

Effect of Fresh *Citrus limon* Juice on Liver Histomorphology of Growing Rabbits (*Oryctolagus cuniculus*)

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Abstract: Histomorphology of the *Citrus limon* was investigated, Twenty four (24) rabbits of mixed breeds aged between 10 – 16 weeks, weighing between 600 – 2185g were used in this experiment to determine the morphological effect of feeding varying dietary levels of *citrus limon* juice on the histomorphology of the liver of growing rabbits. The rabbits were randomly allocated into four treatments containing 0% (control), 25%, 50% and 75% *citrus limon* juice administration respectively. Feed and water were served ad-libitum throughout the 5 weeks experimental period. At the end of the juice administration, Final body weight of the animal were taken, animals were sacrificed using chloroform inhalation in a desiccator, liver tissues were extracted, weighed and placed into Neutral buffered formalin, sent to the laboratory for histopathological analysis Changes in Liver weight was not significant ($P<0.05$) compared to the Body weight while the weight gain in the treatment groups was extremely significant ($P<0.001$). The histopathological results revealed no cellular abnormality in the entire treatment groups as compared to control group. In conclusion, it is shown that *Citrus limon* juice can be included up to 75% in rabbit diets without any adverse or deleterious effect on the histomorphology of the liver of rabbits, as it enhances the cyto-architecture thereby preserving and increasing the cellular profile.

Keywords: Rabbit, Liver. Citrus limon, Histomorphology

INTRODUCTION

Lemon trees are fruit trees that produce small, oval, yellow citrus fruits. Their scientific name is *Citrus limon*. The lemons are a type of berry called a hesperidium. Lemons can be used in various ways in our life. They can be used as preventative and domestic medicine because they are very rich in vitamin C, bioflavonoids, acids and volatile oils that help the body fight infections. Since they are very acidic, they cause an alkalizing effect upon the body. The sweetened juice relieves gingivitis, stomatitis, and inflammation of the tongue. They can also be used for food like cakes, juice and beverages. The taste and scent of the lemon help food better. The lemon peel oil is used in soaps and shampoos. The petitgrain oil, which is from the leaves, twigs, and immature fruits, are important in colognes. The tree's woods are also very useful in furniture[1].

Citrus limon juice is widely known as a diuretic, antiscorbutic, astringent, and febrifuge. In Italy, the sweetened juice is given to relieve gingivitis, stomatitis, and inflammation of the tongue. Lemon juice in hot water has been widely advocated as a daily laxative and preventive of the common cold. Adversely, the thorns of the lemon tree inflict painful punctures and scratches. Lemon peel oil may cause contact

dermatitis, chronic in those who handle, cut and squeeze lemons daily. Parts of the body touched by contaminated hands may show severe reactions after exposure to the sun. People that suck *Citrus limon* may suffer irritation and eruptions around the mouth.

The liver is a vital organ of the digestive system present in vertebrates and some other animals. It has a wide range of functions, including detoxification, protein synthesis, and production of biochemicals necessary for digestion it plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification.

Farm animals are being fed by human with forages, *Citrus limon* juice which may contain certain elements and metals that can have adverse effect on the well being of the animal and in turn influence the digestive/metabolic life of the animals negatively.

Citrus limon can be used in various ways in our life. They can be used as preventative and domestic medicine because they are very rich in vitamin C, bioflavonoids, acids and volatile oil that help the body fight infections[1].

Studies have shown the usage of citrus pulp and peel meal as animal feedstuff without any side effect on the animals. Meanwhile, no significant studies have been carried out on the juice of *Citrus limon* to establish its beneficial effect and possible toxicity in rabbit models, this study was designed to determine possible histopathology and gross morphology effect of *Citrus limon* juice on the liver of growing rabbits

MATERIALS AND METHOD

Materials and Reagents

24 Rabbits, hutches, concentrate feed (grower mash), syringes and hypodermic needles, universal treated bottles, latex hand gloves, weighing balance, centrifuge, Graduated vials, measuring tape, citrus lemon juice, microscope, microtome, oven, paraffin wax, embedding moulds, staining racks, slides, cover slide, chloroform, feeders, drinkers, *Centrosema pubescens*. Reagents used: Turll solution, sodium metabolite, Metaheamoglobin, Sodium chloride, potassium chloride, mountant, Neutral Buffered formalin, Absolute Alcohol, Eosin, Xylene, Paraffin wax.

