

Comparative Study on Effect of Condense Tannins of Supari and Neem Extract on the Liver Function of Rats

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ABSTRACT

Aim: To see the effect of condense tannins of neem extract and supari extract on, the rats and to investigate the effect of condense tannins of supari and neem extract on the liver of rats. Results shows that condense tannins damage the liver of rats. The serum level of AST, ALP, ALT rises and as well as total protein level decreases.

Keywords: Condense tannins, supari, neem extract, AST, ALT, ALP.

INTRODUCTION

Tannins are chemically phenolic compounds have a verity of biological activities. Tannins are widely distributed in plants. They play a role in protection and as well as regulation of plant growth. It also causes dry and puckery feeling in mouth¹.

Tannins can be classified into two main groups. Hydrolysable tannins and Condensed Tannins. The hydrolysable tannins are those who have polyol (glucose) as a central core. The hydroxyl group of the carbohydrates present are partially or totally esterified with phenolic group. These tannins are present low quantity in plants. The condensed tannins are group of naturally occurring Polyphenolic bioflavonoids which form oligomers and polymers. They are wide spread in plants. The condense tannins are more as compare to hydrolysable tannins. Tannins described as a antihyperglycemic agents in diabetic rats^{2,3}.

The word Areca is derived is derived from adakka. It is first originated from srilanka and malaysia². This was also cultivated in subcontinent and some regions of central Africa.

The common name of Areca is supari³. It is chewed at least 10% of total population of the world^{4,5}. Main constituents of this plants is 30% water, 5% protein, 3% fet 47% carbohydrate, and phenolic compounds (flavonols, Tannins). The tannins constituents are highest in unripe, areca and decreased with increase maturity.

The lethal dose of (LD₅₀) of raw areca extract in healthy male and female Wister rats was found to be 2321.96mg/kg and 2257.52mg/kg respectively⁸. Research also has shown that exposure of mice to

aqueous extract of areca nut resulted is sever loss of ultrastructure integrity of cells in the liver lobules. It was also noted that when rats which was occupied the hepatic damage by CCL₄ was not reversed by addition of aqueous extract of supari⁹.

Liver is the largest organ which also occupied the some part of upper abdominal cavity. The liver size varied from age, size and sex. All the life the colour of the liver is reddish brown^{10,11}. Liver for the epithelial cells which are called hepatocytes¹². Liver is also surrounded cells celled phagocytic cells¹³.

The objective of the study was to investigate the effect of extract of condense Tannins of supari and neem extract on the weight of rats and enzymes of liver and levels of protein, calcium and magnesium. Now a days the people are addicted to chew the supari, which is dangerous for health.so present study is to design only to see the lethal effect of condense tannins on rats.

MATERIAL AND METHODS

Fresh growing shoots and leaves of neem plant was collected. The samples were dried in oven at (50-52^oC). The dried sample (500g each) were first pass to a 2mm screen and again pass through 0.5mm screen. The ground samples were stored for determination of Tannins. The samples were extracted to quantitatively for extraction process. aqueous solution of acetone (70%) was used. The finely ground dried sample (200mg) was taken in a glass beaker for approximately 25ml capacity. Ten ml of aqueous acetone (70%) was added and the beaker was suspended in an ultrasonic water bath and subjected to ultrasonic water bath (Branson 3210) and subjected to ultrasonic treatment for 20 min at room temperature. The contents of the beaker was then transferred to centrifuge for 10 min at approximately 300g at 4^oC using refrigerator centrifuge . the supernatant was collected and kept on ice. The pallet left in the tube was transferred to a beaker using two

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portions of 5ml each of 70% Aqueous acetone and again subjected to ultrasonic treatment for 20 min. the supernatant was collected. The method described by Porter *et al*¹⁵ (1986) was followed for the determination of condensed tannins in the extract. The dry matter contents were calculated by the formula given by Porter *et al* (1986).

