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CARDIAC MARKERS INTEGRITY IN WISTAR RATS MODEL FED WITH AQUEOUS EXTRACTS OF PHOENIX DACTYLIFERA, CYPERUS ESCULENTUS AND SOYBEAN WITH COD-LIVER OIL

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ABSTRACT

This study determine the nutritional and physiological role of cyperus esculentus, phoenix dactylifera, soybean aqueous extract, cod liver oil (standared drug) and the combination of both phoenix dactylifera, cyperus esculentus and soybean extracts on the integrity of cardiac markers using wistar rats model. The cardiac markers aimed at in this study includes troponin I, troponinT, CKMB, AST, and myoglobin using enzyme immunoassay, electrophoresis methods measured in pg/mls, U/L, ng/ml respectively. The study was design in such a way that group1 serve as the control while group2 to group4 served as the test groups with the administration of 1500mg/kg cyperus esculentus (gp2) and 700mg/kg phoenix dactylifera extracts. Group3 received 800mg/kg cyperus esculentus, 1600mg/kg phoenix dactylifera and 1200mg/kg soybean extract daily while group4 was administered 400mg/kg standared drug (codliver oil) daily. The results from this study indicate the cardiogenic protection and benefit of these nut fruits to maintain the integrity of cardiac markers and promotes physiological normalcy within the heart that serve as the power house of the cardiovascular system. However we observed an increase in troponin T(123pg/ml) in group2 with a significant difference and myoglobin (16.20ng/ml) in group4 though not statistically significant compared with other cardiac markers. A strong positive correlation between troponin I versus troponin T and CKMB was

also observed. The deccrease in cardiac markers concentration/level are vivid manifestation of these nut fruits ability to promote cardiac health integrity and other cardiovascular parameters for smooth oxygen supply to satisfy all tissues demand in the body.

KEYWORDS: Cardiac markers, Troponin, Myoglobin, Creatinine kinase, CKMB.

INTRODUCTION

In-spite of the growing knowledge from researchers in medical science, mortality rate from infarction of the myocardial cells continue to be on a global rise. The permanent loss of myocardial cells resulting from ischemic injury can cause irreversible damage of myocardial contraction rate and decrease in ventricular performance or the initiation of dilated heart failure development (Alhaider *et al.*, 2017). Cardiac markers are road map to the diagnosis of myocardial infarction with the troponins being the most commonly used markers.

Insufficient physiological understanding of cardiac troponins metabolism diagnostic potentials to cardiac markers remains unlock to the management of cardiovascular diseases (Chaulin, 2022). The troponin complex comprises of three sub-units namely troponins C,T, and I that regulate the sliding of actin on myosin. Troponin T is responsible for binding the complex to tropomyosin wrapping around actin filaments, troponin I block or inhibit actin and myosin sliding while the troponin C bind calcium to regulate the inhibitory sliding action of actin on myosin in both cardiac and skeletal muscles (Nicholas, 2015).

The main mechanism for the release of cardiac markers in myocardial cells and its circulation in normal healthy people and the destruction of troponins from blood stream is yet to be fully unlock (Katrukha, 2013). The mechanism for the contraction of cardiac muscles is similar to skeletal muscles except that the cardiac muscles function in syncytium by forming a threshold and all contract from the stimulation of a singular myocardial cell. The generation of action potentials result in the release of calcium from the sarcoplasmic reticulum into the sarcoplasm making calcium to bind with the inhibitory protein troponin and tropomyosin. This allows actin to slide with myosin which are contractile proteins in myocardial cells (Chaulin, 2021).; Henderson *et al.*, 2017).

The amount of calcium available to inhibit troponin is directly proportional to the rate and amount of myocardial tension developed (Sokolow and Mcllroy, 1986). The three major stages in the metabolic pathway of troponins are 1 the release of troponins from myocardial

cells, 2 circulation of troponins in the blood plasma and 3 the removal of troponins from the blood stream with each of these stages having a significant effect on the level of troponins in the serum. The specific mechanism leading to the release of cardiac troponins into the blood stream from the myocardium are poorly understood due to physiological conditions such as stress, circadian fluctuation rhythm, physical-exertion, tissue injuries, anemia, hypotension, tachycardia and non ischemic pathologies that may affect cardiac Troponin concentration in the blood.

