

## Biochemical Effect of Citrus lemon Juice on the Liver of Growing Rabbits (*Oryctolagus cuniculus*)

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**Abstract:** Twenty four rabbits of mixed breeds aged between 10 – 16 weeks, weighing between 600 – 2185g were used in this experiment to determine the effect of feeding varying dietary levels of citrus lemon juice on the biochemical parameters of growing rabbits. The rabbits were randomly allocated into four treatments containing 0% (control), 25%, 50% and 75% fresh citrus lemon juice respectively. Feed and water were served ad-libitum throughout the 5 weeks experimental period. At the end of the juice administration, animals were sacrificed using chloroform inhalation in a desiccators, Blood samples were collected in plain bottles, centrifuged and serum were taken for Liver function tests. Biochemical indices of liver function determined include serum total protein, albumin, conjugated and total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities. The result revealed extremely significant ( $P < 0.001$ ) influence of the juice on the biochemical parameters. In conclusion, it is shown that Citrus lemon juice does not alter or impair biochemical activities of the liver, it can be included up to 75% in rabbit diets without any adverse or deleterious effect on the Liver function activities of growing rabbits.

**Keywords:** Rabbits, Alkaline Transaminase, Alanine Transferees, Alkaline Phosphatase, Conjugated Bilirubin, Total Bilirubin, Citrus lemon

### INTRODUCTION

Citrus lemon is a small tree in the Rutaceae (citrus family), it is thought to have arisen as a hybrid between other Citrus species with lineage including lime (*C. aurantifolia* or *C. latifolia*), pomelo (*C. maxima*), and citron (*C. medica*) or hybrid between sour orange (*C. aurantium*) and citron. It has a long history of cultivation in South East Asia and China, but arrived the Mediterranean during Roman times, and was brought to the New World in the 16th century. In commercial cultivation, *C. lemon* is often grafted onto rootstock of the hardy rough lemon, *C. jambhiri*. [1-4].

Lemons are used in various ways in our life; they are 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 are 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 petit grain oil, which is from the leaves, twigs, and immature fruits, are important in colognes. The tree's woods are also very useful in furniture[5].



Fig-1a: *Citrus limon*. Source [6].



Fig-1b: Rabbit (*Oryctolagus cuniculus*). Source[7]

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 bio-chemicals necessary for digestion. The liver is necessary for survival; 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.

Liver function tests (LFT) are a helpful screening tool, which are an effective modality to detect hepatic dysfunction. Since the liver performs a variety of functions, no single test is sufficient to provide complete estimate of function of liver [8]. Research 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 lemon to establish its beneficial effect and possible toxicity in rabbit models, thus justifying this study being designed to determine the biochemical effect of citrus lemon juice on the liver functioning activities 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,. Reagents used: Turll solution, sodium metabolite, Metaheamoglobin, Sodium chloride, potassium chloride, mountant, formalin, Absolute Alcohol, ALP, ALT, AST, Total Bilirubin, and Conjugated Bilirubin Panels and Kits for Rabbit monoclonals from Biochain Inc. USA.

### **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 lemon 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, and Akwa Ibom States situated on latitude 5<sup>o</sup>02'32 N and longitude 7<sup>o</sup>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 lemon Juice Administration**

The experiment was designed to have four treatments.

- Treatment 1 - Distilled water for 5 weeks
- Treatment 2 - 25% Citrus lemon juice concentration + 75ml of Water for 5 weeks
- Treatment 3 - 50% Citrus lemon concentration + 50ml of Water for 5 weeks
- Treatment 4 - 75% Citrus lemon concentration + 25ml of Water for 5 weeks

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

Blood was collected from the heart of the dissected animal using syringes and needles. The collected blood samples were put into well labeled sterile plain bottles. The blood samples were centrifuged using the centrifuge machine and serum was collected into well labeled plain bottles, while the blood cells were discarded. The serum was taken to the laboratory for liver function analysis.