Experimental Animals and Management

Twenty four (24) rabbits, aged 10-16 weeks old were purchased from reputable farms in Uyo metropolis for the experiment. They were acclimatized for two weeks before the administration of the treatment (*Citrus limon* juice). The rabbits were divided into four groups; each group comprised of 6 animals, 3 males and 3 females. Two mild strains of rabbits were used; the chinchilla and New Zealand white. The experiment was carried out at the rabbitry unit of the teaching and research farm of Animal Science Department, University of Uyo, Akwa Ibom States situated on latitude 5°02'32 N and longitude 7°54'06E and lies at altitude of 120metres above sea level with average rainfall of 1500mm. The state is in the south-south geopolitical zone, Nigeria.

Experimental plan and Fresh *Citrus limon* Juice Administration

The experiment was designed to have four treatments.

Treatment 1 - Distilled water for 5 weeks

Treatment 2 - 25% *Citrus limon* juice concentration + 75ml of Water for 5 weeks

Treatment 3 - 50% *Citrus limon* concentration + 50ml of Water for 5 weeks

Treatment 4 - 75% *Citrus limon* concentration + 25ml of Water for 5 weeks

The variety of *Citrus limon* used was the rough lemon bought from a reliable source at Itam Market, Uyo. Akwa Ibom state, Nigeria. The rabbits were weighed before the administration, progressive administration was given with time. The *Citrus limon*

were peeled and the juice squeezed out into a clean container manually. The extract was filtered using a clean sieve and the filtrate collected into clean bottles.

Sample Collection for Histopathological analysis

The rabbits were anesthetized with chloroform vapour soaked in cotton wool in a dessicator 24 hours after the last juice administration. The animals were dissected and the liver of each rabbit was carefully cut out, trimmed of all fat and connective tissue and weighed immediately. The tissues were immediately fixed in neutral buffered formalin in well labelled Petri-dish. The extracted liver tissues were placed in 10% neutral buffered formalin (fixation), transferred to a graded series of ethanol (Dehydration). On day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene (clearing). Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax (impregnation) at one-hour intervals was made, after which the tissues were embedded (embedding) in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicularly to the long axis of the tissues. The sections were designated "vertical sections". Serial sections of 5µm thick were obtained from a solid block of tissue (microtomy), fixed on clean albuminized slides to prevent sections coming off the slides and later stained with haematoxylin and eosin staining techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mounted in DPX mountant, allowed to dry at room temperature and observed histopathologically under digital light microscope.

Gross Morphological and statistical Analysis

The initial and final weight of each rabbits and the weight of each liver in each group were taken using sensitive weighing balance graduated at 0.000g. Analysis was carried out using Graphpad prism 6 version 2; all data were expressed as mean ± SEM. One way analysis of variance (ANOVA) was used to test for difference among the groups. Dunnet's multiple range tests was used to test for significant differences among treatment means. P < 0.001 was considered extremely significant, P < 0.01 was very significant and P > 0.05 was non – significant.

Photomicrography

Histological results were obtained by photomicrography using digital photomicrographic microscope at the Gross Anatomy Research Laboratory, Department of Human Anatomy, College of Health sciences, University of Uyo, Uyo, Akwa- Ibom state, Nigeria as illustrated in Plate 1 to 4.

RESULT

Gross Morphological Changes

Table 2: Weight Gain and Liver weight of Rabbits as affected by the *Citrus limon*.

