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*International Journal of Current Research*
*Vol. 9, Issue, 07, pp.54638-54643, July, 2017*

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH**

**ISSN: 0975-833X**

## REVIEW ARTICLE

# HISTOLOGICAL EFFECT OF TUNKABEIN DRINK ON THE LIVER AND STOMACH OF ADULT WISTAR RATS

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**ARTICLE INFO**

***Article History:***
Received 09th April, 2017
Received in revised form
24th May, 2017
Accepted 16th June, 2017
Published online 31st July, 2017

***Key words:***

Histological, Stomach,
Liver, tunkabein,
Wistar rats, alcohol.

**ABSTRACT**

Tunkabein drink is the local name for a locally prepared alcoholic beverage made up of alcohol (ogogoro) and *Phyllantusamarus.* In some of the Ijaw Communities, especially Amassoma, Bayelsa State of Nigeria. Twenty four(24) adult wistar rats of both sexes weighing 171g-250g were used for the study. They were assigned to four experimental groups of six rats each;group A(high dose group), groupB (low dose roup), group C(alcohol group) and group D(control group).The rats were sacrificed at the 15th and 22nd day.Histological result of the liver reveals that animals in group A showed extension of the portal tract into the parenchyma of the tissue with the infiltration of inflammatory cells,group B showed inflammatory cell and slight presence of Mallory bodies, group C showed dilated sinusoid and numerous Mallory bodies while group D showed normal liver tissue. Histological result of the stomach reveal that animal in group A show gastritis and superficial erosion, while group B show cysticaly dilated gastric gland with edematous stroma, group C showed distorted gastric architecture and slightly atrophic gland, however group D show normal histology of the stomach. The varying degree of damage done to the liver and stomach is due to the masking effect of thevarious concentration of alcohol over Phyllantusamarus (hepatoprotective and gastroprotective). Tunkabein is both hepatoxic and gastrotoxic and thus chronic consumption should be avoided.



**Citation: Eric, U. E., Okoye, F.B.C. and Owajaniro, M. G. 2017.** "Histological effect of tunkabein drink on the liver and stomach of adult wistar rats", *International Journal of Current Research*, 9, (07), 54638-54643.

## INTRODUCTION

Tunkabein drink is the local name for a locally prepared alcoholic beverage made up of alcohol (ogogoro) and *Phyllantusamarus.* In some of the Ijaw Communities, especially Amassoma, Bayelsa State of Nigeria, Tunkanbein drink is used to cure malaria and other ailments. The aerial part of the plant is mostly used. *Phyllanthusamarus* is widely known and used as a medicinal plant that cures a variety of diseases. Traditionally, it is known by many different names: carry me seed, Gulf leaf flower, hurricane weed, stone breaker, shatterstone, tunkabein, etc. It belongs to the family Euphorbiaceae (Phyllanthaceae) (Kassuya*et al.*, 2005). With its origin from tropical America, it has spread widely as a weed across the tropics to the subtropics. It is well known and explored across Africa and Indian Ocean islands (Burkill., 1994). Nigeria has also gotten a fair share of its numerous usages. *Phyllanthusamarus* grown as a medicinal crop is found mostly in waste ground, open localities, grassy scrub vegetation as well as dry deciduous forest.

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*Phyllanthusamarus* basically contains phyllanthine and hypophyllanthine collectively known as lignans (Sharma *et al*., 1993; Somanabandhu *et al*., 1993) geraniin and 5 flavonaoids which includes quercertin, astralgin, , isoquercitrin, rutin and quercertrin.It is a monoecious plant.This study is of a great significance. It will help health policy makers to formulate policies on alcohol consumption. If *Phyllanthusamarus* is found to have inhibitory effect on alcohol toxicity, then consumers of alcohol will be encouraged to add *Phyllanthusamarus* to the alcohol before consumption. It will also trigger economic policies favourable to traditional alcoholic beverages which will influence the government on lifting the ban on ogogoro. But if on the contrary, alcohol consumption will be strongly discouraged. The central purpose of this study is to investigate the histopathological effect of oral administration of Tunkabein drink (alcoholic beverage containing phyllanthusamarus) on the liver and stomach of adult wistar rat.