The overall increase of troponins in the heart is not the sole reason for Troponin being more sensitive than CK-MB. The initial increase in sensitivity reflect the Troponin percentage released reaching the blood after cardiac injury is more for Troponin than for CK-MB. Increase level of Troponin will continue due to its slow release and destruction of the structural pool. Therefore since the half life of troponins lasted for about two hours, its elevation will permit for more clinical detection in case of cardiac events (Luciano and Allan, 2005; Tanaka *et al.,* 1997; Jaffe *et al., 1996).*

Creatine kinase MB is one of the most biomarker used for the diagnosis of myocardial injury due to its distribution in normal healthy human serum is as low as 0-6% but become elevated during myocardial infarction. Creatine kinase (CK-MB) is a cytosolic enzyme that increase the mobilization of high phosphate energy into and out of the mitochondrial. It is abundant in many tissues but mostly predominant in cardiac muscular cells and thus can be a useful marker for an early myocardial infarction and not late diagnosis due to its short half life (Chi-Chun *et al.,* 2015). Note that the level of CK-MB do not rise immediately following heart attack within four to six hours. Higher levels may indicate heart problems such as infarction, inflammation of heart muscles or sac surrounding the heart during cardiac defibrillation when using an electric shock in fixing heart rhythms (Abbot *et al.,* 1984). However false elevation of CK-MB has been reported in the sera of patients with malignant tumors and muscular damages elsewhere in the body.

Myoglobin –small protein found in blood plasma following myocardial infarction used as a cardiac marker (Said *et al.,* 2016).

AST-Aspatate transaminase is a synthesize enzyme by the liver, though the heart, brain, muscles and kidneys provide lesser amount of this enzyme as well. The normal physiological concentration in the blood is low but become elevated heart and liver muscle damage. The

clinical manifestation of yellow eyes and skin (jaundice) may warrant this test (WebMD 2023). Animal restraint techniques resulting in mild muscular injury may also increase the concentration of AST in non clinical studies (Aulbach and Amuzie, 2017).

The main objective for troponin assay is the quantification of a very small amount of troponins release during early onset of cardiac injury when a reduced number of myocardial cells are affected (Eugene *et al*., 2007). It has been established that cardio toxin induce, increases cardiac troponins concentration in wistar rats but data related to the concentration of these proteins in healthy wistar rats fed with nut fruits compared with cod liver oil are very scanty and unavailable. Hence values for the detection of cardiac troponins in animals can be made available by developing sensitive and accurate immunoassays (Herman *et al*., 199.2001, 2006).

MATERIALS AND METHODS

Research Animals

Thirty two male wistar rats were purchased from the departmental animal house divided into four groups of eight each for this research study.

Figure 1: Some Research Animals used in this Study.

Table 1: Photochemical Constituents of Cyperus Esculentus.

Constituents	Extracts
Glycosides	3^+
Alkaloids	3^+
Saponins	3^+
Reducing sugar	2^+
Flavonoids	3^+
Steroids	2^+
Resins	-
Phenols	
Tannins	2^+

Key: 3^+ high constituents, 2^+ moderate constituents, - not present

Table 2: Photochemical Constituents of Phoenix Dactylifera.

Constituents	Extracts
Terpenoids	+
Alkaloids	+
Saponins	+
Tannins	+
Flavonoids	++
Anthraquinones	+
Phenols	++

Key: += present ++ = highly present,

The presence of phenols, Flavonoids, Saponins, phytosterols, sphingolipids and it's flavones were also detected from the aqueous extract of the soy bean.

Measurement of weight

Weight of individual animal in their respective groups was taken weekly using (Golden Meter USA) scale balance calibrated in grammes for five weeks (35) days period of the study. The average weight of individual groups was done by the addition of sum total weight multiplied by the number of animals in a group.

Experimental Design

The animals were grouped randomly into four (4) groups as follows:

Group 1 received normal feeds and water ad libitum as the normal control.

Group 2 received aqueous extracts of cyperus esculentus and phoenix dactylifera daily at 1500mg/kg and 700mg/kg body weight

Group 3 received cyperus esculentus at 1600mg/kg, phoenix dactylifera at 800mg/kg and soybean at 1200mg/kg body weight of aqueous extracts respectively.

Group 4 received a standard drug at 400mg/kg body weight of liquid cod liver oil daily.

Collection of blood samples: Blood samples were collected via cardiac puncture after the animals were anesthetized using ketamin injection and then introduced into plain and EDTA bottles for the analysis of the required cardiac parameters for the study.