Chemicals and drugs used

1. Condensed Tannins of Supari was purchased from purchase from pharmaceutical company, Islamabad.
2. Gum acacia was procured from a local pharmacy at Faisalabad.
3. Ethanol from Merck Germany

Diagnosis kit

1. AST (SGOT) kit catalog no BD-117000-02 manufactured by Biocon, Germany.
2. ALT (SGPT) kit Catalog No BD 118000-04 manufactured by Biocon Germany
3. ALP kit catalog no BD-162200-23 manufactured by Biocon Germany.
4. Bilirubin Merck Germany
5. Magnesium, Merck Germany
6. Calcium, Merck Germany
7. Total protein

Experimental Animals Used; Healthy albino Wistar rats (200-300g) were obtained from the NIH, Islamabad. 5 animals per cage were housed in metal cages. The animals had free access to food and tap water. Animals were kept under observation for one week before experimentation, under usual management conditions at 30°C (environmental temperature), in the animal room of the physiology and Pharmacology Department, University of Agriculture, Faisalabad.

Preparation of extracts

Gum acacia solution: 2% Gum acacia in distilled water was prepared. After 48 hours it was shaken well.

Preparation of Condensed Tannins of Supari Extract for activity: 100mg/kg Condensed Tannins was dissolved in equal amount was dissolved in gum acacia (2% solution) to prepare the doses

Preparation of Condensed Tannins of Native Neem Extract for activity: 100mg/kg Condensed Tannins of fresh growing shoots, leaves of neem extract were 2% gum acacia solution.

Collection of Sample: For getting the sample, the animals were anaesthetized with ether followed by putting it into a desiccator. 5 ml of blood was collected by cardiac puncture using sterile disposable syringe. The used syringe was damaged and discarded according to proper precautions. Serum was

separated for determination of SGPT, SGOT, Alk. P., Total Bilirubin, Ca, Mg and total protein by using kits.

RESULTS

1. Effect of Condensed Tannins of Supari on liver enzymes, total bilirubin, calcium, magnesium and total protein.
2. Effect of Condensed Tannins of Neem Extract on liver enzymes, total bilirubin, calcium, magnesium and total protein.

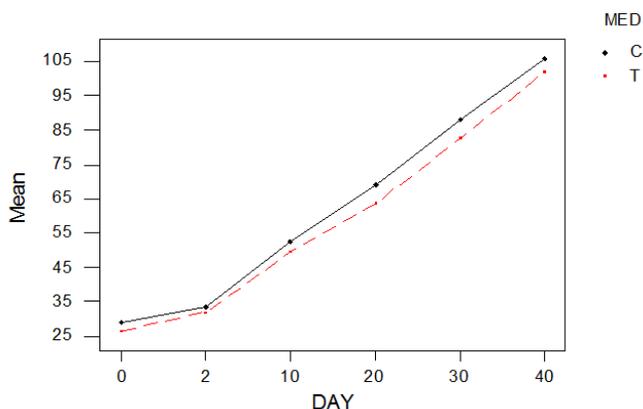
Effect of Condensed Tannins of Supari (C) and Condensed Tannins of Neem Extract (T) on liver enzymes Ca, Mg and total protein:

a. Effect of Condensed Tannins of Supari and Condensed Tannins of Neemon SGPT

Results showed the effect of on liver enzyme. SGPT levels have been presented in fig 1 of 0-40 days. The values were not in normal range & long term treatment showed cause of severe toxicity.

The results have shown in fig 7, that the level of serum enzymes and bilirubin were slightly increased and the level of total protein was decreased. All the values were not in normal range but data showed that long term treatment of condensed tannins can be harmful for liver. These results also show that the effect of condensed tannins are time dependent. All the results analyzed included the analysis of variance.