## MATERIALS AND METHODS

**Location of study:** This study was carried out in the Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta

University located on Wilberforce Island, Amassoma, Bayelsa state of Nigeria.

**Duration of study**

The study which encompasses the purchase and acclimatization of the animals; collection of plants and administration together with the tissue processing, and histological studies, spanned through the months of August and November, 2016.

**Animals/care**

Twenty four adult wistar rats of both sexes weighing between 171-250g were used for this work. They were bought from the animal house of the Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State of Nigeria. They were randomly assigned into four groups (A, B, C and D) of six each and were put in their respective cages of aluminium frame with metal nettings cover allowing them acclimatize for two weeks. They were fed with pelletized feed manufactured by Grand Cereals limited, Jos, Plateau State and were given water ad libitum. The animals were exposed to natural room temperature and lighting conditions (12 hour light-dark cycle), and were housed under standard condition at 25±2$^{o}$C(constant temperature), a relative humidity of 60± 5% and also handled according to standard protocols for the use of laboratory animals (College of Health Sciences animal ethics Committee ). Their cages were cleaned at interval of two days. All the animals were checked for abnormal behavior and illnesses.

**Collection/confirmation of plants**

Fresh *Phyllanthusamarus* plants (aerial parts) were collected at different locations in Amassoma town, Southern Ijaw Local Government Area of Bayelsa State. The plant was identified and authenticated in the Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, Niger Delta University, Wilberforce Island of Bayelsa State by the Head of Department, Prof. Kolawole KayodeAjibesin. A voucher specimen was deposited in the Herbarim of Niger Delta University. It was washed and the water allowed to air dry at room temperature but still maintaining its freshness.

**Preparation of tunkabein drink**

The fresh aerial parts of *P. amarus*were weighed with the aid of Chimadzu (ELB600, JAPAN) digital weighing balance with the weights at 29g and 34.2g respectively. It was put into clean plastic bottles labeled 'A' and 'B' respectively. Concentrations of 311mls of 30% ethanol and 264mls of 60% of ethanol were prepared and put into their respective containers as labeled. 264mls of 60% ethanol was added to container 'A' containing 29g of *P. amarus* while 311mls of 30% ethanol was added to container 'B' containing 34.2g of same plant. The mixtures were vigorously shaken and allowed to stand for 24hours before administration.

**Experimental design**

Twenty four adult wistar rats weighing between 171-250g were randomly assigned into four groups of six (6) each. Group A and B serve as the treatment groups while C and D serves as positive and negative control respectively. The treatment groups received 1ml of Tunkabein drink each for 14days and 21days respectively using improvised orogastric tube. The administration of the drink is as indicated below:

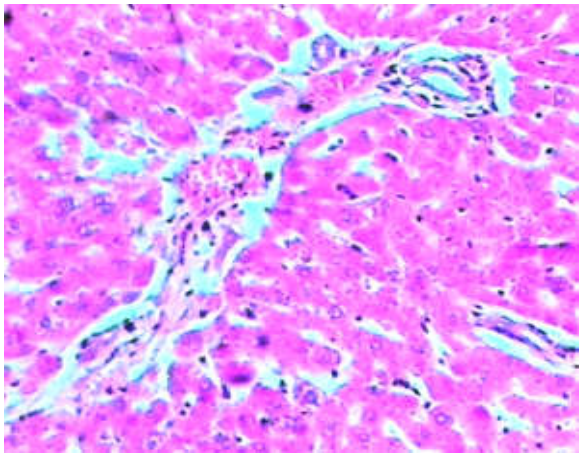
- **Group A**: Received 1ml of Tunkabein drink (29g of *P. amarus* in 264mls of 60% ethanol)
- **Group B**: Received 1ml of Tunkabein drink (34.2g Of *P. amarus* in 311mls of 30% ethanol)
- **Group C**: Received 1ml of 60% ethanol.
- **Group D**: Was fed with water and feeds only.

