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### HISTOPATHOLOGICAL STUDY OF TOXICITY MIXTURE OF CASHEW APPLE JUICE (*Anacardium occidentale*), COW MILK AND YOGURT ON THE WISTAR RAT

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#### KEYWORDS

Dairy products

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Toxicity

Histopathology

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#### ABSTRACT

The purpose of this study was to confirm or to deny the general opinion Benin people that the simultaneous consumption of dairy products, cow milk, yogurt (fermented cow's milk) with cashew apple juice is toxic to human and the animal. Histopathological examinations were performed on thirty Wistar rats consist of ten batches. Each batch is consisted of three rats. The first batch has served as a control and the remaining nine were treated differently. Cow milk, yogurt and cashew apple juice either alone or mixed with each others were administered to each rat at the rate of 2.0 ml / 200g of body weight. Histological observation of removed organs (stomach, liver and kidneys) within the treated batch showed that the organs of rats treated with cashew apple juice / cow milk /yogurt (respectively batch 2, 3, and 4) was not showing any structural abnormality. Similar types of findings were reported for the rat group treated with a mixture of juice and milk. However, it was reported that the juice-yogurt association has some action on the liver but it did affect the functioning or anatomy of other analyzed organs. It can be conclude that the juice and cow milk mixture are not toxic to animal cells, but the juice-yogurt mixture has some toxic effect on the liver cells only.

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## 1 Introduction

The cashew (*Anacardium occidentale*) is a multi-virtues tree and its fruit is rich in nutrient elements such as vitamins, proteins and fermentable sugars (Cirad-Gret, 2002; Arouna et al., 2010). Besides its agroforestry importance, the fruit (cashew nuts & cashew apple) of cashew tree provide specific products which are highly sought outside the Benin (Aina, 1996; Gagnon, 1998; Arouna et al., 2010). Fruit of cashew tree contains about 47% of oil which is quite close to the almond (RIC-International, 2004). Beyond the nuts, cashew tree also offers cashew apple. This is edible and constitutes the fleshy part of the fruit. But it is often neglected during the harvest of nuts and rots in vain, yet it contains five times more vitamin C than an orange and is very rich in carbohydrates, fiber, mineral salts and vitamins (Assunção & Mercadante, 2003). The cashew apple juice is rich in polyphenolic compounds and also has diversify carotenoids profile (Assunção & Mercadante, 2003; Abreu et al., 2005; de Brito et al., 2007; Michodjehoun-Mestres, 2009; Michodjehoun-Mestres et al., 2009; Pinto de Abreu 2012). More than 450,000 tons of cashew apples were left to rot in Benin; this is a great loss ( Adégbola et al., 2011; Padonou et al., 2015). It was cultivated by about 200,000 households and contributes in average to 24.87% in the total income of farm producers (SNV-AFP-ICCO, 2012). In Benin it is an interesting alternative and is the second agricultural export product after cotton (Degla, 2015). Set in 10th place worldwide with only 2% of the volume of exported nuts and 2nd of West African exporting countries after Guinea Bissau, Benin has experienced over the five year period from 1997 to 2001 an annual growth rate of exports of 33% nuts in value and 40% in volume (Aïvodji & Anasside, 2009). Cashew apple can be used directly for food. Its juice is an ideal medium for culturing yeast for the production of bioethanol (Bokossa-Yaou, 1993; Gbohaïda et al., 2015; Gbohaïda et al., 2016). Many farmers reported the occurrence of poisoning in cattle promoted with cashew apples (Filho & Soto-Blanco, 2012). In Benin, Ivory Coast and several other African countries, the consumption of cashew apple with milk is considered incompatible (Soro, 2012; Lacroix, 2003). This belief has a negative impact on the consumption of cashew apple juice; what hinders the development of this apple. Therefore present study was undertaken to check the statements that fall within the popular belief in Wistar rats.

## 2 Materials & Methods

### 2.1 Preparation of Cashew apple juice

The plant material cashew apple variety red was harvested from the Dassa-Zoumè local area, at the center of Benin. Cashew apple fruits were cut into small pieces and crushed them under laboratory conditions, the resulting mash was pressed using a press to extract the juice. The animal products cow milk were collected from farmers and then sterilized in the laboratory; yogurt was made from the same milk (cow milk fermented). Wistar albino rats were selected in the pet of

Human Biology Unit of the Faculty of Health Sciences of Cotonou.

