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EFFECTS OF NATURAL PRODUCTS ON BODY WEIGHT AND BIOCHEMICAL PARAMETERS IN HEALTHY RATS

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ABSTRACT

The present study was undertaken to evaluate effect of natural products i.e. *Anthocephalus indicus*; KADAM, roots, *Hibiscus rosa sinensis roots, Tinospora cordifolia* stem and *Cassia tora* seeds in normal healthy rats. In this study ethanol extract of above mentioned medicinal plants had macerated with aqueous gum acacia (2%, w/v) suspension and fed orally (500 mg/kg bw p.o.) to male adult healthy normal rats of *Charles Foster* strain for 30 days. Results of this study showing that alcoholic extracts caused no any significant reduction in blood glucose, total cholesterol, triglyceride, phospholipids, free fatty acid, lipid peroxide and no significant increased in post heparin lipolytic activity, but on the other hand as per pre-existing data and my published studies in diabetic patients and diabetic experimental animals showed that extracts exerting all above effects significantly. That's why it is very clear here if healthy person will take natural products, it never cause hypoglycemia, hypolipidemia and under weight. Natural products also not cause any significant change on hepato-specific parameters. Thus from this study we conclude that, natural products are safe, non toxic and free from side effects, in comparison to synthetic drugs.

KEY WORDS: Natural Products, Hepato-specific parameters, Post heparin lipolytic activity, plant antioxidant.

INTRODUCTION

During Vedic period Aryans compiled their work related to herbal remedy in holy Vedas when they came to north India. References about a number of herbal remedies have been mentioned in *'Rig-Veda'* (about 200 B.C.). In *'Atharva-Veda'* (about 200 B.C.) description of medicinal plants has been made under separate chapter *'Ayurveda'*. It was *Charak* (about 600 B.C.) who made the scientific classification of herbal drugs on remedial properties in his renowned treatise 'Charak Samhita' (A compendium of general medicine). *Anthocephalus indicus;* KADAM, *Hibiscus rosa sinensis, Tinospora cordifolia* and *Cassia tora* are some of them. (1-3) Design of newer, safer and effective chemotherapeutic agents is one-way of addressing this situation. This in turn enforces the need to develop protocols to evaluate the efficacy of herbal drugs. (4) But effects of above *Anthocephalus indicus;* KADAM, *Hibiscus rosa sinensis, Tinospora cordifolia* and *Cassia tora* in healthy rats had not studied in detail. In view of the above considerations the present study was designed to investigate the adverse effects of *Anthocephalus indicus, Hibiscus rosa sinenss, C. tora and T. cordifolia* in normal healthy rats.

ANTHOCEPHALUS INDICUS

Anthocephalus cadamba Miq., Syn. *A. indicus,* (Family-Rubiaceae) commonly called kadamba enjoys a hallowed position in Ayurveda- an Indian indigenous system of medicine. It is also named as Kadam. (5-10)

Tree of *A. indicus*

Flowers of *A. indicus*

Fruits of *A. indicus*

HIBISCUS ROSA SINENSIS:

The herb *Hibiscus rosa-sinensis* Linn [Malvaceae] has antidiabetic and so many beneficial effects. (11-15)

Flower of Hibiscus *Roots of Hibiscus*

TINOSPORA CORDIFOLIA

India is bestowed with enormous biodiversity of medicinal plants. Among them *Tinospora cordifolia* has a wide array of bioactive principles as well as it has been proven medicinally important plant, have not received considerable scientific attention. (16-18)