Determination of Creatine kinase (CK-MB) and Myoglobin

Tthis parameters were analysed using dry cell by placing the blood on the test kit and introduced into the machine and the results obtained within ten minutes.

Determination of AST (Reitman and Frankel Method)

→Principle: (AST) x – Oxoglutarate + L – aspartate GOT L - glutamate oxalogcetate. AST is measures by monitoring the conc of oxoloacete hydrazones formed with 2,4 dinitrophenyl –hydrazine.

→ALT: x- oxogluterate + L- alanme GPT L- glutamate + pyruvate. ALT is measured by monitoring the conc of pyruvate hydrazone formed with 2,4 dinitrophynyl-hydrazine.

- Procedure: The procedure for ALT and AST are the same
- Label the test tube as sample blank and sample.
- Pipette 0.5ml of rgt 1 (R1) into all the test tubes.
- Add 0.1ml of the sample to the tubes labeled sample.
- Mix and incubation for exactly 30mins of 37’c.
- Pipette 0.5ml of rgt 2 (R_2) to all the test tubes.
- Add 0.1ml of the sample to the tube labeled sample blank.
- Mix and allow to stand for exactly 20mins at 25’c
- Pipette 5.0ml of NaoH to all the test tubes.
- Mix and read the absorbance of the sample against the sample blank after 5mins at 540mm. unit u/l

RESULTS

Table 3: Mean Values of Cardiac Markers in The Study Groups.

Groupings	Trop I (pg/ml)	Trop T (pg/ml)	CKMB (pg/ml)	Myo (ng/ml)	AST (U/L)	Sig
GP 1	0.75±0.71	92.50±6.36	1.36±0.15	12.85±6.58	34. 5±	0.01
GP 2	0.99±0.49	123.00±5.65	2.15±0.28	10.85±0.92	31.00	0.00
GP 3	0.62±0.06	76.50±6.36	1.25±0.10	10.70±1.84	28.51	0.01
GP 4	0.91±0.06	112.50±7.78	2.01±0.13	16.20±0.85	33.01	0.45

Key: Trop I =Troponin I, Trop T= Troponin T, Myo = Myoglobin, AST=Aspatate transaminase.

Significant differences are present within the groups from the above table using ANOVA in gp1,2 and 3 whereas no significant difference exist in the group 4 with other parameters.

Table 4: Individual group Cardiac markers compared with other groups.

Dependent Variables	Individual Groups	Joint Groups	Mean diff (1-3)	Standard Error	Sig-
Troponin I (ng/ml)	1	3(2)	-0.23	0.06	0.10
		4(3)	0.13	0.06	0.59
		4(2)	-0.15	0.06	0.37
	3(2)	1	0.23	0.06	0.10
		4(3)	0.36	0.06	0.02
		4(3)	0.08	0.06	1.00
Troponin T (pg/ml)	1	3(2)	-30.50	6.58	0.05
		4(3)	16.00	6.58	0.43
		4(3)	-20.00	6.58	0.23
	3(2)	1	30.50	6.58	0.05
		4(3)	46.50	6.58	0.01
		4(3)	10.50	6.58	1.00
CKMB (pg/ml)	1	3(2)	-0.79	0.18	0.07
		3(2)	0.11	0.18	1.00
		4(3)	-0.65	0.18	0.05

Table 5: Correlation Between Trop I vs other parameters.

Variables		r- values	Sig
Trop I (pg/ml)	Trop T (pg/ml)	0.998*	0.00
Trop I (pg/ml)	CKMB (pg/ml)	0.929*	0.00
Trop I (pg/ml)	Myo (ng/ml)	0.344	0.40 not sig

Table 6: Pearson Correlation Between CKMB versus other Cardiac Markers.

Variables		R-value	Sig
CKMB (pg/ml)	Trop (pg/ml)	0.932	0.00
CKMB (pg/ml)	Myoglobin (ng/ml)	0.280	0.50 not sig

** Correlation is significant at 0.01 level (2 tailed)

A strong positive correlation is observed between Troponin I, Troponin T and CKMB and vice versa among the cardiac markers studied expect in the Myoglobin.

Table 7: Mean Weight Difference Between Control and Test Groups.