### 2.2 Experimental setup

Ten sets of 3 rats each were formed among these the first set was used as control rat while the rest nine sets of rat had received various treatments of 2ml for 200g body weight. Among these, the set 2, 3 and 4 have been given only cow's milk, juice and the yogurt respectively. While the rats of set 5, 6 and 7 have received a mixture of juice and cow milk in different proportions. Finally 8, 9 and 10 set were treated at the mixture of juice-yogurt (Table 1). All set of rats were acclimated for fourteen days to farming conditions from the pet of the Human Biology Unit of Cotonou's Faculty Health Sciences. Ethical guide line proposed by University was followed strictly and before being sacrificed, rats were anesthetized with chloroform.

Table 1 Various treatments imposed to the Wistar of rats

Batches of the rats	Treatment
Batch 1	Control
Batch 2	Cashew apple juice (2 ml/200g of body weight)
Batch 3	Milk (2 ml/200g of body weight)
Batch 4	Yogurt (2 ml/200g of body weight)
Batch 5	0.5ml milk +1.5ml juice/200g of body weight
Batch 6	0.75ml milk +1.25ml juice/200g of body weight
Batch 7	1ml milk + 1ml juice/200g of body weight
Batch 8	0.5ml yogurt + 1.5ml juice/200g of body weight
Batch 9	0.75ml yogurt +1.25ml juice/200g of body weight
Batch 10	1ml yogurt + 1ml juice/200g of body weight

The abdominal cavity of each rat has been incised and the organs such as stomach, liver and kidneys were removed and fixed in formaldehyde at 10%. These organs were then cut and then placed in numbered cassettes. Finally, the tapes were immersed in a formaldehyde solution at 10% for 72 hours to be fixed. Traffic operations, coating, cutting with a microtome, spread, dyeing and setting were performed according to classic histological techniques (Beaumont & Cassier, 1970; Maynard et al., 2014).

## 3 Results and Discussion

Table 1 shows the experimental device which used for histopathological analyzes. Results of histopathological analysis revealed that the treatments containing juice, milk and yogurt, singly or combination of juice and milk did not have any impact on the integrity of the stomach cells, kidney and hepatic and the conditions of stomach, liver and kidney are identical to control rats (figure 1-7).

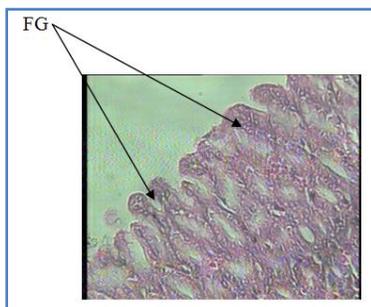


Figure 1 Histological structure of the stomach of the control rats FG - glandular sheet.

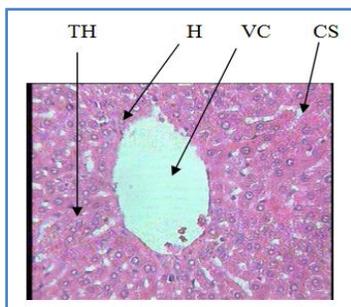


Figure 2 Histological structure of the liver of the control rats TH - Hepatic bays; H - Hepatocytes; VC - Centrilobular vein; CS = Sinusoids capillary.

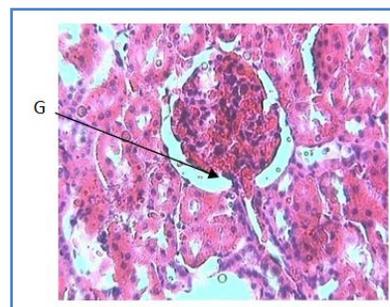


Figure 3 Histological structure of the kidney of the control rat G - Glomerule

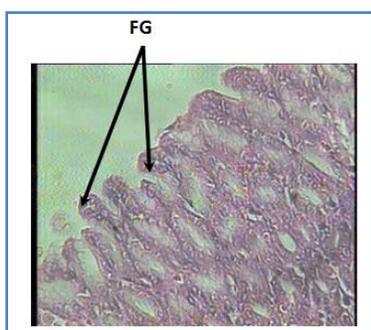


Figure 4 Histological structure of the stomach of the rats after 14 days of the treatment of juice - milk mixture

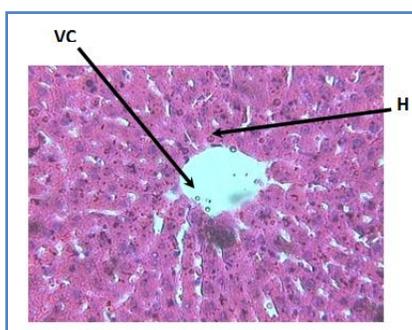


Figure 5 Histological structure of the liver of the rats after 14 days of the treatment of juice - milk mixture