Different parts of Tinospora cordifolia

CASSIA TORA

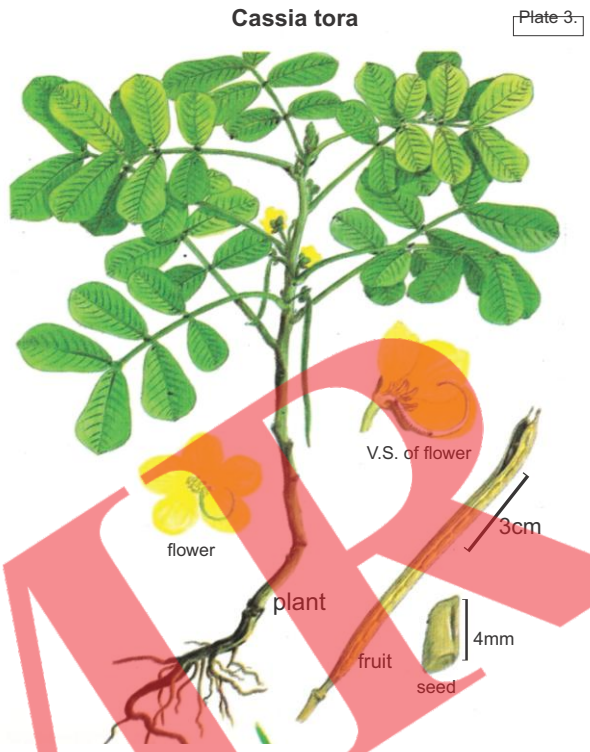
In ancient system of medicine, *C. tora* was used to treat a variety of medical complications like bronchitis, constipation, conjunctivitis, ulcer, hypertension, hypercholestrolemic, liver damage. (19-21)

Material and Methods

ANIMAL TRIAL

Collection of plant material

Anthocephalus indicus, Hibiscus rosa sinensis, Tinospora cordifolia and *Cassia tora* were collected from the local area of Lucknow and identified taxonomically by the Department of Pharmacology, Era's Lucknow Medical College & Hospital, Lucknow and a voucher specimen were also submitted. (21)



Preparation of extracts

Anthocephalus indicus (root & fruits), *Hibiscus rosa sinensis* (root), *Tinospora cordifolia* (stem) and *Cassia tora* (seeds) were dried under shade and made into fine powder using laboratory mill. Powder (1000g) was extracted thrice with 3x2000 ml portions of 95% ethyl alcohol in a laboratory percolator at room temperature. Time allowed for each extraction was 8 hr. The extract obtained after third extraction was colorless. All the extracts were mixed; alcohol was distilled out at reduced temperature (20 0C) and reduced pressure (100 psi) in a rotor evaporator. This yielded 15g, 20g, 18g, and 20g (2% w/w) of crude extract, which was used for *in vivo* study. (21)

Preparation of doses

A quantity of 50 mg each (*Anthocephalus indicus* (root), *Hibiscus rosa sinensis* (root), *Tinospora cordifolia* (stem) and *Cassia tora* (seeds)) extract were suspended /ml tripled distilled water (TDW) containing 2 % (w/v) gum acacia. The suspension were given in a volume of 1ml/100g animal bw (500 mg drug /kg bw) by oral intubation. (21)

Chemicals

Blood glucose (BLG), Total cholesterol (TC), triglycerides (TG), Phospholipid (PL) were analyzed using standard kits from Erba Diagnostic, (Mannheim GmbH, Germany) by an auto analyzer (Erba Mannheim, EM 360, Germany). Intralipid from victrum AB, In the Kabivitrum Group, Stockholm, Sweden. (21)

Experimental animals

Healthy male adult rats of *Charles Foster* strain (200-250g) bred in the animal house of the Central Drug Research Institute, Lucknow were used. The animals were kept in controlled conditions; temperature 25 - 26˚C, relative humidity 60-70% and 12/12 hrs light / dark cycle (light from 08:00 AM to 08:00PM), provide with standard pellet diet (Lipton India Ltd.), and *water adlibitum*. (21)

Experimental design

The rats were divided in five groups having six animals in each as follows: Group 1: normal control rats (on normal saline); Group 2, *Anthocephalus indicus* treated normal rats rats; Group 3, *Hibiscus rosa sinensis* treated normal rats; Group 4, *Cassia tora* treated normal rats; Group 5 *Tinospora cordifolia* treated normal rats. (21)

