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BIOCHEMICAL ALTERATIONS DUE TO COMBINED METHANOLIC EXTRACT OF *DALBERGIA SISSOO* AND *AZADIRACHTA INDICA* ON LIVER AND KIDNEY OF SPRAGUE DAWLEY RATS

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ABSTRACT

Dalbergia sissoo and *Azadirachta indica* are commonly known trees of India. They belong to family leguminoseae (fabaceae) and meliaceae respectively. They are broadly used in folk medicine for several diseases. The present study is carried out on biochemical parameters i.e. on content of protein and glycogen with standardized method in liver and kidney of Sprague-dawley rats. Due to combined extract of these two plants at single and daily doses of 250 mg/kg b.wt. results revealed significantly decreased ($P \leq 0.05$) content of both protein and glycogen as compared to their respective control groups.

KEYWORDS: ***Dalbergia sissoo*, *Azadirachta indica*, Liver, Kidney, Protein, Glycogen**

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INTRODUCTION

Dalbergia sissoo belongs to the Leguminosae family and is widely planted outside its natural range. The sissoo plant is a folk remedy for excoriations, gonorrhea and skin ailments[1]. Ayurvedics prescribe the leaf juice for eye ailments, considering the wood and bark abortifacient, anthelmintic, antipyretic, aperitif, aphrodisiacs, expectorant and refrigerant. They use the wood and bark for anal disorders, blood diseases, burning sensations and dysentery, dyspepsia, Leucoderma and skin ailments. The alternative wood has been used in India for days, eruptions, leprosy and nausea[2]. The leaves and trunk exudates of *Dalbergia sissoo* contain various compounds like dalbergenone, dalbergin and methyl dalbergin, 4-phenyl chromium, dalbergichromene[3]. *Azadirachta indica* (Neem tree) has attracted worldwide prominence in recent years, owing to its wide range of medicinal properties. The plant belongs to family meliaceae. Neem has been extensively used in Ayurveda, Unani and Homeopathic medicine and has become a cynosure of modern medicine. Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex. More than 140 compounds have been isolated from different parts of Neem[4]. All parts of the neem tree, including leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. Neem leaves and its constituents have been found to exhibit immunomodulatory, anti-inflammatory[5], antiulcer[6], antimalarial[7], antifungal[8], antibacterial[9], antiviral[10], antioxidant, antimutagenic and anticarcinogenic properties[11]. Aqueous extract of neem leaves showed both ulcer protective and ulcer healing effect. The medicinal importance of these plants encourages us to carry out the biochemical alteration in the liver and kidney of Sprague Dawley rats with the quantitative analysis of protein and glycogen contents.

MATERIALS AND METHODS

Collection of the plant and preparation of extract

The leaves and small twigs were collected from Bundelkhand University Campus, Shivaji Nagar Colony and adjacent areas of Jhansi. The plant is air dried and grounded to powdered form. The powdered plant material is extracted with methanol in a soxhlet extractor to dryness yielding a semisolid mass.

Animals

The study was conducted in sexually mature, female Sprague Dawley rats having a body weight of 150±10 mg, purchased from CDRI (Central Drug Research Institute), Lucknow. Prior to study, the clearance was obtained from the Animal Ethical Committee (ICMR, Government of India) under approval number IAEC/CPCSEA 716/02/9/CPCSEA. The animals were acclimatized to the experimental room at a temperature of 25-30 ˚C, controlled humidity conditions (50-55% RH) and 12 hours light and 12 hours dark cycle. They were fed with a standard pelleted diet (Amrut Brand Sangli) and water ad-libitum. For experimentation, animals were randomly distributed into two groups. One group is meant for experimental and other serves as control. Both groups were having equal number of rats. The experimental group received a dose (single and daily) concentration of 250 mg/kg b.wt. and control received vehicle only. Autopsy of both groups was performed on the same day after 7, 14 and 21 days, which is done by chloroform if the rat is to be sacrificed and by diethyl ether if to keep alive.

Biochemical estimation

Protein content was estimated by the Lowry *et al* (1951) method and glycogen content was estimated by Siefter *et al* (1950) method.

Statistical Analysis

The results were expressed as Mean±S.E. Statistical analysis of data was performed using Student's t-test to study the differences amongst the means. $P \leq 0.05$ was considered as statistically significant.