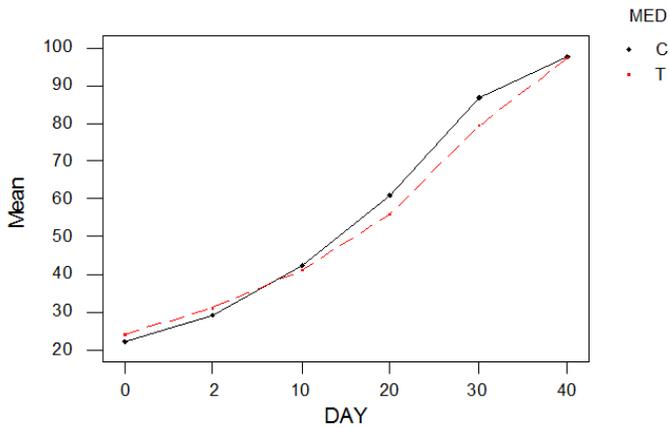
Interaction Plot - Data Means for SGPT



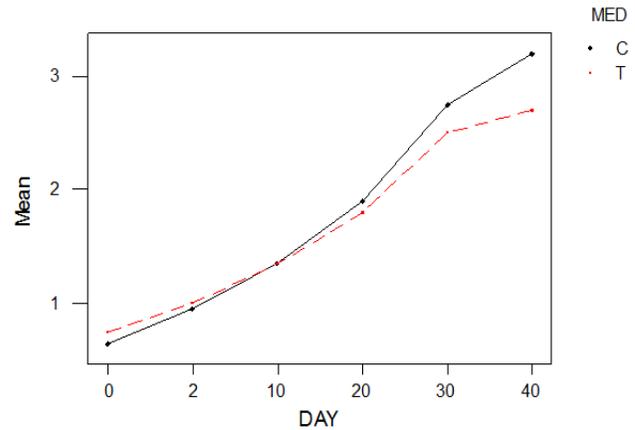
b. Effect of Condensed Tannins of Supari and Condensed Tannins of Neemon SGOT

SGOT level was increased. The results in Fig. 2 showed that longer time treatment caused severe hepatotoxicity.

Interaction Plot - Data Means for SGOT



Interaction Plot - Data Means for BT



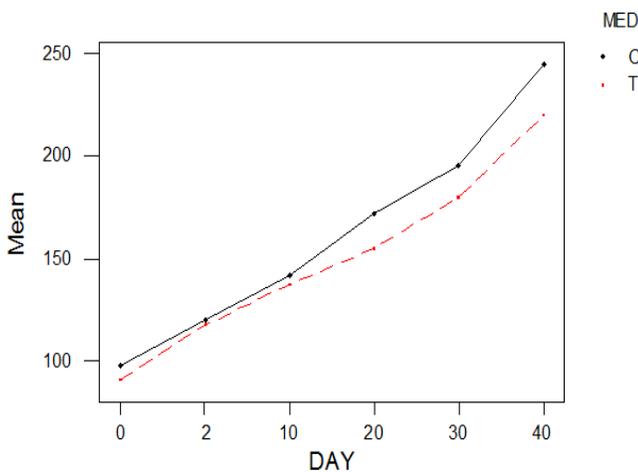
c. Effect of Condense Tannins of Supari and Condense Tannins of Neem serum Alk.P level

As shown in fig3, the Alk.P level from 0-40 days. A increase in enzyme activity level was noted by the use of Condense Tannins of Supari and Neem after40 days, which shows that longer term use cause severe toxicity to liver.

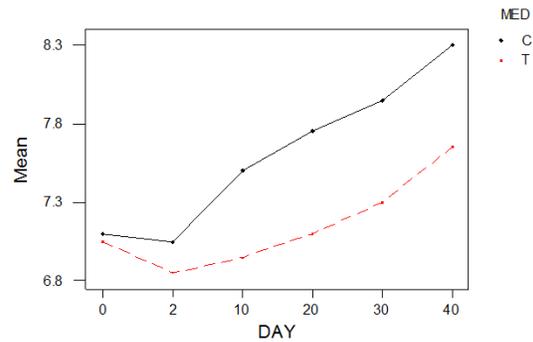
e. Effect of Condense Tannins of Supari and Condense Tannins of Neemon Ca & Mg

The level of calcium and magnesium was not in normal range throughout the period of 0-40 days fig 5, 6.