Assessment of biochemical parameters

The blood was centrifuged and plasma was separated. The fasting blood sugar (FBS) was analyzed in plasma, Super oxide dismutase (SOD), Catalase (CAT), Hepatic triglyceride lipase (TGL) and lipoprotein lipase (LPL) were estimated in liver homogenate. Serum total cholesterol (TC), triglyceride (TG), high density lipoprotein total cholesterol (HDL-TC), serum bilirubin, SGPT, SGOT and alkaline phosphatase were assayed by standard spectrophotometric methods. Low density lipoprotein total cholesterol (LDL-TC) andvery low density lipoprotein total cholesterol (VLDL-TC) were calculated by Friedewald's equation. Serum was also used for the assay of lecithin cholesterol acyl transferase activity (LCAT), lipid peroxide (LPO), and reduced glutathione (GSH). A portion of serum was fractionated into very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) by polyanionic precipitation methods. Lipoproteins were measured for their total cholesterol (TC), phospholipids (PL), triglyceride (TG) and apoprotein by standard spectrophotometric methods. were analysed in liver homogenate. (22-38)

Statistical analysis: One-way-analysis of variance (ANOVA- Newman's Student t-test) was performed by comparison of values for normal treated group with normal healthy control. All hypothesis testing were two-tailed. P <0.05 was considered statistically significant and the results were expressed as mean ± SD. The Graph pad INSTAT 3.0 software was used to carried out the statistical analysis. (39)

RESULTS

Effect of drug treatment on body weight of drug treated normal rats after 1 month with respect to Normal Control without drug treated

Weight of all experimental rats (Normal Control

Group	Body Weight (gm)		Percent change with respect to normal control	'p' value
Normal Control+ 2 % Aqueous gum acasia	Study Day 0 (Beginning)	242.50±33.90	-	-
	Study Day 30 (End of study)	360±23.56	-	-
Normal Control + *A. indicus* treated	Study Day 0	251.16±18.32	+3.71	NS
	Study Day 30	345±20.70	-4.16	NS
Normal Control + *Hibiscus* treated	Study Day 0	246.50±28.44	+1.65	NS
	Study Day 30	320±27.66	-11.00	NS
Normal Control + *Cassia tora* treated	Study Day 0	247.16±27.31	+2.00	NS
	Study Day 30	351±126.44	-2.50	NS
Normal Control + *T. cordifolia* treated	Study Day 0	240.00±21.72	-.82	NS
	Study Day 30	348±23.65	-3.00	NS

Table 1: Effect of drug treatment on body weight of normal rats after 1

Values expressed as gram (gm) are mean ± SD of six rats, drug treated groups were compared with Normal Control. Percent change is with respect to Normal Control .NS = Not significant.

without drug treated and with drug treated) was record in beginning of study and after completion of the study. Percent change in weight was calculated with respect to healthy control in end of this study. No any significant change in weight of drug treated normal rats was found with respect to normal control without drug treated, after one month, in end of the study (Table 1).

EFFECT OF *A. INDICUS, H. ROSA SINENSIS, C. TORA* AND *T. CORDIFOLIA* ON BLOOD GLUCOSE, SERUM LIPID AND LIPOPROTEIN PROFILE IN HEALTHY CONTROL RATS (NORMAL RATS).

In normal rats, administration of *A. indicus, H. rosa sinensis, C. tora* and *T. cordifolia* at the dose of 500 mg/kg b.w. orally once daily for 30 days lowered the levels of blood glucose (0.5 – 4.0%), TC (2-4%), PL (2-4%), TG (0.3-2.0%) and increase in total serum protein (1-3%) Table-2. Furthermore, treatment with test natural products the lipid and protein components of serum lipoproteins remains almost the same to that of Control (Table-2).