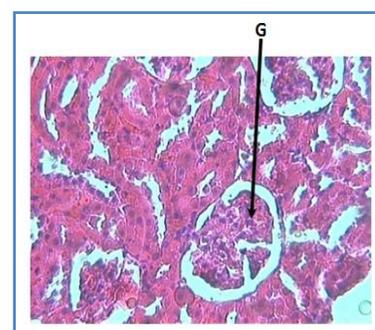


Figure 6 Histological structure of the kidney of the rats after 14 days of the treatment of juice - milk mixture

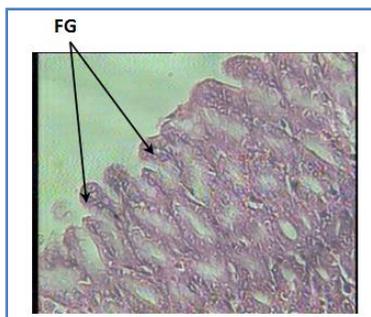


Figure 7 Histological structure of the stomach of the rats after 14 days of the treatment of juice - yoghurt mixture

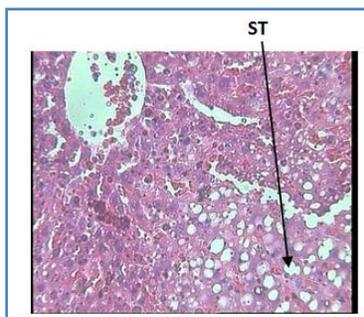


Figure 8 Histological structure of the liver of the rats after 14 days of the treatment of juice - yoghurt mixture, showing periportal steatosis (ST)

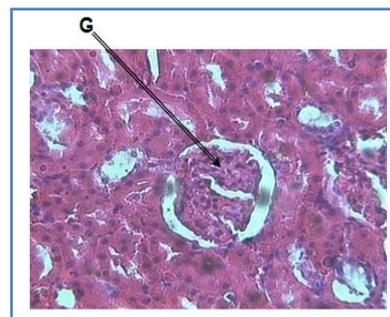


Figure 9 Histological structure of the kidney of the rats after 14 days of the treatment of juice - yoghurt mixture

There is no literature on the effects of the consumption of cow's milk and cashew apple juice mixture. On the other hand, figures 8 and 10 showed some differences in the state of the liver cell of the rat than the control rats. Group 8, 9 and 10 were treated with juice and yogurt mixture and the liver of these groups rat showed an anomaly when compare with the control group (Figures 8 & 10). After 14 days of treatment a periportal steatosis in one hand is observed for a mixture of 1 mL of juice + 1mL of yoghurt to 200g of body weight (Figure 8) and a periportal necrosis on the other hand, to a mixture of

1.25 mL of juice + 0.75 mL of yoghurt to 200g of body weight (Figure 10).

The association yogurt + juice then translated a frank hepatic cytolysis for rats of batch 9 and 10. According to Fromenty et al. (2012), this periportal steatosis may be because of the accumulation of lipid in hepatocytes mainly as triglycerides. This accumulation of triglycerides is often caused by obesity, diabetes of type 2, alcohol poisoning and drugs.

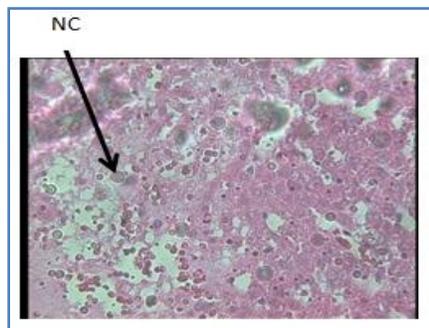


Figure 10 Histological structure of the liver of the rats after 14 days of the treatment of juice - yoghurt mixture, showing the destruction of hepatics bays and necrosis cells (NC)

Yogurt also contains lipids and its association with the cashew apple juice would therefore be a favorable factor in the formation of triglycerides. The rats of these same batches didn't show any abnormalities in case of stomach and kidney (Figures 7 & 9). This study checked the hepatic toxicity of cashew apple juice and yogurt mixture. In the other hand, the cashew apple juice, cow's milk and yogurt eaten alone didn't show any detectable toxicity in present study. Results of study suggested the precaution of avoiding the combination of cashew apple and yogurt can ensure that apple consumption in complete safety. These are the results are a preliminary study that may be the basis of further research, exploration of the toxicity of this mixture in animal model is required.

#### Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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