Groups	Weight Gain (g)	Liver weight (g)
Group 1 (Control)	456.7±33.09	57.0±6.037
Group 2 (25%)	386.0±29.11 ^{ns}	51.20±1.878 ^{ns}
Group 3 (50%)	390.3±58.84 ^{ns}	58.30±0.491 ^{ns}
Group 4 (75%)	335.2±57.27 ^{ns}	68.95±4.494 ^{ns}

Means ± SEM (n=6). ns = Not-significant (P < 0.05)

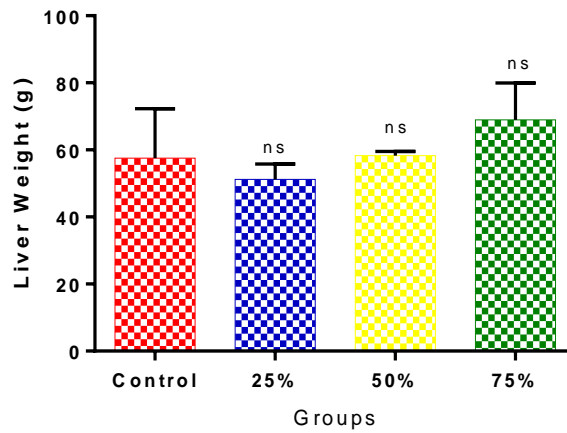


Fig- 1: Liver body weight of animal among the treatment groups.

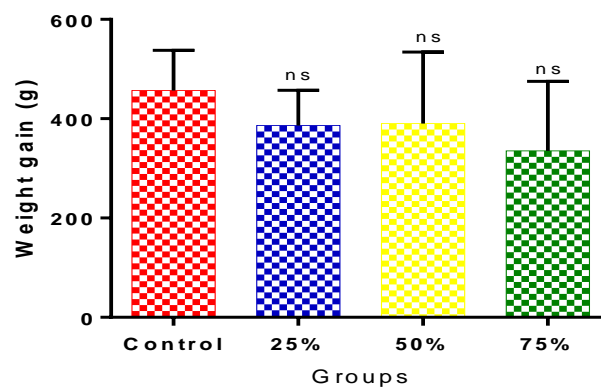


Fig-2: Weight gain of animal among the treatment groups.

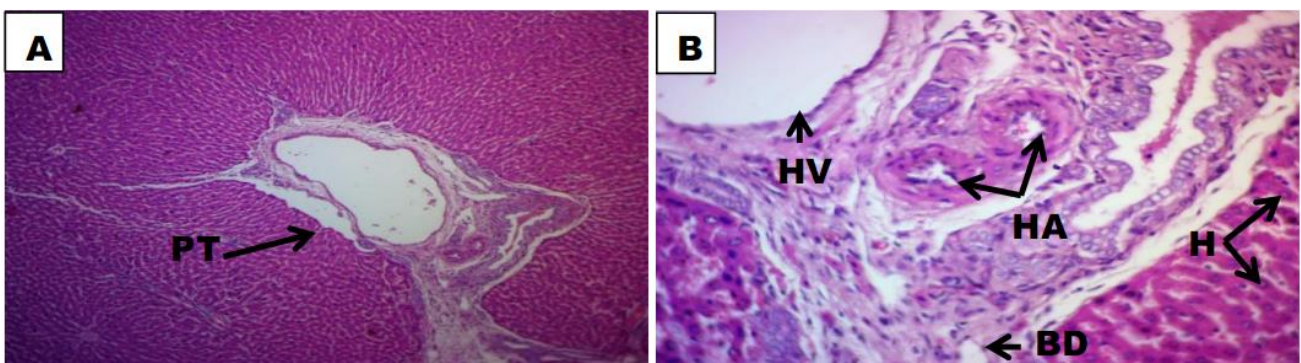


PLATE A (100X) and B (400X) Control Liver without treatment stained with H&E method

