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Research Article

Priapism-Induced Remote Alterations on Liver Histology, Liver Biomarker Enzymes and Haematological Parameters of Animal Models and the Attenuating Role of Ascorbic Acid

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Abstract: The remote effect of priapism on haematological indices and liver function is still obscured. We evaluated the reversal effect of vitamin C on remote changes in blood parameters and liver function of Sprague – Dawley rats. 25 male rats were randomly divided into 5 groups of 5 rats each. Group 1 served as the control and was treated with 5ml/kg body weight normal saline. Group 2 rats were induced with priapism. Group 3 was induced with priapism and then treated with 25 mg/kg body weight of vitamin C. Group 4 was induced with priapism and then treated with 2.5mg/kg body weight of testosterone. Group 5 was induced with priapism but were treated with both vitamin C and testosterone. Priapism was induced for one week followed by 6 hours post priapism administration of testosterone and vitamin C. Results showed markedly elevated liver enzymes in treated groups especially in groups 2 and 4. The haematological indices also revealed anaemia and leucopaenia in various treated groups. Although the role of both testosterone and Vitamin C was evaluated, only vitamin C treated groups showed improvement in blood parameters and liver function of Sprague-Dawley rats.
Keywords: priapism, liver function, Vitamin C, testosterone.

INTRODUCTION

Priapism is defined as an acute medical condition characterised by persistent and painful erection without sexual stimulation. It basically involve only the corpora carvernosa and usually last longer than 4h to 6h despite orgasm. Although the duration time of a normal erection before it is classified as priapism is still controversial, penile erections for more than 6 hours could lead to irreversible fibrosis with endothelial and trabecula destruction of the erectile tissue and consequently in unending erectile dysfunction and impotence [1-4].

It has been reported that Ischemia-reperfusion injury can lead to destruction in both local and remote tissues such as the liver and blood by generation of free radicals [5-6]. Ischemic reperfusion injury post treatment with various antioxidants has been evaluated in many organs [7]. Excessive generation of free radicals which exceeds the natural antioxidant defence mechanisms in the body results to oxidative damage of tissues [6, 8-9].

Several studies have indicated that additional vitamin C supplies in the body may improve the antioxidative defence. Vitamin C is a powerful antioxidant with the ability to slow or prevent the oxidation of other molecules [10]. Vitamin C as an antioxidant terminates the chain of the reaction by removing free radicals and inhibits other oxidation reaction by oxidizing themselves [11-12]. Antioxidant enhancements or antioxidant rich-foods may be used in dipping oxidative degeneration by free radicals [13].

The present study was aimed at evaluating the attenuating effects of vitamin C and testosterone on the liver histology, biomarker enzymes and some haematological parameters in animal models.

MATERIALS AND METHODS

Animals

Thirty adult male Sprague–Dawley rats weighing between 250 and 300g were housed in solid plastic cages in animal house of Anatomy department of the College of Medicine, University of Lagos and allowed to acclimatize for 2 weeks. The animals were later grouped into five rats per cage and maintained at temperatures between 25 – 28^{o}c. The rats were allowed to eat standard rodent chow and water *ad libitum* throughout the experimental period.

Experimental Protocol

Induction of Priapism

It is a delicate procedure that demands intensive precautionary measures. The penis of rat is a very tender organ and could be easily injured. When traumatised, either by the use of too tight constriction band or rough handling could strangulate the penis and impair micturiction process resulting in damming of urine within the urinary bladder, ureter or hydronephrosis and uraemia. This destabilises the kidneys and becomes a source of infection and renal failure which could lead to death of the rats.

Undoubtedly, the following procedure occurred earlier in this index study and 90% of all the first set of rats died. The subsequent use of 2mm slices of size 16F catheter and minimal trauma to the penis was successful.