**Histological study**

Three rats in each group were sacrificed on the 15$^{th}$ day using chloroform inhalation method while the rest were sacrificed on the 22$^{nd}$ day during which the organs of interest, liver and stomach were harvested. Specimen of liver and stomach were fixed in 10% formal saline, dehydrated in ascending grades of alcohol, cleared in xylene, impregnated and embedded in paraffin wax. Serial sections of 5microns thick were obtained using rotary microtome (LEICA RM 2125 RTS). The sections were deparaffinized and stained routinely using haematoxylin and eosin (H&E) method (Ochei and Kolhatkar, 2000). Photomicrographs were taken using research microscope (Olympus) in the Department of Anatomical Pathology, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State.

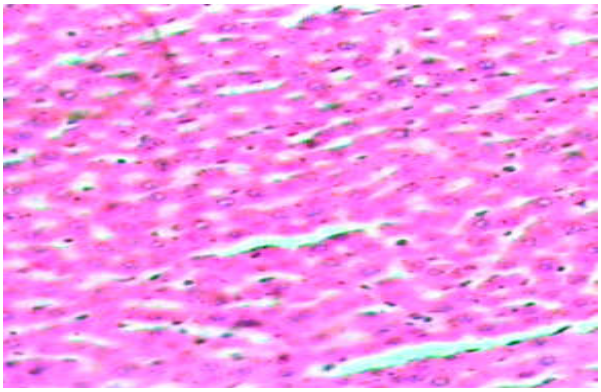
## RESULTS

**Histological Studies:** The histological photomicrograph of the liver of adult wistar rat, slide 1 (Group A) shows the extension of the portal tract into the parenchyma of the tissue with the infiltration of inflammatory cells, mostly neutrophils. Slides 2a (Group B) revealeddilated sinusoids and vacuolated hepatocytes while slide 2b (Group B) shows liver tissue with occluded central vein with fibrinous substances. The parenchyma shows inflammatory cells with little Mallory bodies. Slide 3(Group C) shows a liver tissue with dilated sinusoids and numerous Mallory bodies. Slide 4 (Group D-Normal Control) shows normal liver tissue morphology. The section shows normal hepatocytes radiating towards the normal central vein, sinusoids and blood vessel are nomal.

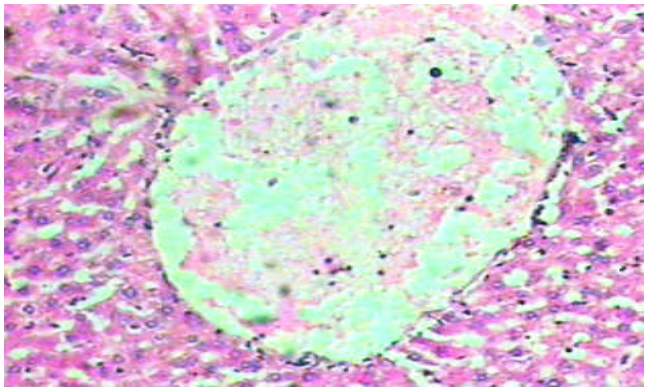


**Slide 1. (Photomicrograph of the Liver - Group A, ×100) (60% Alcohol + *P. amarus*) showing the extension of the portal tract into the parenchyma of the tissue with the infiltration of inflammatory cells**