It was seen that *A. indicus. H. rosa sinensis, C. tora* and *T. cordifolia* caused decrease in the level of -Lipoprotein TC (2-8%), PL (2-8%), TG (0.82-6.0%) and apolipoproteins (0.3-1.3%) respectively. However, herbal preparations increase lipid and protein contents of -Lipoprotein HDL by (0.25-6%) (Table-3).

EFFECTS OF *A. INDICUS, H. ROSA SINENSIS, C. TORA* AND *T. CORDIFOLIA* ON SERUM FREE FATTY ACIDS, HEPATIC TRIGLYCERIDE LIPASE AND TOTAL HEPATIC LIPOPROTEIN LIPASE ACTIVITY STATUS IN NORMAL RATS.

In normal rats administration of *A. indicus, H. sinensis, C. tora* and *T. cordifolia* for 30 days lowered the levels of free fatty acid (0.7-15%) hepatic triglyceride lipase (0.47-4%) and total hepatic lipoprotein lipase activity (0.25-4%) respectively (Table 4).

Experimental schedule	Blood glucose (mg/dl)	TC (mg/dl)	PL (mg/dl)	TG (mg/dl)	Protein (g/dl)
Normal Control	89.79±10.30	87.50±11.16	75.96±7.36	82.21±5.80	7.00±0.55
*A. indicus*treated	88.20±1060 (-2%)	85.65±11.69 (-2%)	74.62±7.53 (-2%)	81.61±5.76 (-1%)	7.09±0.56 (+1%)
H. rosa sinensis treated	86.64±10.09 (-4%)	85.88±11.55 (-2%)	74.95±8.09 (-1%)	81.35±5.86 (-1%)	7.11±0.58 (+2%)
C. tora treated	89.27±10.8 (-0.57%)	84.45±11.46 (-4%)	74.77±7.34 (-2%)	80.29±5.49 (-2%)	7.10±0.58 (-1%)
T. cordifolia treated	88.76 ^{NS} ±10.15 (-1%)	84.67±11.60 (-3%)	74.89±7.46 (-2%)	81.89±6.04 (-0.38%)	7.18±0.53 (+3%)

Table 2:Effects of A. indicus, H. rosa sinensis, C. tora and T. cordifolia on blood glucose and serum lipids in normal rats

Values expressed are mean ± SD of six rats. Values with parenthesis are percentage change. Drug treated groups are compared with control (change are non significant)

Experimental schedule	Very low density lipoprotein (VLDL)				Low density lipoprotein (LDL)				High density lipoprotein (HDL)			
	TC	PL	TG	Apo-protein	TC	PL	TG	Apo-protein	TC	PL	TG	Apo-protein
Normal Control	8.11 ±0.66	16.80 ±2.06	40.10 ±3.29	7.02 ±0.99	17.87 ±1.73	11.97 ±1.43	20.60 ±1.76	16.87 ±2.37	51.64 ±5.59	41.18 ±3.36	14.90 ±1.51	173.82 ±11.63
A. indicus treated	7.71 ±0.54 (-5%)	16.06 ±2.12 (-4%)	39.77 ±3.43 (0.82%)	7.01 ±0.73 (-0.14%)	17.40 ±1.66 (-3%)	11.07 ±1.25 (-8%)	19.90 ±1.42 (-3%)	16.92 ±2.42 (+0.6%)	54.78 ±8.27 (+6%)	41.95 ±3.32 (+2%)	15.07 ±1.49 (+1%)	174.08 ±11.59 (+0.14%)

Table 3: Effect of A. indicus, H. rosa sinensis, C. tora and T. cordifolia on lipoprotein profile in normal rats