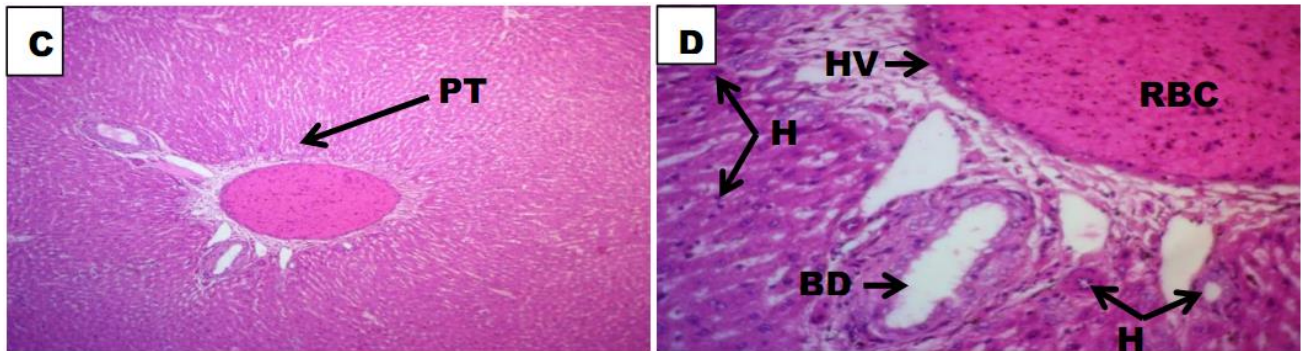


PLATE C (100X) and D (400X) Liver treated with 25% C. Limon juice stained with H&E method

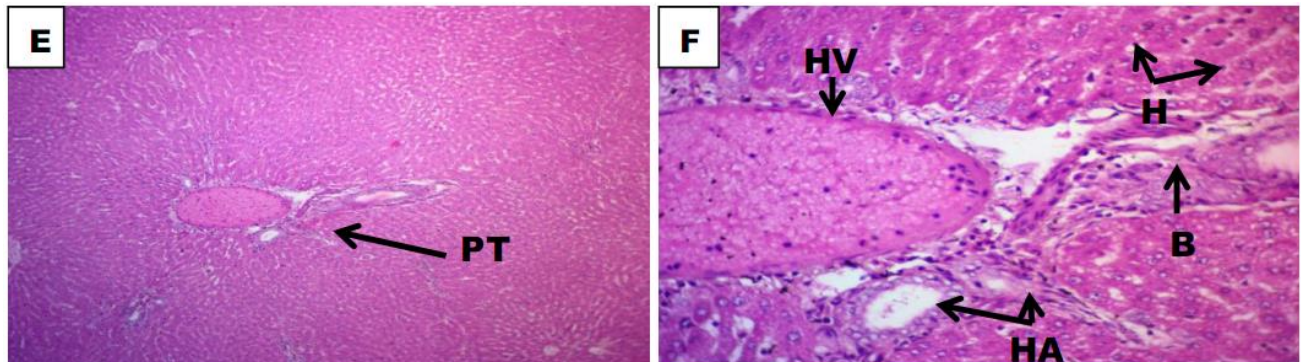


PLATE E (100X) and F (400X) Liver treated with 50% C. Limon juice stained with H&E method