All operations were performed under sterile conditions. The animals were anesthetized with ketamine injection (50 mg/kg, ip). Priapism was induced with the method described by Sanli *et al*. [14]. The tip of a 60-cc syringe was applied to the base of the flaccid penis, so a vacuum erection device was created. Before the application of vacuum to the penis, a constriction band, which was cut from 16 Fr Foley catheter in 2-mm slices, loaded around the tip of the vacuum erection device. Then the tip of the syringe was placed at the base of the penis and withdrawn gently to induce erection in the rat penis. After induction of erection in sufficient grade, the constriction band was then placed at the base of the penis by slipping off the syringe. Testosterone was administered intramuscularly at 2.5mg/kg body weight three times weekly [15] and oral Vitamin C at 25mg/kg body weight daily [16] throughout the one week of priapism induction.

Animal groupings

The animals were divided into five groups containing 5 rats each.

Group 1: Served as the control and was treated with 5ml/kg body weight of normal saline

Group 2: In this group, priapism was induced for one week but no drug was administered

Group 3: Priapism was induced and 6 hours later 25 mg/kg of Vitamin C was injected intraperitoneally.

Group 4: Priapism was induced and 6hrs later 2.5mg/kg body weight of testosterone was injected intramuscularly at three times weekly.

Group 5: Priapism was induced and 2.5mg/kg body weight of testosterone and 25mg/kg body weight of vitamin C injected via same route as above.

The animals were sacrificed one week after priapism induction. The penis and the seminiferous tubules were harvested for histology and seminal fluid analysis.

Tissue preparation for histological analysis

All rats were penectomized 1 week after the induction of priapism and the penis were fixed in Bouin's solution for 24 hours and then dehydrated by passing through ascending grades of alcohol (70%, 80%, 90% and absolute alcohol). After dehydration, tissues were cleared in xylene, infiltrated, and then embedded in paraffin wax. Each penis was sectioned along horizontal axis in 5um thickness. Two sections from each rat were blocked in paraffin. Two sections of each block (total 4 sections for each testis) were stained with Haematoxylin and Eosin (H & E) according to routine light microscopic procedures.

Statistics

Statistical analysis was done using SPSS statistical software (version 7). Paired T test was used to compare mean values between the control group and other groups in the study. The comparison was done for liver enzymes and haematological indices.

RESULT AND DISCUSSION

The liver enzymes – alanine transaminase, aspartate transaminase and alkaline phosphatase were elevated in both the Priapsm-alone group and Priapsm + Testosterone group. However, Priapsm + vitamin C group and Priapsm + vitamin C + Testosterone group showed normal levels of liver enzymes (Table 1). This indicates that Vitamin C as an antioxidant has protective effects on the liver metabolism and enzymes.

The haematological indices revealed marked anaemia especially in Priapsm group and Priapsm + Testosterone group where antioxidants were not administered. Priapsm + Vitamin C and Priapsm + Vitamin C + Testosterone group had marginally normal levels of packed cell volume (Table 2). All the groups revealed lymphocytosis with neutropaenia except for control group with normal lymphocyte and neutrophil counts. Total white cell count, monocyte and eosinophil counts were normal for all groups.

Ischemia-reperfusion injury associated with priapsm is a complex phenomenon that results in local and remote tissue degeneration through generation of free radicals [5-6]. Ascorbic acid biosynthetic pathway in rat involves the liver as a site of synthesis [17-18]. However, natural antioxidant defence mechanisms in

the body can be exceeded by excess production of free radicals resulting in oxidative stress [6, 8-9, 18]. Vitamin C is a powerful hydrosoluble antioxidant in body fluids [10], scavenging reactive oxygen and nitrogen species [19]. However, it is possible that additional vitamin C supplies may improve the antioxidative defense of these animals.

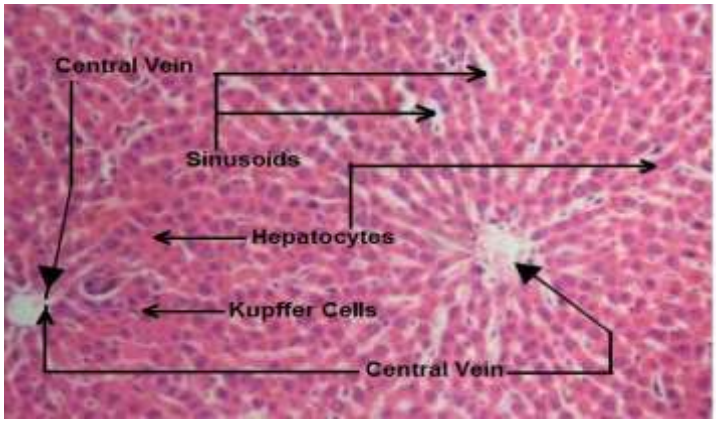

Fig. 1: A section of the Liver from Control group showing normal liver architecture (H&E x400)