### **Biochemical Analytical Techniques**

Liver function analysis were carried out to determine the serum concentrations of total protein, albumin, conjugated and total bilirubin, and the activities of liver enzymes such as AST, ALT and ALP using diagnostic kits (Biochain Inc. USA). Total protein was determined by the Biuret method [9], albumin by the bromocresol green method [10], bilirubin was estimated by the method described by Jendrassik and Grof [11]. Alanine and aspartate aminotransferase were determined based on the colorimetric measurement of hydrazone formed with 2, 4 dinitrophenyl hydrazine [12], alkaline phosphatase by the phenolphthalein monophosphate method [13].

### **Statistical Analysis**

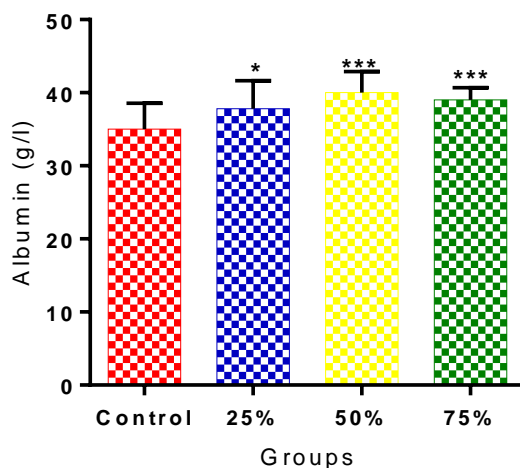
Analysis was carried out using Graph pad prism 6 version 2; all data were expressed as mean ± SEM. One way analysis of variance was used to test for difference among the groups. Dunnet's multiple range test was used to compare the significant differences among the means. P<0.001 was considered extremely statistically, P<0.01 was very significant, P<0.05 was significant and P>0.05 was non – significant.

## RESULTS

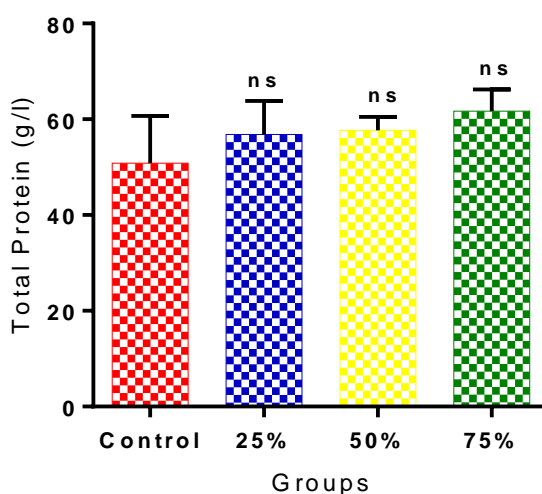
**Table 1: Biochemical indices as affected by fresh Citrus lemon in rabbits**

PARAMETERS	CONTROL	25%	50%	75%
Aspartate Aminotransferase	74.000 ± 0.707	58.000 ± 1.581***	53.000 ± 0.707***	44.000 ± 1.000***
Alanine Aminotransferase	124.00 ± 1.414	109.00 ± 1.140***	96.000 ± 1.140***	89.000 ± 1.140***
Alkaline Phosphatase	22.100 ± 0.100	18.400 ± 0.141***	14.300 ± 0.114***	9.800 ± 0.141***
Total Bilirubin	10.400 ± 0.141	6.700 ± 0.114***	5.300 ± 0.071***	4.700 ± 0.071***
Conjugated Bilirubin	0.746 ± 0.012	0.400 ± 0.071***	0.290 ± 0.071***	0.222 ± 0.086***
Albumin	31.50 ± 0.991	37.83 ± 1.558*	40.00 ± 1.183***	39.00 ± 0.683***
Total Protein	50.83 ± 4.020	56.83 ± 2.845 <sup>ns</sup>	57.67 ± 1.145 <sup>ns</sup>	61.67 ± 1.856*