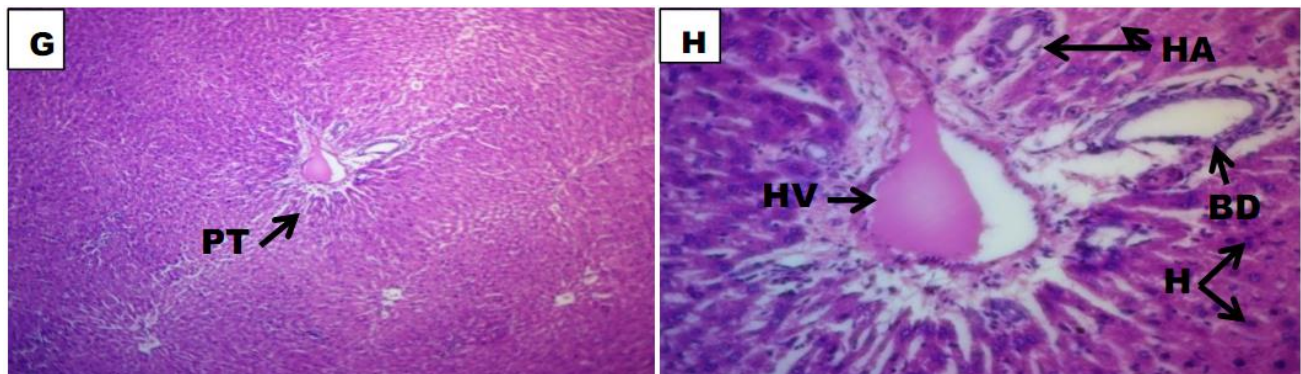


PLATE G (100X) and H (400X) Liver treated with 75% C. Limon juice stained with H&E method

Keys: Portal triad (PT) Bile duct (BD), Hepatic Artery (HA), Hepatic vein (HV), Hepatocytes (H) and Nucleus (N)

PLATE A and B: Control Liver without treatment revealed intact architecture, the cellular profile revealed good portal triad containing hepatic artery, hepatic vein and bile duct. The hepatocytes are well shown, radiating from sinusoidal lining

PLATE C and D: Liver treated 25% concentration of *Citrus limon* juice revealed normal cellular profile, no abnormality seen as compared to control group

PLATE E and F: Liver treated 50% concentration of *Citrus limon* juice revealed normal cellular profile, no abnormality seen as compared to control group

PLATE G and H: Liver treated 75% concentration of *Citrus limon* juice revealed normal with improved cellular architecture as compared to control group.

DISCUSSION

The microscopic appearance of the liver has shown to be extremely useful in diagnosis [2]. From the study, it is observed that there is no significant difference in the row factor (initial body weight and final body weight of the rabbits), though the body weight appeared to be decrease per dose of the *Citrus limon*. The decrease in the final body weight of the experimental animals agrees with Johnson *et al.*, [3] who stated that saponins (a phytonutrient in *C. limon*) cause hypocholesteralaemia by binding cholesterol, making it unavailable for absorption. According to livestrong.com [4], lemon's high vitamin C content helps in weight loss. It is reported that vitamin C is inversely related to your body mass [5]. The researcher reported that having enough vitamin C intakes as opposed to being vitamin C deficient, helps the body set

off a series of chemical reactions that break down your body fat for use as energy. Lemon polyphenols (molecules found in plants with anti-oxidant qualities) “significantly suppressed” weight gain, accumulation of fat pads, excessive fat in your bloodstream, high blood sugar and insulin resistance. *Citrus limon* contain flavonoids that have many other weight-related benefits, including decreasing liver’s production of cholesterol and triglycerides[6-7].

The weight of the liver in the 25%, 50% and 75% *C.limon* juice treatment groups were not significantly different when compared to the control group.

This agrees with findings in livestrong.com [3] that the liver is much heavier in young animals than in older ones as it atrophies with age. According to a recent medical report from livestrong.com, lemons and other citrus fruits contain a pigment called cryptoxanthin that may significantly improve liver health[3].

Flavonoid is very important as it enhances the effects of vitamin C and provides a powerful defense against oxidative stress. They also have powerful anti-inflammatory benefits, they may also act as chemical messengers, physiological regulators and cells cycle inhibitors[8].

CONCLUSION

Liver is the major organ in the body which is responsible for a lot of metabolic activities including detoxification, this study revealed no abnormality seen in the liver tissue when exposed to fresh juice of *Citrus limon*. The findings showed that *Citrus limon* can be fed to rabbit at up to 75% level of inclusion without any detrimental effect on the cyto- architectural components of the liver of growing rabbits.

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