BIOLOGICAL STUDIES OF INDIGENOUS MEDICINAL PLANTS-II: Effects of *Aplotaxis lappa* Dcne on various parameters of liver metabolism in rabbits

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SUMMARY : The effects of Aplotaxis lappa Dcne roots and its ethanol extract were studies on serum glutamate oxalacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (AP), lactate dehydrogenase (LDH), and biochemical parameters such as serum total proteins, glucose, cholesterol, total lipids and uric acid levels. The crude drug significantly decreased the SGPT and LDH activities, while its ethanol extract did not disturb the activities of these enzymes. Serum alkaline phosphatase was also not effected by crude powdered drug; whereas its ethanolic extract significantly decreased it. The SGOT was also not effected by both the crude powder and its ethanol extract. A significant hyperglycemia was produced by both of the crude drug and its ethanol extract. The cholesterol and total lipid contents were decreased by the crude drug only; whereas total proteins and uric acid were not effected. The possible mechanisms of these effects have been discussed.

Key words : Aplotaxis lappa, SGOT, SGPT, LDH.

INTRODUCTION

Aplotaxis lappa is a mesophytic herbaceous species of family Composite, locally known as "Kust-i Shirin" (1). The medicinal value of this plant is attributed mainly due to its roots, which is well documented (1, 2). The essential oil is pharmacologically active and was used for a long time in bronchial asthma especially of vagotonic type, as anthelmitic, diuretic and in the treatment of malaria, leprosy, persistent hiccough and rheumatism (1, 2). The roots of this plant are frequently used in traditional folk medicine for various purposes (3).

This communication is in continuation of our work on the biopharmaceutical studies of indigenous crude drugs (4, 5). The present report is aimed at studying biochemical effect of crude *A. lappa* and its ethanol extract on liver of rabbits. Besides some enzymatic activities, viz., SGOT, SGPT, AP and LDH, the following biochemical components were selected as liver function tests: total proteins, glucose, cholesterol, total lipids and uric acid contents.

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MATERIALS AND METHODS

Plant materials

Dried crude roots of *A. lappa* were obtained from Muzaffarabad (Azad Kashmir) and after proper identification, these were finely pulverized and passed through a sieve of 200 mesh.

Chemicals

Unless otherwise stated, all the chemical used were of analytical grade.

Extraction

Powdered roots (300 g) were macerated in 95% ethanol (600 ml) for 48 hours and filtered off. The filtrate was evaporated under reduced pressure and the resinous residue was weighed and mixed with known quantity of starch to facilitate the encapsulation.

Animals

Twelve adult male rabbits (*Oryctolagus cumiculus*) of albino strain weighing 0.9 to 1.3 kg were acclimatized in the animal house for a period of one week and were fed on fresh green fodder and tap water *ad libitum*. They were divided into two groups by random selection and six animals in each group were labeled as 'A' and 'B' respectively. The blood samples of all the

experimental animals collected on zero day prior to the beginning of any experimental schedule. Zero hour was considered as their control material.

Administration of drug

A. lappa root powder was encapsulated. These capsules were administered orally to group 'A'. Each animal received a dose of 0.1 g/kg of the body weight daily for five consecutive days. The animals of second group "B" received ethanolic extract which was 1 g equivalent to crude drug per kg of body weight for five consecutive days.

Blood sampling

The blood sampling were done from ear vein of the rabbits at zero hour (i.e. prior to the dosing). After the last dose, the blood was drawn at the intervals of 6, 12, 24, 48, 96, 144 and 192 hours. 4 ml of blood was collected each time in blood sampling vials. Each blood sample was centrifuged for about 20 minutes at 2000 RPM. The clear supernatant serum was separated and kept for analyses.

Enzymological and biochemical parameters of blood serum

The blood serum samples were analyzed first at zero hour (i.e. before any treatment) and then at the intervals of 6, 12, 24, 48, 96, 144 and 192 hours after the administration of last dose.

All the spectrophotometric determinations were performed on Hitachi 139-vs-vis-spectrophotometer. The activities of serum enzymes and other serum parameters were determined according to the following methods.

