



EFFECT OF KOLA NUT (*COLA NITIDA*) EXTRACT ON SOME BIOCHEMICAL LIVER MARKER USING RAT MODEL

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Abstract

Cola nitida is native to west Africa and now cultivated around the world for its seeds called kola nut . The daily intraperitoneal (i.p.) injection of (10mg/kg body wt) Kola nut extract for 60 days caused a significant increase in : aspartate aminotransferase (AST) , alanine aminotransferase (ALT) , alkaline phosphatase (ALP) , gamma-glutamyl transferase (GGT) , albumin and total bilirubin in all time intervals . The increase still significant after the withdrawal of the extract . This is may be due to the presence of caffeine which caused increase activation of : AST, ALT, ALP, GGT, albumin and total bilirubin . kola nut may a harmful effect , So prolong usage must be avoided .

Key words : Kola nut , AST , ALT , ALP ,GGT , bilirubin , albumin .

Introduction

Cola nitida is an evergreen forest plant that is common in tropical region of Africa. It's a member of the tropical family *Sterculiaceae* (Russel , 1955) . Its fruits contain seeds known as kola nuts (Jayeola , 2001) .In West Africa and Sudan, kola nuts are popular masticatory agents, eaten as a principal stimulant to keep awake and withstand fatigue. Besides the fact that they contain caffeine and act as a stimulant and anti-depressant, they are also thought to reduce hunger, aid digestion, and work as an aphrodisiac(Hatasaka and Goldstain , 2001) . As long ago as 12th century , an Arab physician recommended kola for the relief of various stomach complains (Abaka , 2000) and by the 16th century , it was incorporated into the matière medical of Islamic science (Lovejoy , 2007) , it has been used also in folk medicine as an aphrodisiac and an appetite suppressant (Burdock *et al .* , 2009) . Previous reports have shown that administration of kola nut extract stimulates the central nervous system activities (

Scottoet *et al .* , 1987; Abdel-Rahman and Waggas,2004 ; Imam-Fulani *et al .* , 2018) . increases the cardiacmuscle concentration (chukwu *et al .* ,2006) , increases glucose uptake skeletal muscle in dogs (Salahdeen and Alada , 2009). The biological and physiological effects of kola nut extract have been attributed to its caffeine content (Osim *et al .* , 1991 ; Carrillo and Bennitez , 2000) even when the caffeine content in kola nut extract has not been characterized (Salahdeen *et al .* , 2015).Also toxicological studies on caffeine consumption have shown lots of clinical symptoms , such as : nervousness , insomnia to sensory disturbances , diuresis arrhythmia , elevated respiration and gastrointestinal disturbances (Nawrot *et al .* , 2003 ; Udenbuani *et al .* , 2017) . It was observed that there is a lake of study on the effect of kola nut on liver function , So the present study was therefore designed to evaluate the effect of daily injection of kola nut extract on activity of serum liver enzymes , total bilirubin and total albumin level using rat model .

Materials and methods

Preparation of the extract

Kola nut were bought from the open market in Saudi Arabia (Jeddah) and were extracted according to the method described by Ajarem (1990) .

Animals and experimental design.

The experimental animals used in this study were adult male albino rats , *Rattus rattus* (160-180 g) , the animals were selected from animal house of king Fahd of medical researchers in Jeddah , Saudi Arabia . The animals were housed under controlled environment conditions (12 h light / dark cycle) at a temperature of 25+10 C and humidity of 60+5 % and fed standard diet and water *ad libitum* for the experimental period . The animals were randomly divided into two groups .

The first group (n=36) was divided into sex subgroups each of 6 rats . The animals were daily injected (i.p.) with 10 mg/kg of kola nut (Ajarem , 1990 ; Abdel-Rahman and Waggas , 2004 , Obidike *et al .* , 2011) , and one subgroup was decapitated after 15 , 30 and 45 and 60 days . To examine the withdrawal effect , the animals were decapitated after 15 and 30 days of stopping the administration of cola nut extract . The second group divided as the first group , the rats were injected with saline vehicle and served as control .

Sample preparation and biochemical analysis

Blood was collected by retro bulbar method in heparinized tubes and centrifuged at 2500 rpm for 15 min and plasma was separated and stored at -4°C for analysis. Serum level of AST and ALT were estimated by the method of Reitman and Frankel (1957) . Serum ALP were determined by the method Kind and King (1954) .GGT activity was assayed according to Persijin and Van Der Slik (1976) and Klin (1974) . Total Bilirubin was carried out using method of Jendrassik (1938) . Serum total Albumin activity were determined according to Eastham (1976.) by using commercial kits (mybiosource company, USA) .