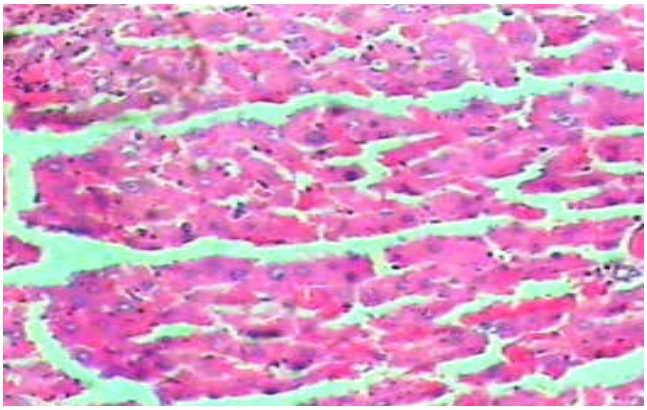
The hepatocytes show normal blue stained nuclei. The histological photomicrograph of the stomach of adult wistar rat, slide 5 (Group A) shows acute gastritis with superficial erosion, it consists of inflammatory cells and fibrin on the surface of the inflammatory cells with loss of covering epithelial layers. Slide 6a (Group B) shows cystically dilated gasric glands with edematous stroma while Slide6b revealed distorted submucosa and ulcerated mucosa, Slide7 (Group C) shows distorted gastric architecture and slightly atrophic glands. Finally, slide 8(Group D-normal control) shows a normal histology of the stomach of adult wistar rat. The mucosal lining is intact.



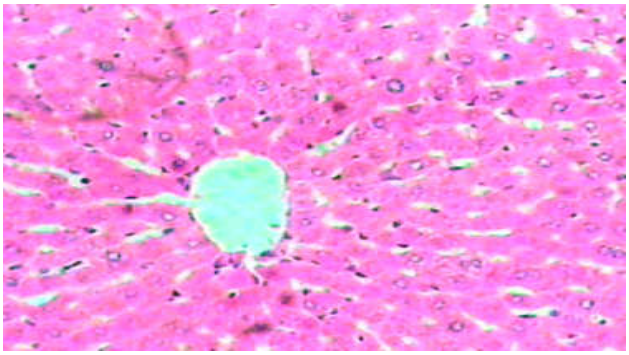
**Slide 2a. (Photomicrograph of the Liver - Group B, ×100) (30% Alcohol + *P. amarus*) at 14days of administration showing liver tissue with dilated sinusoids and vacuolated hepatocyes**



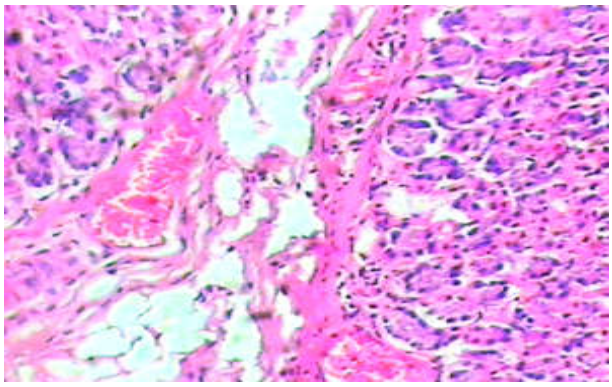
**Slide 2b. (Photomicrograph of the Liver - Group B, ×100) (30% Alcohol + *P. amarus*) at 21days of administration showing liver tissue with occluded central vein with fibrinous substances. The parenchyma shows inflammatory cells with little Mallory bodies**



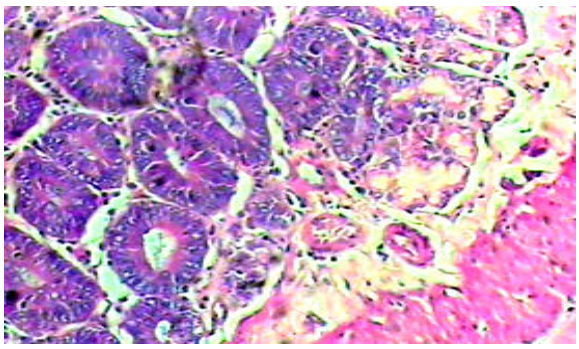
**Slide 3. (Photomicrograph of the Liver - Group C, ×100) (60% Alcohol) showing a liver tissue with dilated sinusoids and numerous Mallory bo**