Glutamate Oxaloacetate Transaminase (SGOT)	Reitman and Frankel (6)
Glutamate Pyruvate Transaminase (SGPT)	Reitman and Frankel (6)
Alkaline phosphatase (AP)	Bergmeyer (7)
Lactate dehydrogenase (LDH)	Bergmeyer (7)
Total proteins	Doumas (8)
Glucose	Hultman (9)
Cholesterol	Watson (10)
Total lipids	Zoellner and Warnock (11)
Uric Acid	Reiner (12)

Statistical analysis

Mean levels and standard errors of all the parameters were calculated and the data was expressed as M±S.E. Analysis of variance (ANOVA) and least significant differences (1sd) tests were used to check the significant differences between various parameters of factorial experimental designs (13). Further to compare the values within a parameter, student 't' test was used (13).

RESULTS AND DISCUSSION

The effects of oral administration of dried *Aplotaxis lappa Dcne.* root and its ethanolic extract to adult male rabbits were studied in respect to these enzymological and biochemical parameters viz. Serum glutamate oxaloacetate transaminase (SGOT) serum glutamate pyruvate transaminase (SGPT), serum alkaline phos-

Table 1: Physicochemical characteristics of EtOH extract of *A. lappa* root powder.

Properties	Observations	Chemical Nature
Yield	12.83%	
Consistency	Thick viscous oily material	
Color	Reddish brown	
Specific gravity	1.028	
Acid value	15.4	
Sponification value	10.2	
Reaction with Acetic anhydride/H_2SO_4	Reddish brown color	Steroids and triterpenes
Reaction with Acetic anhydride/H_2SO_4/Heat.	Dark violet color	Phenols/terpenes
Reaction with Antimony chloride/Acetic acid	Yellow color change to blue violet	Steroids/terpenes/flavonoids
Reaction with Conc. HNO_3	Dark brown color	Lactone
Reaction with $FeCl_3$/HCl hydrazine in HCl	Bluish green color	Phendic group
Reaction with 2,4, dinitrophenyl hydrazine in HCl	Light olive green color	Aldehydic group
Reaction with Acidic $KMnO_4$	Decolourization	Unsaturation
Reaction with Bromine water	Decolourization	Unsaturation
IR spectrum	Strong absorption at 3600, 1720, 1600 cm^{-1}	$^-$ OH, esters, acidic, $^-$ CHO, C=O groups

phatase (SAP), serum lactate dehydrogenase (LDH), serum total protein levels, serum glucose, serum cholesterol, serum total lipids and serum uric acid levels. The physicochemical characteristics of ethanolic extract has been outlined in Table 1. The activities of some major enzymes were studied in the present investigation to determine the functional indices of liver, heart and skeletal muscles. The enzymatic activity of SGOT and SGPT along with the other enzymes such as alkaline phosphatase and LDH were studied to evaluate liver and heart malfunctions in animals.

SGOT is found nearly in every tissue of the body including RBC. It is present in high concentration in muscle, myocardium, and liver (14). The serum SGOT concentrations increase shortly after the myocardial infraction and hepatic parenchymal injury. The measurement of the serum SGOT is therefore helpful for the diagnosis of myocardial infraction, hepatocellular diseases and skeletal muscles disorders (14). It was observed that crude powdered drug and its ethanolic extract produced no significant changes in the serum SGOT level in both groups of rabbits (Tables 2-4).

Table 2: Means levels of various serum parameters at various time intervals after oral administration of powdered *A. lappa* root (1 g/kg of body weight).

Blood Parameters	Time intervals (hours)							
	0	6	12	24	48	96	144	192
SGOT (mlu/ml)	20.26±2.83	14.2±3.50	20.11±1.38	18.87±2.29	21.61±2.00	21.45±1.94	15.90±4.24	22.58±2.15
SGPT (mlu/ml)	32.52±4.29	25.16±3.61	25.14±2.06	17.83*±2.81	24.05±1.90	32.01±2.60	54.25±3.22	39.51±3.81
SAP (mlu/ml)	20.31±1.79	20.07±4.01	34.02±4.14	28.07±2.25	27.02±2.05	26.18±2.36	27.12±3.04	27.37±1.4
LDH (mlu/ml)	242.64±16.35	90.72**±15.86	138.23±17.62	125.39±17.05	157.32±15.19	223.73±9.08	219.85±16.85	268.04±17.84
TP (G/1)	3.21±0.105	3.23±0.068	3.32±0.099	3.25±0.035	3.09±0.156	2.90±0.14	3.41±0.11	3.11±0.075
SG (mg/100 ml)	105.06±5.80	130.88±7.076	165.39***±12.72	112.09±5.93	94.76±46.36	97.81±3.26	90.55±7.28	105.35±3.41
SC (mg/100 ml)	64.77±5.02	75.72±5.93	90.29±5.88	131.48****±8.09	92.15±6.02	68.98±2.34	65.11±4.09	69.77±4.59
TL (mg/100 ml)	401.48±18.34	353.75±13.29	329.47±13.06	271.74±19.93	225.84±16.67	215.15±19.57	247.18±24.92	371.36±14.47
SUA (mg/100 ml)	2.39±0.43	2.26±0.36	2.15±0.49	1.95±0.29	2.25±0.30	2.29±0.24	2.14±0.42	2.08±0.32