RESULTS

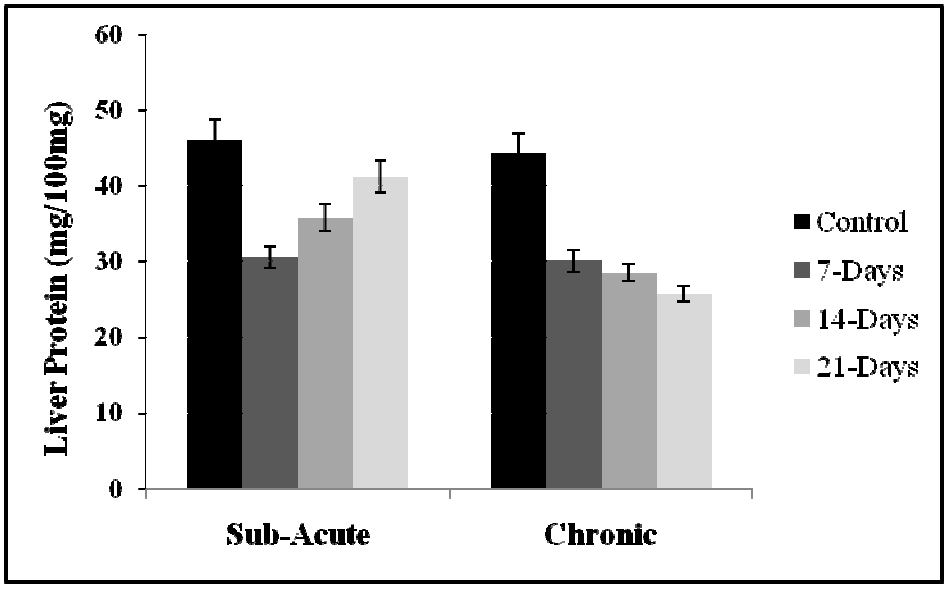
Effect on protein content

During single administration of the dose, the protein content significantly decreases at shorter duration, but at longer duration it recouped to the normal level as compared to the control group. During daily administration, the protein content decreases both at shorter as well as longer duration. (Table 1, Graph 1 & 2).

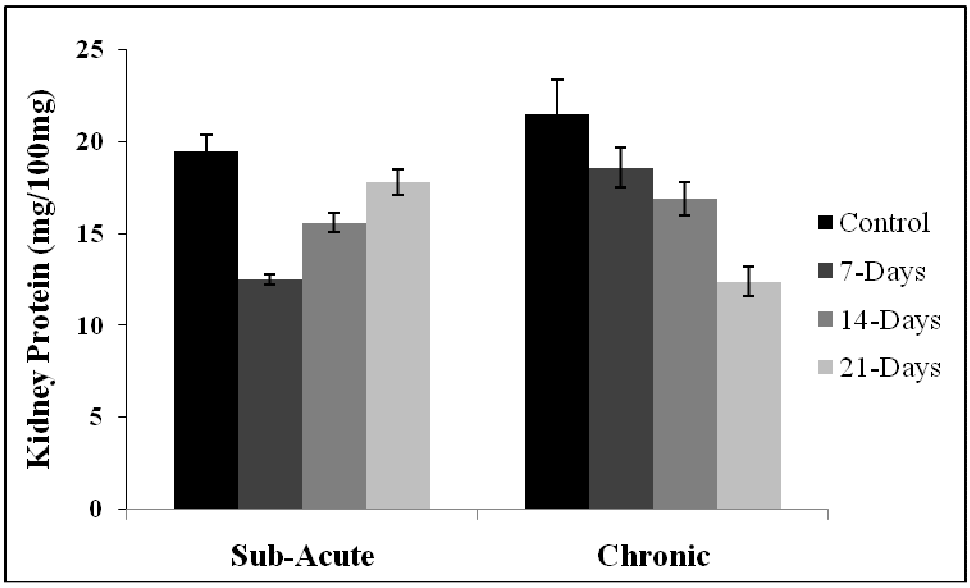
Table 1

Showing the effect of single and daily administration of Dalbergia sissoo and Azadirachta indica on the liver and kidney of female albino rats indicating content of protein (mg/100mg).