Number of weeks	Gp1(g)	Gp2(g)	Gp3(g)	Gp4(g)	sig
WK 1	97.25±66.83	187.50±17.08	163.25 ±109.05	232.00± 37.10	0.08
WK 2	123.00±82.50	155.75±103.91	176.00	241.75±	0.00

			117.60	45.38	
WK 3	123.50±83.14	164.75±109.87	188.50± 126.06	250.00± 49.60	0.03
WK 4	119.00±81.65	116.00±134.03	177.50± 118.67	178.75± 124.16	0.00
WK 5	128.75±86.59	110.00±127.14	171.50± 114.63	173.25± 119.45	0.02

DISCUSSION

Weight: An increase in body weight and elevated blood presure are esterblished fact known to cause cardiovascular diseases. This fact was observed by Solomon *et al* (2017) from his research study on human subjects having positive correlation with blood pressures in Bayelsa state Nigeria. The experimental animals weight were higher in group4 (232.00g), followed by group2 (187.50g) and group3 (163.25g) compared with the control (97.25g) prior to the administration of the variuos aqueouse extracts. However the control group had an increase weight of (123g) while group3 administered 1600mg/kg cyperus escuentus, 800mg/kg phoenix dactylifera fruit and1200mg/kg soybean daily weighed (176g) and group4 that received 400mg/kg standard drug (codliver oil) daily weighed 240g compared with a decrease weight in group2 (155g) administered 1500mg/kg and 700mg/kg body weight aqueous extract of cyperus esculentus and phoenix dactylifera fruit. There was a significant increase in the weight of both control and the test groups during the third week of the research study compared with the second week.

The weight of the experimental animals also decreases significantly among the test groups compared with the control group during the fourth week but thus dereases steadily to the fifth week compared with an increase weight in the control (128g) group.

Cardiac markers: The level of troponins, CKMB, Myoglobin and aspatate aminotransferase were observed to be significantly lower in group3 that 1600mg/kg tigernut, 800mg/kg date fruit and 1200mg/kg soybean extracts respectively compared with other groups and the control. The troponins concentration among group2 treated at 1500mg/kg and 700mg/kg body weight daily with the extract of tigernut and date fruit has the highest levels when copared to other groups though troponin I level (0.99pg/ml), AST (31.00u/l) appears to be within normal range in group2 (Loeb et al., 1999).

However troponin T was abnormally elevated significantly (123pg/ml) in group2 while myoglobin level was also increased in group4 (16.20ng/ml) though not significant. Notable

causes of cardiac troponins increase include plaque formations within the intima of cardiovascular vessels, thrombosis, imbalance in delevery of oxygen demand, acute myocardial injury, chronic myocardial injury, myocarditis, persistent tarchicardia, acute heart failures, structural pathology of the heart, severe anemia, chronic kidney disease etc (stavroulakis *et al*., 2020) (Chauin 2021). Further more the cardiac markers including AST were within normal range except troponin T (112pg/ml) with higher level in group4 compared with the control (92pg/ml) gruop.

Comparative analysis of troponin T in group1 with group2 indicate a significant p-value of 0.05. Additionally the troponon T in group2 compared with group3 shows 0.01 significant p-value. More so troponi I in group2 compared with group3 indicate a significant value of 0.02.

There was a strong positive correlation between troponin I versus troponin T and CKMB cardiac markers with r-values of 0.988, 0.929 and a significant values of 0.00 in both markers.

The guidelines applicable in the diagnosis of non-ST segment currently in myocardial infarction are mostly based on elevated troponin levels. Rather taking single absolute value of CKMB characterize with false positive elevation level than trusted elevation has thrown major weight on cardiac troponins which are more sensitive and more reliable for interpretation in the context of clinical conditions (Mohammed et al., 2011). Cardiac troponins are physiologically involved in mediating calcium interactions between the filament of actin and myocin coded by specific genes unique to myocardial cells. Most molecules serve as drug binding site used for the diagnosis of diseases in the laboratory. In clinical practice the capability of cardiac troponins are much connected with the fundamental principles of troponins metabolism (Aleksey 2022).

CONCLUSION

The results from this study have shown a remarkable improvement in cardiac markers integrity to promote normal physiological condition of the heart. We therefor recommend the intake of these nut fruits as suppliment to curb the menace of cardiovascular diseases. However additional criteria for the establishment of MI will include pathological Q-waves on serial ECG and evidence from imaging indicating loss of myocardial viable region that fails to contract when there is no known ischemic cause.

CONFLICT OF INTEREST: There is no any form of conflict of interest among the authors.

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