Mean of six readings with standard error shown as M±SE

* Significant, compared with zero (t = 17.84; P<0.02), ** Significant lowering compared with zero hour (t = 6.67; P<0.001), *** Significant increase compared with zero hour (t = 4.92; P<0.001), **** Significant increase compared with zero hour (t = 7.00; P<0.001).

SGOT=Serum Glutamate Oxalacete Transaminase,

SGPT=Serum Glutamate Pyruvate Transaminase,

SAP=Serum Alkaline Phosphatase

LDH=Serum Lactate dehydrogenase,

TP=Total Protein,

SG=Serum Glucose,

SC=Serum Cholesterol,

TL=Serum Total Lipids,

SUA=Serum Uric Acid.

The concentration of SGPT in the tissue is often lower than SGOT. It is present in high concentration in cardiac and skeletal muscles and in low concentration in other tissues (14,15). The crude drug decreased the SGPT activities after 24 hours (Table 2). The decline of SGPT activities is possibly due to specific inhibition of synthesis of enzyme or substrate inhibition or due to fall in concentration of coenzymes; whereas ethanolic extract had shown no significant effect (Table 3). It is possible that the principal compound responsible for lowering the serum SGPT level may not be extractable in ethanol or may be destroyed during extraction.

The crude drug and its ethanolic extract seemed to have no significant effects on the serum alkaline phosphatase. It is possible that the crude drug either does not have any active principle which produce changes in serum alkaline phosphatase or have some antagonistic compound which may be quite active in inhibiting the drug to have such effect (Table 2 and 3).

LDH is an important enzyme of glucose metabolism and is also widely distributed in body tissue. This enzyme is often important in diagnosing the myocardial infraction or liver disorders (14,15). The powdered crude drug produced a significant fall in serum LDH activities after 6

Table 3: Mean levels of various blood parameters at various time intervals after oral administration ethanolic extract of *A. lappa* root (1 g/kg of body weight equivalent to crude drug).

Blood Parameters	Time intervals (hours)							
	0	6	12	24	48	96	144	192
SGOT (mlu/ml)	15.26±1.87	12.78±2.39	15.35±0.98	14.30±1.58	11.09±2.30	13.65±1.16	13.52±3.25	15.06±1.024
SGPT (mlu/ml)	27.75±5.07	24.11±4.16	26.63±2.8	33.67±6.27	25.33±3.72	26.79±3.72	29.06±3.57	24.05±4.69
SAP (mlu/ml)	32.72±4.45	25.91±2.25	17.61±2.16	25.69±2.58	31.35±2.61	33.67±1.53	31.43±1.06	35.37±2.22
LDH (mlu/ml)	258.47±15.96	232.97±7.49	247.72±6.32	245.64±9.82	237.52±7.67	258.85±1.73	256.6±4.91	257.49±5.34
TP (G/1)	3.10±0.13	3.34±0.13	3.65±0.50	3.20±0.31	2.87±0.20	3.76±0.39	4.21±0.65	3.54±0.20
SG (mg/100 ml)	95.11±4.60	167.12*±6.60	87.39±9.51	101.08±8.00	86.39±2.83	103.38±5.29	86.28±2.96	96.24±6.02
SC (mg/100 ml)	95.74±13.40	67.72±10.19	81.72±9.71	85.71±9.39	77.26±6.9	71.43±7.27	75.74±5.41	73.37±10.87
TL (mg/100 ml)	356.52±20.98	356.44±16.59	331.57±22.67	327.15±17.02	327.34±10.72	325.22±11.46	370.23±43.12	344.53±13.42
SUA (mg/100 ml)	2.68±0.02	2.22±0.66	4.33±0.58	4.28±0.80	3.05±0.62	2.61±0.45	3.43±0.35	2.22±0.45

Mean of six readings with standard error shown as M±SE

* Significant increase compared with zero hours (t=8.95; P<0.001).

