rabbits compared with the other groups T_1 and T_3 that had similar levels indicating that hypothermia had effect on BUN. In other words, there was no significant difference in the group that received oral vitamin E (T_3) compared with control group (T_1), thus, indicating that vitamin E reduced BUN level in the serum of rabbits during hypothermia; thereby improved kidney function. This finding agrees with that of [15] who investigated the effect of oral vitamin E supplementation on oxidative stress in guinea pigs with hypothermia and found that hypothermia increased BUN but vitamin E aided its reduction and hence improved kidney function of the guinea pig during hypothermic condition.Creatinine is produced in the body during creatine metabolism and excreted via urine. It is the by-product of creatine phosphate in the muscle and they are fairly produced constantly by the body [9]. Therefore, increased level of creatinine in the serum is majorly due to the infiltration rate of the kidney indicating kidney failure or malfunction. The finding in this study showed that hypothermia had effect on creatinine level. Animals of T_2 had significantly increased levels of serum creatinine compared with the other two groups (T_1 and T_3) that had similar levels. This observation is an indication that vitamin E was able to reduce levels of creatinine in T_3 rabbits to the T_1 levels in the presence of hypothermia. It is also an indication that vitamin E can restore to normal the function of the kidney based on its effect on creatinine levels in the animal serum in hypothermic condition. This finding agrees with that of [16] who demonstrated that vitamin E supplementation on

haematological and plasma biochemical parameters during oxidative stress in goats induced by long term exposure to arsenic found that there was a decrease in creatinine level in the serum of goats with vitamin E dietary supplementation. However, this finding did not agree with that of [17], who investigated the effect of different vitamin E sources and levels on selected oxidative stress indices in blood and tissues as well as on rearing performance of slaughter turkey hens and found that there were no significant differences in their serum levels. This might partially be due to the level of vitamin E supplemented in their diet was not adequate to up-regulate the function of the kidney, as vitamin E was administered at 45mg/kg of body weight in that study.

5 CONCLUSION

It was concluded that vitamin E supplementation can up-regulate liver and kidney health statuses and thus better support their functions in rabbits during hypothermic condition based on the serum levels of known biomarkers of the liver and the kidney in the rabbit serum. Thus vitamin E can be used to improve liver and kidney functions in rabbits during hypothermic seasons during production.

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