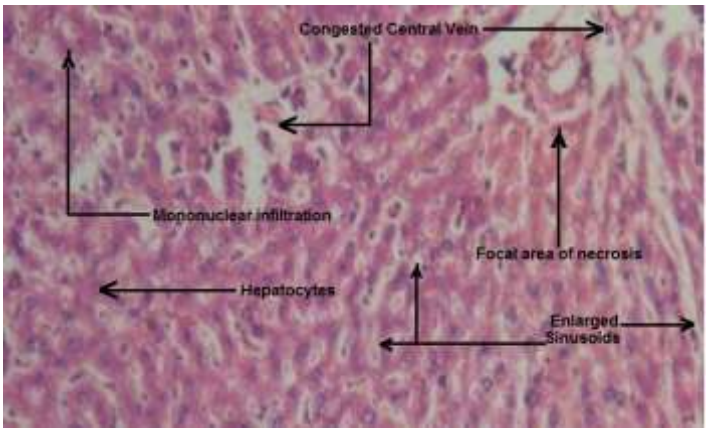

Fig. 2: A section of the Liver from Priapsm-alone group showing degeneration of liver architecture (H&E x400)

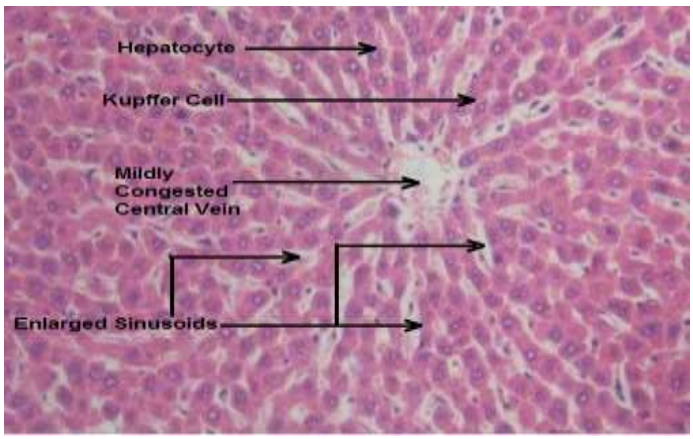

Fig. 3: A section of the Liver from Priapsm+ Vitamin C group showing liver architecture comparable to the control group (H&E x400)

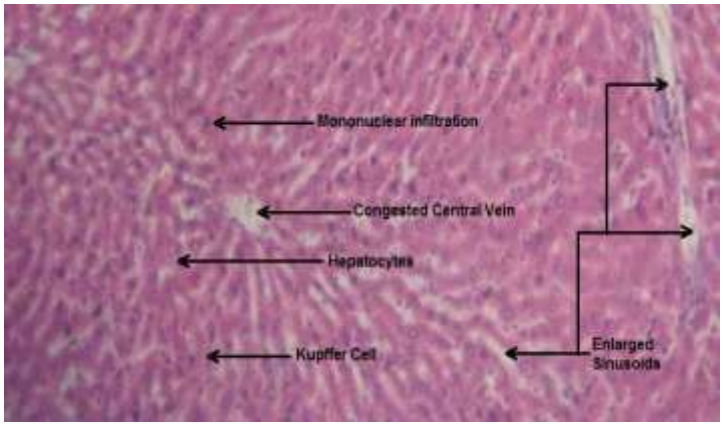

Fig. 4: A section of the Liver from Priapsm+ Testosterone group showing liver architecture comparable to that of Priapsm-alone group (H&E x400)