SGOT=Serum Glutamate Oxalacete Transaminase,

SGPT=Serum Glutamate Pyruvate Transaminase,

SAP=Serum Alkaline Phosphatase,

LDH= Serum Lactate dehydrogenase,

TP=Total Protein,

SG=Serum Glucose,

SC=Serum Cholesterol,

TL=Serum Total Lipids,

SUA=Serum Uric Acid

hours (Table 2); whereas its ethanolic extract exhibited no significant change in serum LDH level (Table 3). It could be concluded that certain active principles are not extractable in ethanol from crude drug.

The effect of *A. lappa* root powder and its ethanolic extract on biochemical parameters such as total proteins, glucose, cholesterol, total lipids and uric acid were also investigated. Any change in the concentration of serum protein indicate a change in the normal liver functions. It was found that both the forms of drug produced no significant change in serum total protein level (Table 2 and 3).

Glucose level in the serum was studied to find out the effect on carbohydrate metabolism. The crude *A. lappa* root as well as the ethanolic extract caused significant increase in serum glucose level. An acute hyperglycemic response was observed with crude drug at 12 hours (Table 2). Similarly glucose concentration, with ethanolic extract showed a significant hyperglycemic response at 6 hours (Table 3). Increase in the serum glucose level is possibly due to the following reasons.

An increase in growth hormones, increase in Epinephrine (Adrenaline) or an increase in glycogen or thyroxin level or an increase in cortisol or other 11-ozysteroids in the body. The high level of hyperglycemia possibly increases the extra cellular osmotic pressure that causes cellular dehydration.

The cholesterol level is also increased significantly by crude drug after 24 hours; whereas ethanolic extract had no effect. The increase in serum cholesterol is possibly due to hypothyroidism, nephrotic syndrome, obstruction of bile ducts or hyperlipidemia. The serum cholesterol mostly elevated in xanthomatosis (14,15). The crude *A. lappa* root may produce hypothyroidism which is possibly responsible for an increase in the serum cholesterol level. Hypothyroidism may also be produced by drug (by disturbing the central regulation of thyroid gland or by disturbing the hormonal control of thyroid gland, by effecting the CNS).

It was found that the crude drug decreased the total lipid count, whereas its ethanolic extract did not show any effect. The decrease was maximum in a sample taken at 96 hours (Table 2). The decline was similar to insulin effects. The administration of insulin is often followed by a fall in plasma circulating free fatty acids form the adipose tissue by enhancing lipogenesis and the synthesis of acylglucerol and finally increasing the rate of oxidation of glucose to CO_2 via hexose monophate shunt (16).

The powdered *A. lappa* root and its ethanolic extract produced no significant changes on the serum uric acid level in both animal groups.

The analysis of variance (ANOVA) of the data indicated that as a whole, the effects of blood parameters are significant i.e. their effects highly differ (Table 4). There is no variation in the effects of *A. lappa* root and its ethanolic extract and also no variation among various time intervals (Tables 4 and 5). Statistical analysis also indicated that all the factors (i.e. The drug and its ethanolic extract, time intervals and various blood parameters) have no relationship. They are independent variables (Tables 4 and 5).

Further work is planned to isolate and characterize the active constituents responsible for such activities. Moreover, the mechanisms of their action need an amplification to understand structure-activity relationship of the isolated compounds of *A. lappa* root.

Table 4: Analysis of variance (ANOVA) of powdered *A. lappa* root, its ethanolic extract; various time intervals and various blood parameters.

Sources of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F. Ratio	P Value
Drugs (A)	1	22898.51	22898.51	0.93	0.10
Time (B)	7	30779.11	4397.01	0.18	0.10
Blood parameters (C)	8	9434001.87	1179250.23	48.10	0.01
AB	7	21473.25	3067.61	0.13	0.10
BC	56	276592.31	4939.15	0.20	0.10
AC	8	132593.68	16574.21	0.68	0.10
ABC	56	178906.87	3194.76	0.13	0.10
Error	720	17652923.83	24517.95		
Total	863	27750169.43			

Table 5: Least significant differences between various blood parameter's means.

Blood Parameters	SUA	TP	SGOT	SAP	SGPT	SC	SG	LDH	TL
Mean*	2.71	3.33	16.62	28.04	30.27	80.51	108.22	214.77	323.15

* Underlined blood parameters are identical.

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