Statistical analysis

All data are presented as mean \pm S.E. Statistical analyses between control and treated animals were performed by using Student's *t*-test . Values of $P < 0.05$ were regarded as significant.

Results

The single daily i.p. injection of 10 mg/kg of Kola nut extract significantly increased the level of AST and ALT enzymes after 15 , 30 , 45 and 60 days . AST and ALT enzymes level still significantly ($P < 0.05$) increased 30 days after the withdrawal of the extract . figure (1,2) .

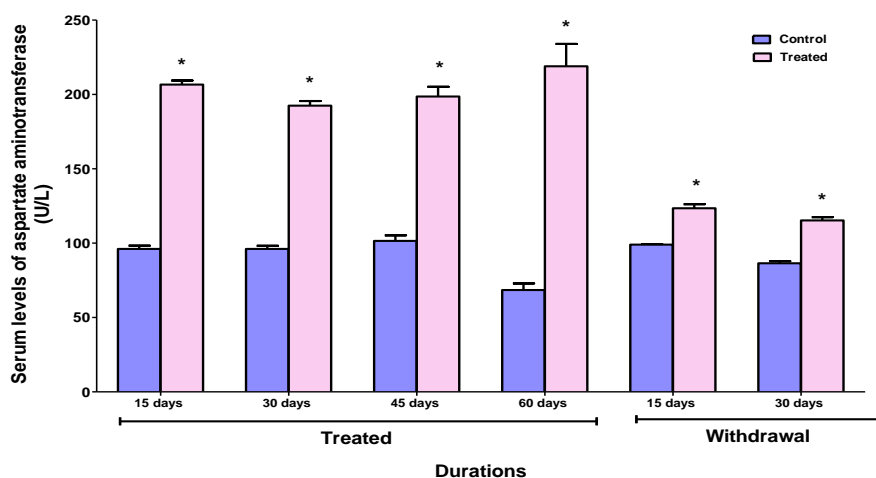


Figure (1): Effect of daily injection of Kola- nut extract (10 mg/kg ,i.p.) and subsequent Withdrawal on serum levels of aspartate aminotransferase (AST) enzyme (U/L) of male albino rat .
* significant at $P < 0.05$.

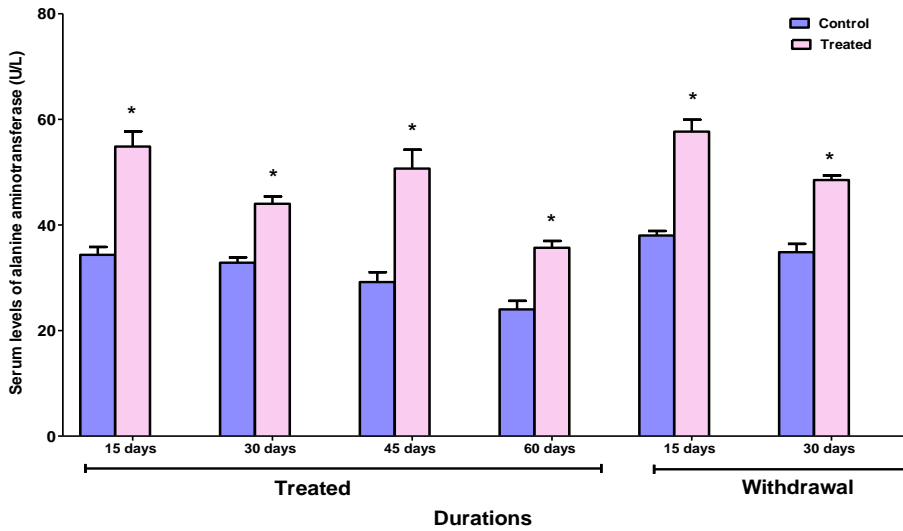


Figure (2): Effect of daily injection of Kola- nut extract (10 mg/kg ,i.p.) and subsequent Withdrawal on serum levels in alanine aminotransferase ALT enzyme (U/L) of male albino rat .
* significant at P <0.05

Figure 3 and 4 shows that 10 mg /kg of Kola nut extract induced a significant increase in ALP and GGT enzymes level after 15 , 30 , 45 and 60 days . ALP and GGT enzymes level persisted its significant increase 30 days after the withdrawal of the extract (P <0.05) .