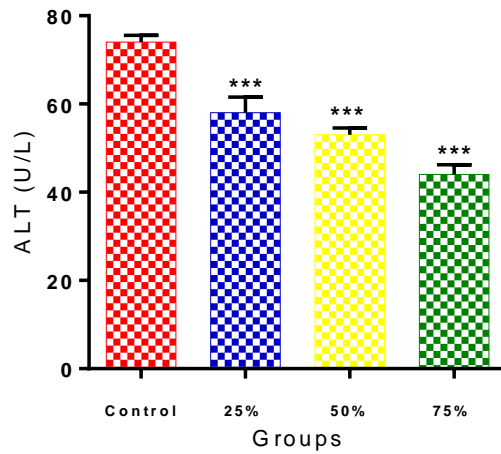
Means ± SEM (n=6). \*\*\* = Extremely significant (P < 0.001), \*\* = very significant (P < 0.01). \* = significant (P < 0.05), ns = Non-significant (P>0.05)



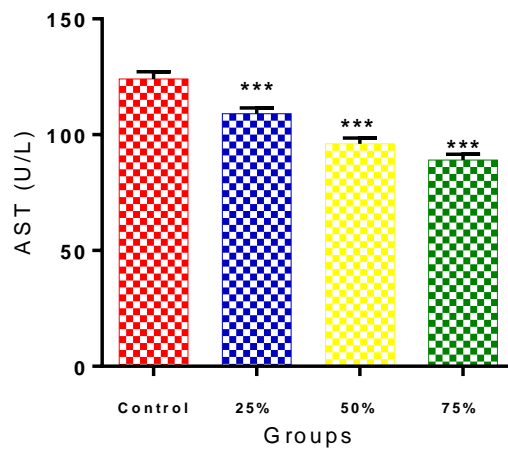
**Fig-2: Albumin concentration among the treatment groups**



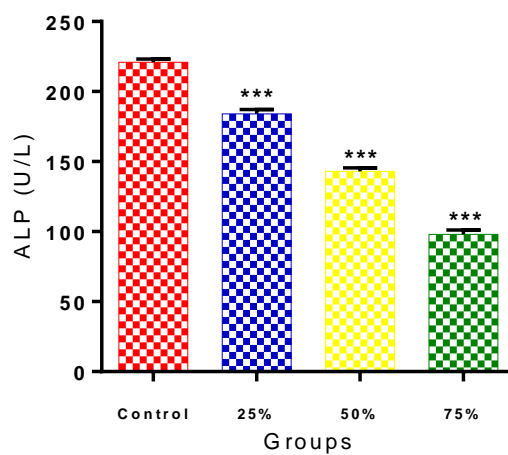
**Fig-3: Total Protein levels among the treatment groups.**



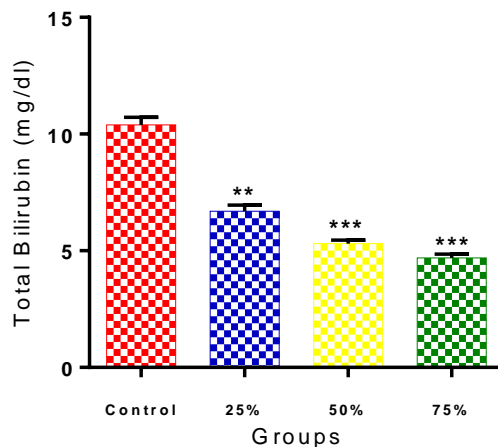
**Fig-4: Alkaline Transaminase level among the treatment groups.**



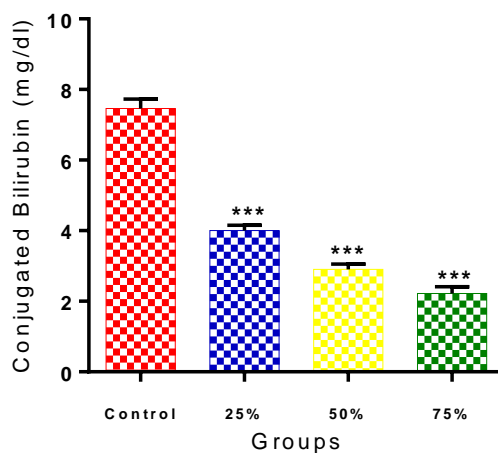
**Fig-5: Alanine Aminotransferase levels among the treatment groups.**