H. rosa sinensis treated	7.72 ±0.50 (-5%)	15.93 ±2.18 (-5%)	39.72 ±3.39 (0.82%)	6.94 ±1.05 (-1.13%)	17.51 ±1.79 (-2%)	11.55 ±1.39 (-4%)	20.02 ±1.67 (-3%)	16.82 ±2.43 (-0.3%)	51.91 ±5.67 (+0.25%)	41.72 ±3.30 (+1.0%)	15.08 ±1.47 (+1%)	174.21 ±11.85 (+0.22%)
C. tora treated	7.58 ±0.53 (-7%)	16.46 ±2.09 (-2%)	39.37 ±3.42 (1.82%)	7.02 ±0.87 (-0% No change	17.19 ±1.71 (-4%)	11.46 ±1.57 (-4.25%)	19.30 ±27.03 (-6%)	17.08 ±2.25 (-1.2%)	53.50 ±8.78 (+4.0%)	42.25 ±3.29 (+3%)	14.89 ±1.10 (+0%)	172.17 ±14.65 (-0.94%)
T. cordifolia treated	7.44 ±0.52 (-8%)	16.43 ±1.89 (-2%)	39.74 ±3.35 (-0.82%)	7.00 ±0.89 (+0.85%)	17.57 ±2.17 (-2%)	10.98 ±1.21 (-8%)	19.94 ±1.69 (-3%)	16.95 ±2.35 (-0.5%)	53.94 ±4.36 (+4%)	42.21 ±3.24 (+2%)	15.06 ±0.94 (+1%)	172.30 ±14.71 (0.9%)

Cont.....Table 3: Effect of A. indicus, H. rosa sinensis, C. tora and T. cordifolia on lipoprotein profile in normal rats

Values expressed are mean SD of six rats. Values with parenthesis are percentage change.Drug treated groups are compared with control (changes were no significant)

Experimental Schedule	Serum Free Fatty Acid[a]	Hepatic Liprotein LipaseActivity[b]	Total Hepatic Lipoprotein Lipase Activity[b]
Normal Control	1.67±0.18	71.78±7.58	85.01±0.06
A. indicus treated	1.65±0.12(-1.0%)	74.65±7.48 (+4%)	88.53±4.58 (+4.0%)
H. rosa sinensis treated	1.61±0.18(4.0%)	74.20±7.83 (+3%)	85.30±8.98(+0.33%)
C. tora treated	1.62±0.19(-3.0%)	72.12±7.55 (+0.47%)	85.23±9.08(+0.25%)
T. cordifolia treated	1.60±0.19(-4.0%)	74.87±7.96(+4%)	88.70±5.12(+4%)

Table 4: Effect of A. indicus, H. rosa sinensis, C. tora and T. cordifolia on serum free fatty acid total hepatic Lipoprotein lipase and hepatic Trigleceride lipase activity in normal rats.

Values are expressed as mean SD of six rats Drug treated groups are compared with control. (The changes are non significant); a=molFFA/L, b=molFFA released hr/mg protein

Experimental schedule	Serum Lipid peroxide[a]	Hepatic SOD[b]	Hepatic CAT[b]
Normal Control	2.91±0.42	2.78±0.19	3853±251.36
A. indicus treated	2.67±0.39 (8.0%)	2.88±0.18 (+4%)	3910±267.08 (+1.47%)
H. rosa sinensis treated	2.48±0.42 (-15%)	2.91±0.23 (+5%)	3916±302.23 (+1.5%)
C. tora treated	2.89±0.43 (-0.7%)	2.82±0.16 (+1.36%)	3929±331.42 (+2%)
T. cordifolia treated	2.46±0.39 (-15%)	2.89±0.17 (+4%)	3944±146.40 (+2.36%)

Table 5: Effect of A. indicus, H. rosa sinensis, C. tora and T. cordifolia on serum MDA and hepatic SOD, hepatic Catalase activity in normal rats

Values are expressed as mean SD of six rats. Drug treated groups are compared with control. (The changes are non significant); a=molMDA/ml, b=units/min /mg protein