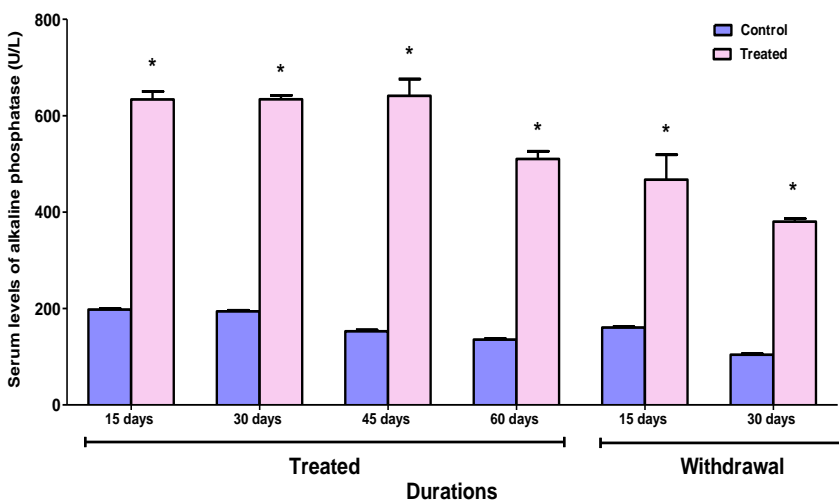


Figure (3): Effect of daily injection of Kola- nut extract (10 mg/kg ,i.p.) and subsequent Withdrawal on serum levels of alkaline phosphatase ALP enzyme (U/L) of male albino rat .
* significant at P <0.05

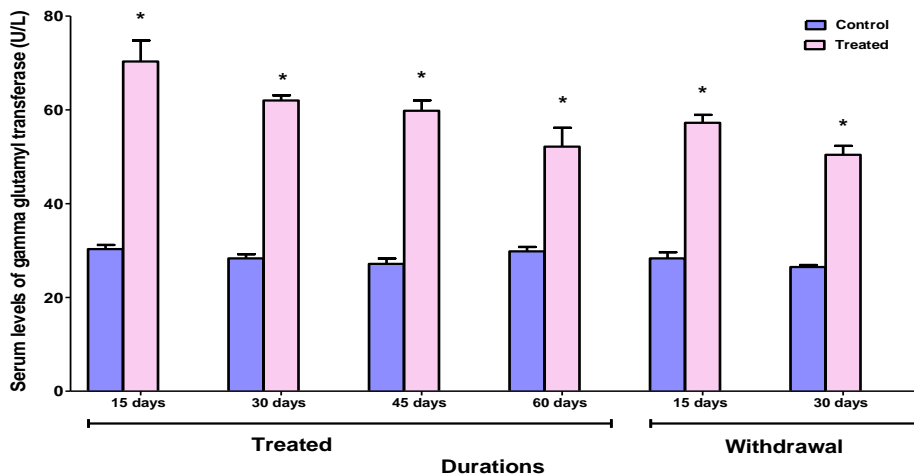


Figure (4): Effect of daily injection of Kola- nut extract (10 mg/kg ,i.p.) and subsequent Withdrawal on serum levels of gamma-glutamyl transferase GGT enzyme (U/L) of male albino rat .
* significant at P < 0.05

In experimental animals , the mean serum activities of albumin were significantly ((P < 0.05) increase compared to control groups of all tested intervals (Figur 5)

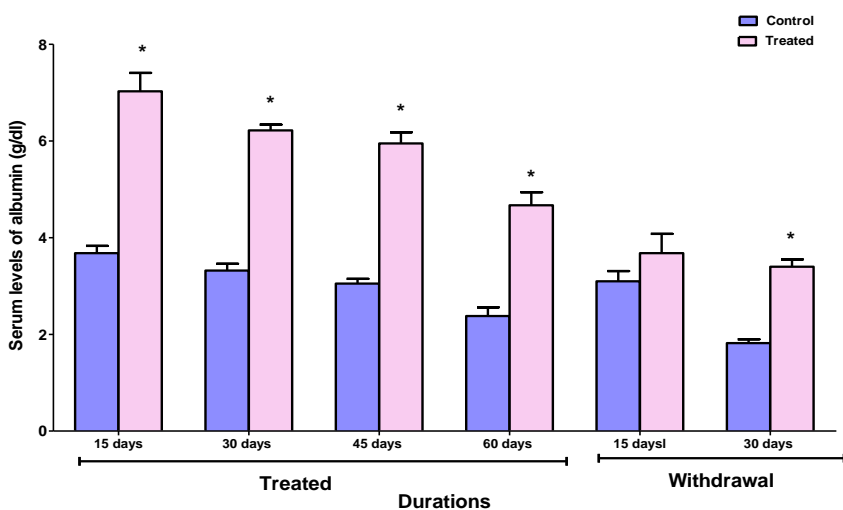


Figure (5): Effect of daily injection of Kola- nut extract (10 mg/kg ,i.p.) and subsequent Withdrawal on serum levels of albumin (g/dl) of male albino rat . * significant at P < 0.05