Protein content (mg/100mg) in Liver			
S.No.	**Duration**	**Single administration**	**Daily administration**
1.	Control	46.1±2.7	44.4±2.5
2.	7-Days	30.6±1.5	30.1±1.4
3.	14-Days	35.8±1.8	28.5±1.1*
4.	21-Days	41.2±2.1	25.8±1.0*
Protein content (mg/100mg) in Kidney			
S.No.	**Duration**	**Single administration**	**Daily administration**
1.	Control	19.5±0.9	21.5±1.9
2.	7-Days	12.5±0.3*	18.6±1.1
3.	14-Days	15.6±0.5	16.9±0.9
4.	21-Days	17.8±0.7	12.4±0.8*



Graph 1



Graph 2

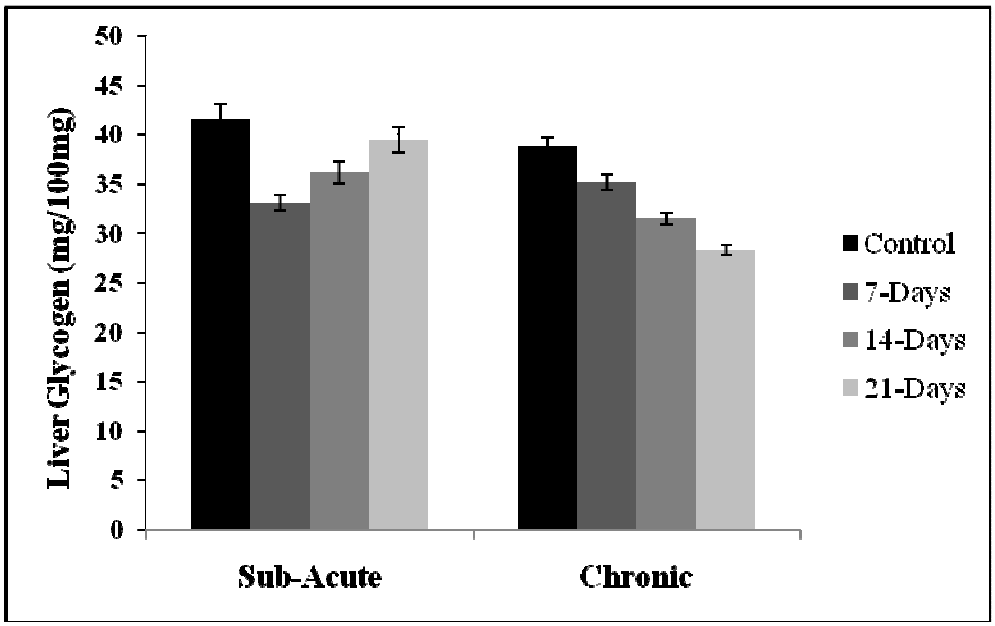
Effect on glycogen content

During single administration of the dose, the glycogen content significantly decreased at shorter duration but at longer duration it recouped to the normal level as compared to the control group. While during daily administration, glycogen content were decreased both at shorter as well as longer duration. (Table 2, Graph 3 & 4).