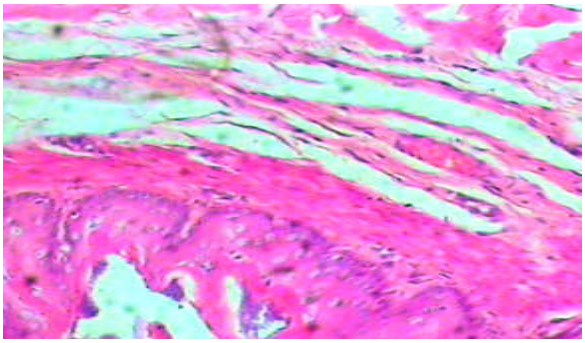
**SLIDE 4: (Photomicrograph of the Liver - Group D, ×100) (Normal Control) showing normal liver tissue morphology. The section shows normal hepatocytes radiating towards the normal central vein, sinusoids and blood vessel are nomal. The hepatocytes show normal bleu stained nuclei.**



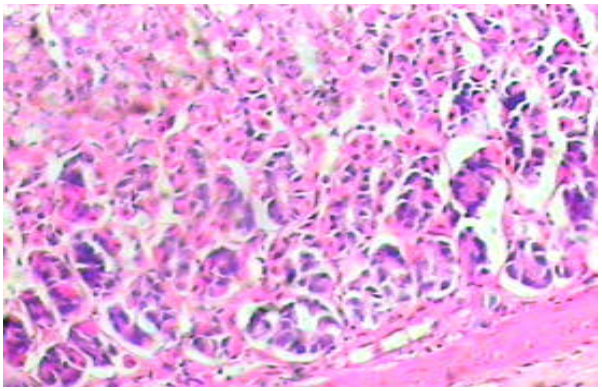
**Slide 5. (Photomicrograph of the Stomach - Group A, ×100) (60% Alcohol + *P. amarus*) showing acute gastritis with superficial erosion, it consists of inflammatory cells and fibrous materials on the surface of the inflammatory cells with loss of covering epithelial layers**



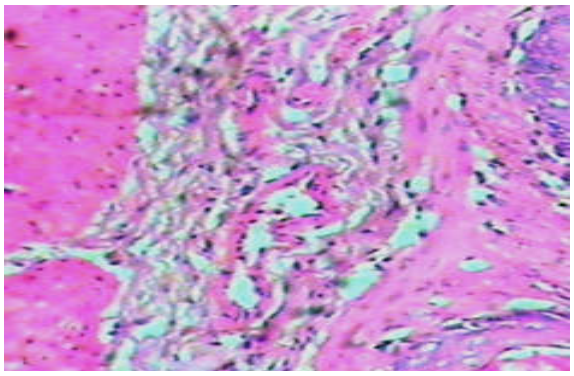
**Slide 6a. (Photomicrograph of the Stomach - Group B, ×100) (30% Alcohol + *P. amarus*) at 14days of administration showing cystically dilated gastric glands with edematous stroma**



**Slide 6b. (Photomicrograph of the Stomach - Group B, ×100) (30% Alcohol + *P. amarus*) at 21days of administration showingextensive submucosal distortion, mucosal ulceration**



**Slide 7. (Photomicrograph of the Stomach - Group C, ×100) (60% Alcohol) shows distorted gastric mucosa architecture with dilated gastric glands**



**Slide 8. (Photomicrograph of the Stomach - Group D, ×100) (Normal Control) shows the normal histology of the stomach with mucosal lining intact**