Moreover figure 6 shows that , the injection of cola nut extract 10 mg/kg significantly increased bilirubin level at all tested intervals . bilirubin level still significantly increase after the withdrawal of the extract

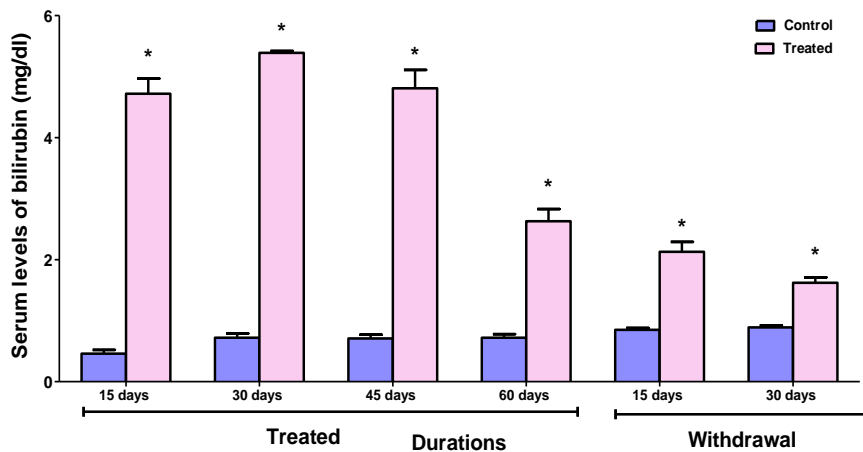


Figure (6): Effect of daily injection of Kola- nut extract (10 mg/kg ,i.p.) and subsequent Withdrawal on serum levels of total bilirubin (mg/dl) of male albino rat. * significant at P <0.05

Presented in table 7 are mean body weight of rats after kola nut exposure , the mean body weight of rats that received 10 mg/kg of kola nut extract were significantly reduced relative to control . There is a significant increased of total body weight after the withdrawal of the extract

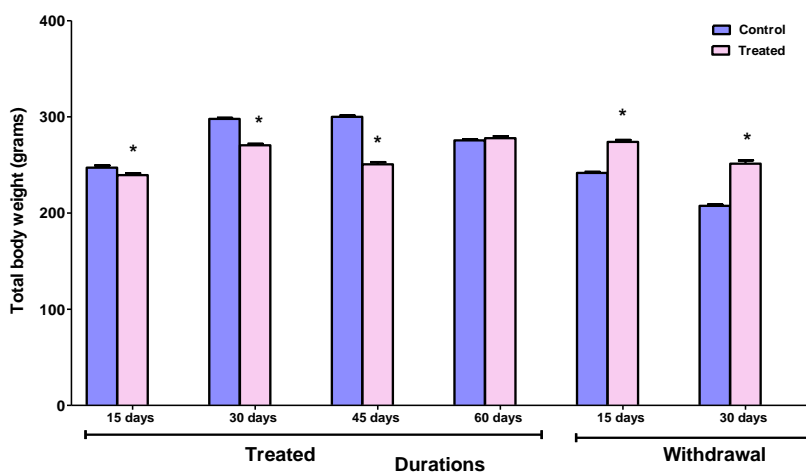


Figure (7):: Effect of daily injection of Kola- nut extract (10 mg/kg ,i.p.) and subsequent Withdrawal on mean total body weights (grams) . * significant at P <0.05.

Discussion

Kola nut contain caffeine among many other principles. It has been hypothesized that liver enzymes are target for caffeine . It is generally believed that most of the action of kola nuts are attributable to caffeine (Casiglla *et al .*, 1993 ; Umoren *et al .*, 2009)

The previous studies indicated extract led to effects similar to those noted following the treatment of caffeine and this similarity is du to the present of caffeine cola nut (Scotto *et al .*, 1987 ; Umoren *et al .*, 2009) . Caffeine rapidly distributed throughout all tissues of the body

(Liguori *et al.* , 1997) . Caffeine is metabolized in liver , through demethylation and oxidation , it forms three dimethylxanthines , and each of these metabolites is further metabolized and then excreted in the urine (Fisone , 2004) .