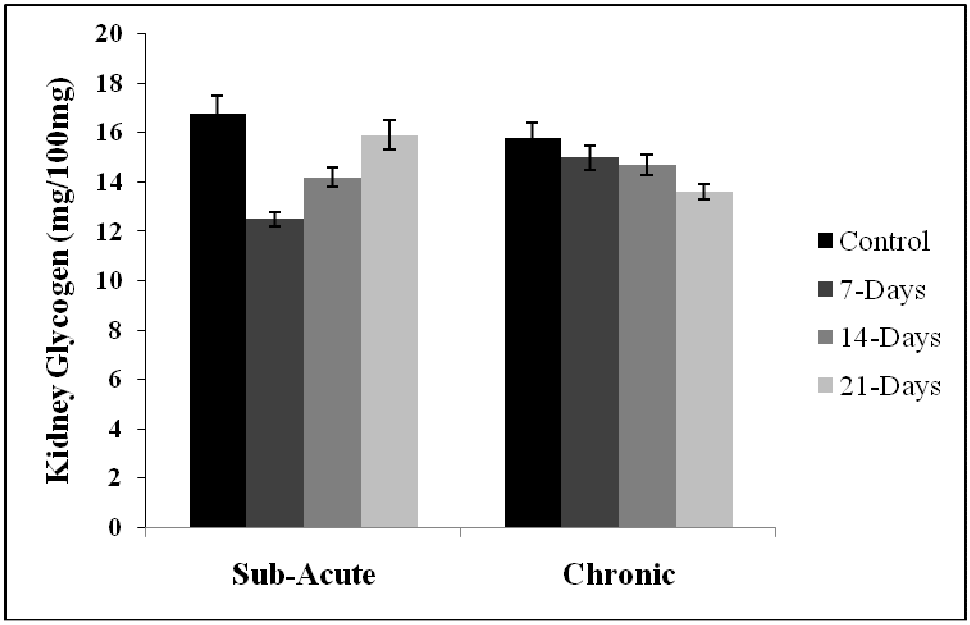
Table 2

Showing effect of single and daily administration of methanolic extract of Dalbergia sissoo and Azadirachta indica on liver and kidney of female albino rats indicating content of glycogen (mg/100mg).

Glycogen content (mg/100mg) in Liver			
S.No.	**Duration**	**Single administration**	**Daily administration**
1.	Control	41.5±1.6	38.8±0.9
2.	7-Days	33.1±0.8*	35.2±0.8
3.	14-Days	36.2±1.1	31.5±0.6
4.	21-Days	39.5±1.3	28.3±0.5*
Glycogen content (mg/100mg) in Kidney			
S.No.	**Duration**	**Single administration**	**Daily administration**
1.	Control	16.8±0.7	15.8±0.6
2.	7-Days	12.5±0.3*	15.0±0.5
3.	14-Days	14.2±0.4	14.7±0.4
4.	21-Days	15.9±0.6	13.6±0.3



Graph 3



Graph 4

DISCUSSION

Proteins are considered as the building blocks of the tissues. They are most abundant intracellular macro-molecules and constitute over half the dry weight of most organisms. Both the plants act as mild antioxidants due to the presence of flavonoids, phenolic compounds and tannins. The decrease in protein content was due to the administration of *Dalbergia sissoo* and *Azadirachta indica* which may alter certain key enzymes which are needed for protein synthesis. *Azadirachta indica* leaf powder and Silymarin standard drug significantly suppressed the increase in plasma activities of AST, ALT and ALP concentration which are considered as markers of liver functional state, resulting in significant decrease

in the total protein and albumin levels after ethyl alcohol treatment[12]. Hypoalbuminemia and a decline in total protein content can be deemed as a useful index of severity of hepatocellular damage. The lowered levels of total protein and albumin recorded in the serum as well as in the liver of ethyl alcohol treated rats revealed the severity of hepatopathy[13]. The ethanolic extract of *Strobilanthes heyneams* (leaves) and morphine hydrochloride caused significant decrease in protein contents in liver and kidney[14]. Similarly, it was investigated that the crude protein composition is either in the matured leaves of *Cissus multistriata* than the young leaves and roots[15]. A number of reports are also available about the increase in the protein contents due to administration of herbal drugs and extracts[16]. Glycogen is an important biochemical component of a cell. It is required to perform various physiological activities being a source of energy. The liver is considered as a metabolic center for the synthesis of glucose from stored glycogen and is used whenever it is required. Administration of *Dalbergia sissoo* and *Azadirachta indica* caused depletion in the glycogen content of liver and kidney. Decreased glycogen contents in liver and kidney certainly reflects its interference with the glycogen synthesis. It is expected that the administration of *Dalbergia sissoo* and *Azadirachta indica* may inhibit the formation of certain key enzymes, which are used in carbohydrate metabolism like glucose-6-phosphate, dehydrogenase and hexokinase[17]. Similarly, it was reported that *Gymnema Sylvestre* causes significant decrease in glucose content in kidney[18]. Even also it was reported that the extract of *Hibiscus rosa-sinensis* caused significant decrease in the glycogen content in the uterus of adult cyclic rats[19].

CONCLUSION

Overall, the results of the present study indicate that the combination of both *Dalbergia sissoo* and *Azadirachta indica* plant demonstrated significant changes in protein and glycogen contents which mean that the active constituents in the plants could cause mild changes to vital organs. Further study is to be done to evaluate the exact mechanism of action of these active phytoconstituents of plants. Thus, at normal therapeutic doses *Dalbergia sissoo* and *Azadirachta indica* is considered to be safe, but for a longer treatment its toxicity will also be considered.

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