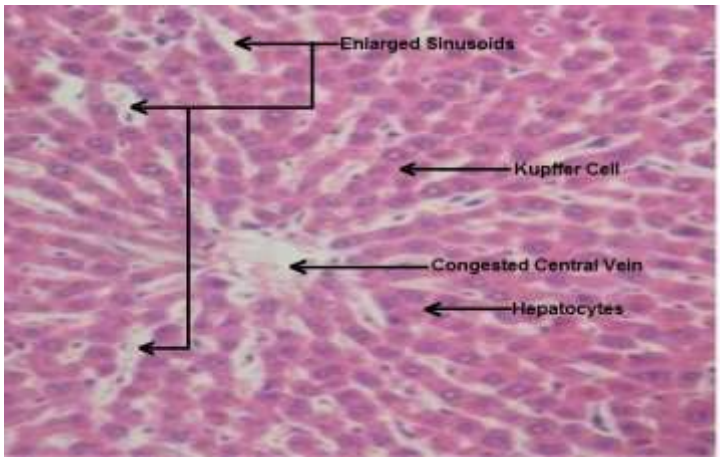

Fig. 5: A section of the Liver from Priapsm+ Testosterone+ Vitamin C group showing liver architecture comparable to that of the control group (H&E x400)

Table 1: Liver Enzymes (U/L) with Mean and Standard Deviation for Various Groups

Group	Alanine Transaminase	Aspartate Transaminase	Alkaline Phosphatase
Group 1	7.80 ± 2.58	7.20 ± 3.11	27.20 ± 4.77
Group 2	15.40 ± 2.07 *	20 ± 1.58 *	43.80 ± 3.03 *
Group 3	6.20 ± 4.43	7.60 ± 3.51	24 ± 4.69
Group 4	18 ± 3.51 *	15.6 ± 2.41 *	41.80 ± 2.86 *
Group 5	6.20 ± 2.59	7 ± 2.24	26.4 ± 3.51

Group 1: Control, Group 2: P-alone), Group 3: P + Vitamin C, Group 4: P + Testosterone, Group 5: P + Vitamin C + Testosterone. Data was expressed as mean ± S.D. Statistics involve the use of t-test *($p<0.05$) and ** ($p<0.005$)

Table 5: Hematological Indices (%) with mean and standard deviation for various groups

Group	PCV (%)	N (%)	L (%)	M (%)	E (%)	WBC (total) ($x10^9$/L)
Group 1	35 ±2.24	60 ± 4.47	40 ± 4.47	2.4 ± 1.14	3 ± 1.58	5.72 ± 1.17
Group 2	25 ± 2.28*	26.6 ± 7.40**	71.2 ± 8.08**	1.4 ± 1.14	1.4 ± 1.14	4.24 ± 0.29
Group 3	31.8±4.15	23 ± 4.90**	77 ± 4.90**	1.6 ± 1.14	1 ± 1	9.5 ± 0.31*
Group 4	22 ± 3.16*	27.2 ± 4.15**	72.8 ± 4.15**	1 ± 1	1 ± 1	5.3±1.05
Group 5	34 ± 3.16	33.6 ± 3.21**	66.4 ± 3.21**	1.4 ± 0.89	0.8 ± 0.84	2.3±0.62*

Group 1: Control, Group 2: P-alone), Group 3: P + Vitamin C, Group 4: P + Testosterone, Group 5: P + Vitamin C + Testosterone, N: Neutrophils, L: Lymphocytes, M: Monocytes, E: Eosinophils. Data was expressed as mean ± S.D. Statistics involve the use of t-test *($p<0.05$) and ** ($p<0.005$)

The scavenging efficacy is from the fact that it is a simple glucose-related carbohydrate with unique electron-donating or accepting properties. This is ensured by the presence of an enediol group in the molecule which bestows its electron lability and making it a member of an oxidation-reduction system [10, 20-21]

CONCLUSION

The results of this study demonstrate that experimentally induced priapsm caused marked anaemia and intractable elevation of the liver enzymes. Post-treatement with vitamin C significantly reversed the changes in all of these parameters in agreement with previous studies [17-18, 21]. Thus, the usage of antioxidant agents in such conditions and clinical implications of antioxidant administration can be improved in the future.

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