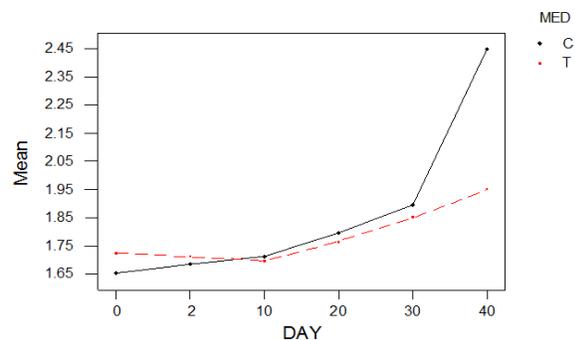
Interaction Plot - Data Means for ALK.P



Interaction Plot - Data Means for CA



Interaction Plot - Data Means for MG

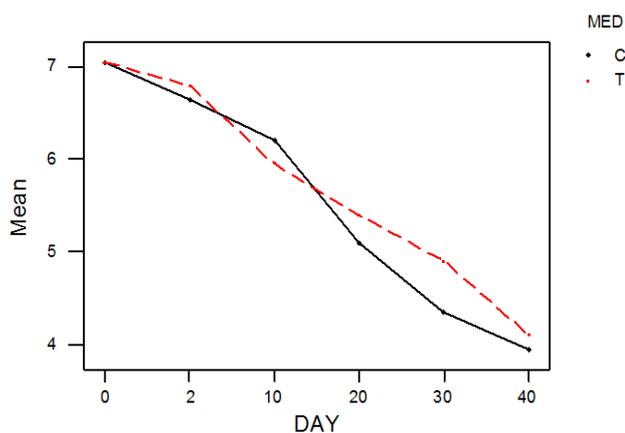


d. Effect of Condense Tannins of Supari and Condense Tannins of Neemon total Bilirubin

The bilirubin level in control group was shown correspondingly from 0-40 days (Fig. 4).

f. Effect of Condense Tannins of Supari and Condense Tannins of Neem on total protein

Interaction Plot - Data Means for PROTIEN



DISCUSSION

The cancer research body of the World Health Organization has categorised areca nut – the main ingredient of both pan masala and gutkha – also as carcinogenic or cancer-causing. Supari is suspected to elevate the risk of cancer of the gums, mouth, throat, lung, liver, stomach, prostate and esophagus. The carcinogenic (cancer causing agents) alkaloids and tannins are even more dangerous by the inclusion of tobacco and lime in gutkha. Specific Supari alkaloids act as competitive inhibitors of GABA receptors and have widespread effects in the body, including actions on the brain, cardiovascular system, lungs, gut and pancreas¹.

The liver is the main hepatic organ. The extent of hepatic damage is assessed by the level of released enzymes like ALP, ALT, AST in blood circulation. Present study revealed that when rats were treated with a dose of 100mg/kg body weight of condense tannins of supari and neem extract, they not only loss their weight but also their LFTs level rises and at the same time their protein level decreased. The present result also supported to some extent by the work of Adeji.J.A¹. *et.al* 2015. They treated the rats with graded dose of 400mg/kg, 800mg/kg, 1200mg/kg of aqueous extract of areca nuts for four weeks induce loss of cytoarchitectural integrity of cells of liver lobules.

Choudhury and Sharan⁷ 2010 also observe that chronic inflammation around periportal hepatitis with a high dose of 1200mg/kg apruva *et.al*. 2014 stated that Areca nut also effect immune system and damage the liver.

CONCLUSION

Result shows that condense tannins of supari and neem extract are very poisonous. They damage the liver, so the liver is unable to revert back.

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