## DISCUSSION

The photomicrograph of Slide 1 revealed the extension of the portal tract into the parenchyma of the tissue with the infiltration of inflammatory cells and prominent hepatocytes. Slides 2a revealeddilated sinusoids and vacuolated hepatocytes and slide2b showed occluded central vein with fibroussubstances. Slides 3 showed dilated sinusoids and numerous Mallory bodies which is a strong indication of alcoholic liver injury. This finding correlates with Chen (2010) in a study that determined the protective effects of quercetin on liver injury induced by ethanol. This result is further confirmed by Lijie*et al*. (2013) who reported necrosis, infammatory cell infiltration, fibrosis and damaged acinihepatis establishing acute alcoholic liver injury. This result strongly corroborates with Hussein *et al.* (2007), Who documented that liver histology of ethanol administered animal showed pathomorphologic alterations in the form of obvious dilatation, congestion of blood vessels and central vein accompanied with marked fibrosis extending from the portal area in-between the hepatocytes. These findings are also supported and explained by those of Charles *et al*. (2003), who stated that alcohol increases hepatic collagen. This leads to cirrhosis, septal and perivenular fibrosis which is in agreement with MacSween and Burt (1986), who observed a spectrum of histological abnormalities in the liver by alcohol administration. Slide 4: (Photomicrograph of the Liver - Group D) (Normal Control) revealed normal liver tissue architecture. The section shows normal hepatocytes radiating towards the normal central vein, sinusoids and blood vessel are normal. The hepatocytes show normal blue stained nuclei. This finding is interestingly similar to Carlos *et al.* (2015). The histological result shows that *P. amarus*, one of component of Tunkabein drink could not inhibit the hepatotoxic activities of alcohol. This may be as result of the high concentration of the alcohol in the drink. The damage is more in the animals that underwent 21days of administration. The gastric mucosa is exposed to various stimuli including ethanol. Its intake has been shown to be associated with marked oxidative damage to gastric mucosa.Photomicrographs of slide 5 (Group A) showed acute gastritis with superficial erosion, it consists of inflammatory cells and fibrous material on the surface of the inflammatory cells with loss of covering epithelial layers. Slide6a (Group B)revealed cystically dilated gasric glands with edematous stroma and slide6b revealed distorted submucosa and ulcerated mucosa. Slide7 (Group C) showed distorted gastric mucosal architecture and slightly atrophic glands. Interestingly, this finding is consistent with Shin *et al*. (2013), who reported that the administration of ethanol induced gastric mucosal damage such as hemorrhage, edema, erosion, ulceration, and loss of epithelial cells.

This result is in agreement with Mahmood*et al.*(2011), who reported that histological observation of ethanol induced gastric lesion showed comparatively extensive damage to the gastric mucosa, oedema and leucocytes infiltration of the submucosal layer. The result also correlates with other studies which reported that alcohol, a major component of Tunkabein drink, causes hemorrhagic gastric lesions characterized by mucosal friability, cellular exfoliation, extensive submucosal edema and inflammatory cell infiltration. (Park *et al.,* 2008). Gastroprotective studies had shown that ethanol could injure the epithelium of stomach. Ethanol may increase the permeability of the vessels and develop edema in submucosal layer of the stomach as well as epithelial lifting. Ethanol also caused dissolution of mucus constituents and reduced the mucus contents (Sener*et al.,* 2004). The gastric damages represented in the result is similar to Chi-Chang *et al*. (2013) who recorded significant and extensive damage in the gastric mucosa, with edema in the submucosal layer which was attributed to many mechanisms, including depletion of gastric mucus and impaired mucosal permeability, and leads to increased leakage of hydrogen ions from the lumen and decreased transluminal membrane potential difference. Slide8 (Group D-Normal control) showed a normal histology of the stomach of adult wistar rat. The mucosal lining is intact. The overall result indicates that Tunkabein drink is gastrotoxic. This toxicity is concentration-related as the injury is more severe in group A and C which received 60% alcohol plus *p. amarus*and 60% of alcohol (without *p. amarus*) respectively as compared to Group B which received 30% alcohol and *p. amarus*. This observation is interestingly confirmed by Karmen*et al.*(1987), who reported that all concentrations of alcohol tested in their study induced injury in the glandular epithelium. This damage, they said, was concentration-related with 25% ethanol eliciting the least damage while 50% ethanol was as damaging as 100% to the gastric mucosa morphologically, a circumstance not previously reported.

### Conclusion

The result of this study shows varying degree of gastric and hepatic damages which may be as a result of the different concentrations of alcohol in the tunkabein drink. *Phyllanthusamarus,* a hepatoprotective and gastroprotective plant seem not to be active in the presence of high

concentration of alcohol. Tunkabein drink is both gastrotoxic and hepatotoxic. It will be important to note that chronic consumption of the drink is likely to cause damage to the liver, stomach and possibly other organs of the consumers.

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