**Fig-6: Alkaline Phosphatase concentration among the treatment groups.**



**Fig-7: Total Bilirubin concentration among the treatment groups.**



**Fig-8: Conjugated Bilirubin concentration among the treatment groups.**

## DISCUSSION

The role of Liver in the body cannot be over emphasized, its major functions include detoxification of waste substances, drugs and metabolism, it is also involved in pathological processes resulting from injuries or disorder, and many other diseases give rise to characteristic changes in the appearance of the liver.

In Table 1, the observation from the ALT results showed that the rabbits treated with 25%, 50% and 75% C. lemon juice were extremely significantly different when compared to the control group at  $P < 0.001$ , considering the values within the reference range of 55 – 260U/L reported by Medirabbit [14].

Slight elevation of serum AST have shown to pose significant effect on the function of the liver provided the AST is within the range since AST and ALT are used basically to detect inflammation due to injury or damage. The AST values obtained for 25%, 50% and 75% treatment were extremely significantly different in comparison with the control at  $P < 0.001$ . The values were within the reference range of 10 –

98U/L for the 50% and 75% treatment groups, but the control group and the 25% group values showed slight elevations from the normal reference range as early reported by Medirabbit [14]. It is important to clarify that ALT and AST levels do not reflect the function of the liver, they are only used to detect inflammation due to injury or damage to the liver from any sources. Even in conditions when AST and ALT are very elevated, the liver still may function properly.

From the ALP analysis, it is indicated that there was high significant difference in the 25%, 50% and 75% treatment groups when compared to the control group at  $P < 0.001$  though values are within the reference range of 10-96U/L reported by Medirabbit [14].

Total Bilirubin findings revealed significant difference in the 25%, 50% and 75% treatment groups compared to the control group at  $P < 0.001$ . The bilirubin values for 25%, 50%, and 75% juice concentration were within the reference range of 0 - 0.75mg/dl as reported by Medirabbit [14]. For the control group, the bilirubin

value was slightly elevated. This agrees with findings reported by mayoclinic.org that elevations in bilirubin in the rabbit could occur with bile obstruction.

The result of the conjugated bilirubin increased in 25%, 50% and 75% treatment groups were extremely significantly different when compared to the control group at  $P < 0.001$  as the values were within the reference range of  $< 12 \mu\text{mol/L}$  ( $< 0.70 \text{mg/dl}$ ) reported by vetstream.com, while the Total protein and albumin revealed extremely significant increase at  $p < 0.0001$  in all the Citrus lemon treated group as compared to the control group, all values observed are within the normal range which indicated the fact that changes in biochemical parameters reflect the physiological status of the animal [15].

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. Farm animals are being fed by human with forages and at times while fruit juices such as Citrus lemon fruit with juices 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. The efficiency and functionality of different organs of farm animals tend to remain uniform throughout the reproductive life of an animal; but significantly altered by such factors as bio-climate, chemicals, hormones, drug and nutrition. Citrus lemon juice is often called the liver's best friend. It is one of the only foods on the planet that has an atomic structure similar to that of digestive juices [16].

Citrus lemon juice supports liver function by strengthening liver enzymes, regulating blood carbohydrate levels and serving as the basis for new enzyme creation. Fresh Citrus lemon juice works better at helping liver to make more enzymes than any other food. In addition, Citrus lemon is a natural antiseptic that kills harmful bacteria. It's also an antioxidant that can stabilize free radicals as they are released into the blood stream during the digestive process.

## 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 lemon. The findings showed that Citrus lemon can be fed to rabbit at up to 75% level of inclusion without any detrimental effect on the biochemical functions of the liver of growing rabbits.

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