	Serum Bilirubin[a]	SGPT[b]	SGOT[b]	ALP[c]
Normal control	0.62±0.06	22.29±2.74	57.22±7.35	17.63±0.9
A. indicus treated	0.50±0.10 (-4.83)	20.73±2.91 (-6.99)	56.65±6.90 (-0.99)	17.34±1.09 (-1.64)
Hibiscus treated	0.59±0.09 (-4.83)	21.28±2.89 (-4.53)	56.71±6.17 (-0.89)	17.43±1.14 (-1.13)
C. tora treated	0.570.08 (-8.06)	20.71±2.91 (-7.08)	56.30±6.92 (-1.60)	17.26±1.07 (-2.09)
T. cordifolia treated	0.58±0.13 (-6.45)	21.87±2.80 (-1.88)	56.03±6.86 (-2.07)	17.37±1.08 (-1.47)

Table 6: Effect of natural products on the hepato specific markers in the serum of normal rats.

Values are expressed as mean±SD of six rats, values in the parenthesis are percent change (changes are non significant). Units a=mg/dl, b=units/l, c=units/dl.

EFFECT OF *A. INDICUS, H. ROSA SINENSIS, C. TORA* AND *T. CORDIFOLIA* ON SERUM LIPID PEROXIDE, HEPATIC SOD AND HEPATIC CATALASE ACTIVITY IN NORMAL RATS

In normal rats administration of *A. indicus, Hibiscus rosa sinensis C. tora* and *T. cordifolia* for 30 days lowered the level of serum lipid peroxide (0.70-15.0%), and increase in the levels of hepatic SOD (1-5%), catalase (1-2.3%) respectively (Table 5).

EFFECT OF A. INDICUS, H. ROSA SINENSIS, C. TORA AND T. CORDIFOLIA ON HEPATO-SPECIFIC MARKER IN NORMAL CONTROL RATS

In normal rats administration of above mentioned herbal preparations for 30 days lowered the levels of Bilirubin, SGPT, SGOT, ALP in serum by (4-8%), (1-6%), (0.6-2.07%) and (1-2%) respectively (Table 6).

DISCUSSION

In normal rats treatment with natural products (*A. indicus, H. Rosa sinensis, C. tora, T. cordifolia)* at the doses of 500 mg/kg body weight orally once in a day for 30 days did not alter significantly, their body weight (Table 1) or blood biochemical parameters namely the levels of glucose, serum protein lipid profile (Table 2), lipoprotein profile (Table 3), free fatty acid with lipolytic enzyme activities (Table 4), lipid peroxide and antioxidant enzymes (Table 5). Also there was no significant change in the hepatospecific parameters like Serum Bilirubin (S. Bil), Serum Glutamate Pyruvate Transminase (SGPT), Serum Glutamate Oxaloacetate Transminase (SGOT) and Alkaline Phosphatase (ALP) (Table 6). This indicated that the herbal preparations as such did not exert any adverse and toxic side effect during treatment in rats. The results of the present study demonstrated that natural products did not cause significant decrease in biochemical parameters in healthy normal rats. That's why it very clear here if healthy person will take natural products it never cause hypoglycemia, hypolipidemia and under weight. Natural products also not cause any significant change on hepato-specific parameters. Thus from this study we conclude that, natural products are safe, non toxic and free from side effects, in comparison to synthetic drugs. (40-42)

CONCLUSION

It should be pointed out here that plant derived natural compounds have established a proven platform for developing new drug synthesis with fewer side effects or free from side effects.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants performed by any of the authors. The study was approved by the Institutional Animal Ethics Committee of Central Drug Research Institute and was carried out in accordance with the current guidelines set by Organization for Economic Co-operation and Development (OECD), received from ***Committee for the Purpose of Control and Supervision of Experiments on Animals*** (CPCSEA), Ministry of Social Justice and Empowerment, Government of India for the care of laboratory animals

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