Utu-Baku *et al.* .(2009) studied the effect of alcohol kola nut constituents on liver aspartate amino transferase and alanine amino transferase enzyme activities , the result indicate that the opposing effect of kola nut alcohol on liver enzymes (AST , ALT) can be related to the neural function and liver function enzymes .

Hussein and Al-Qaisi (2011) studied the effect of caffeine on : ALT , γ -GT , AST ,GOT and GPT enzymes , the results observed that caffeine caused increase in percentage of activation of enzymes ,which play important role in amino acid metabolism and in the urea and tricarboxylic acid cycles .

From the present result and the previous studies it could be concluded that the increase in the liver enzymes content in experimental animals treated with kola nut extract (10mg/kg) may be due to the presence of caffeine

Exposing rat 10 mg/kg of kola nut affected the activity of ALT and AST , which was consonant with earlier observation (Adedapo *et al.* , 2007 ; Al-Qaisi *et al.* , 2011 ; salahdeen *et al.* , 2015) .

AST and ALT more released when the tissue damage (Michel , 1998) . on other hand ALT is more specific in the liver than AST (Marshall , 2000) . Serum Alt is known to increase when there is liver cell damage and it has been employed as a tool for measuring hepatic necrosis (Kaplan , 1986) . AST is not a liver specific enzyme as high levels of the enzyme can also be found in skeletal and cardiac muscle as well as red blood cells (Etuk and Muhammad , 2010).

The increased transaminase levels in this study could be in a part to the presence of caffeine in the extract which caused peroxidative in fatty liver tissue in rats (Dianzani *et al.* , 1991) , on other hand the extract of kola nut caused over secretion of level of these enzymes .

Some of many enzymes found in hepatocytes can be measured in the serum such as the alkaline phosphatase (Reichling and Kaplan , 1988) . ALP levels in the blood are usually a good indicator of bone activities since osteoblasts secrete large quantities of this enzyme (Kuzminskaya and bersan , 1975 ; Rock *et al.* , 1986) .

Obidike *et al.* .(2010) demonstrated that caffeine extract from kola nut in the system of the exposed rats may have led to the disruption in the activity of these osteoblasts, thus leading to increase in the mean ALP value . In this study , ALP level was statistically significant in rats

treated with kola nut extract. This could be evidence of hepatotoxicity caused by caffeine (Casiglia *et al.* , 1993 ; Manne and Saab , 2015) and cholestatic disease (Mayne , 2005).

GGT levels are increased in most forms of liver disease , especially cholestasis , plasma membrane-bound enzyme (Elsevier , 1997; Koenig and seneff , 2015). Also the estimation of GGT is of some value monitoring the progress of acute to chronic hepatitis (Krishnamurthy , 2013).

In this study there is an increased in GGT level after administration of kola nut extract , this result agreement with before studies of the same enzyme (AlQaisi *et al.* (2011; Ogbuji and Ndulaka , 2017) which hypothesized that GGT would be increased as part of the adaptation of cells to oxidative stress after treated with caffeine . on another hand Previous reports have been reported that tissue damage is usually associated with the release of enzymes specific to the affected tissues or organs in circulation (Mathew , 2001; Duru *et al.* , 2012 ; Emmanuel *et al.* , 2017) .

Total albumin and bilirubin were significantly increase in treated animals with kola nut extract in this study agree with other reports (Salahdeen *et al.* , 2015) ,bilirubin strongly increased in cholestatic disease (Mayne , 2005) . A variety of serum proteins and serum bilirubin are evaluated to assess the liver damage (Laker , 1990) , caffeine from kola nut may has considerable effect on synthetic function of the liver .

The result also showed that daily injection of kola nut extract decreased total body weight when compared to the control groups , this result agreement with before reports (Zheng *et al.* , 2004 ; Udebuani *et al.* , 2017) . This observation is very important because the toxicity of chemical compounds in experimental animals is often associated with loss of body weight . Kola nut and its active constituent , caffeine may possess appetite – suppressant effects and this effect produces weight loss (Jessen *et al.* , 2003, 2005 ; Umoren , 2009) . At the same time caffeine increased lipolysis and fat oxidation , So total body weight was decreased .

From the present result and previous study it is clear that injection with kola nut extract for eight weeks induced a significant increase in liver enzyme (ALT, AST , ALP , GGT) , total bilirubin and total albumin , at the same time total body weight was decreased . This is an indication of harmful effects of kola nut on hepatic tissues .

In conclusion , this data suggested that kola nut may effect of liver function and total body weight , this is may be due to the presence of caffeine in the extract . These observation are in line with some previous studies . kola nut may a harmful effect , So prolong usage must be avoided . However , the mechanism of the effect on liver tissue presently remain unclear .

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