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AN EXPERIMENTAL EVALUATION OF AMRUTHA GHRITHA AGAINST CYPERMETHRIN INDUCED HEPATOTOXICITY IN ALBINO RATS

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ABSTRACT

Cypermethrin is a synthetic pyrethroid used extensively in pest control and in many studies it is reported to cause hepatic & renal toxicity[1, 2]. During its metabolism reactive oxygen species (ROS) are generated, these free radicles which cause oxidative stress through peroxidation of the lipid membrane & damage occur in certain tissues & organs[3]. The objective of the present study was to evaluate the hepatoprotective activity of *Amrutha Ghritha* against Cypermethrin induced Hepatoxicity in Albino Rats. The animals were divided into five different groups consisting of six rats each. Groups included for normal control, positive control, ½ TED, TED, TED* 2. The toxicant Cypermethrin 30 mg/kg body weight was administered orally to each group, except the normal control group and then the test drug *Amrutha Ghritha* was administered 1 hour later orally for 28 consecutive days. On 28th day the Biochemical, Histopathological & Antioxidant parameters were estimated. The administration of Cypermethrin caused significant elevation in SGOT, serum Total protein, Urea, Albumin, Uric acid level as compared to normal control. Antioxidant activity is not much significant when compared to hepatoprotective action of the drug. The Histopathology results in Cypermethrin administrative groups shows there was an extensive degenerative changes of moderate severity. The observed changes were cell infiltration at multiple sites, dilatation of the central vein, focal necrosis and cell depletion in hepatocytes. The test drug administrated group shows reversal of important parameters like SGOT, ALP, serum Total protein, Albumin, Globulin & Uric acid. Thus we can conclude the *Amrutha Ghritha* has Hepatoprotective effect and reversed Cypermethrin induced hepatotoxicity.

Keywords: *Amrutha Ghritha*, Cypermethrin, Hepatotoxicity, Oxidative stress

INTRODUCTION

Pesticides are the compounds that are used to kill pests which may be insects, rodents, fungi, ticks etc. India being the second largest producer of fruits and vegetables uses harmful pesticides like Cypermethrin, Malthion, Aldin, Endosulfan[4] etc. Among these pesticides Cypermethrin is Pyrethroid pesticides used extensively in pest controls. During its metabolism reactive oxygen species (ROS) are generated, these free radicles which cause oxidative stress through peroxidation of the lipid membrane &

damage occur in certain tissues & organs. Studies found that Cypermethrin may cause hepatic and renal toxicity. Hepatotoxicity is one of the major causes of morbidity and mortality[5]. The conventional medicine has very little role to play in managing liver diseases, and this lead to fertile role for *Ayurveda*. In *Ayurveda Yakrit vikaras* (Disorder of liver) are explained by different *Acharyas* (Ancient Teacher) along with their treatment, and it is already proven that many of our *Ayurvedic* drugs are potentially hepatoprotective. *Amrutha Ghritha*, explained in *Susrutha Samhitha* (Sanskrit text on medicine and surgery) in the contest of treating *Sthavara visha*[6] (natural toxin like Poison of plant or mineral). As mentioned above the pesticides (*Jaravisha*) used by farmers enters in our body which causes severe damage in human body major from this is hepatotoxicity hence will effect heart. *Amrutha Ghritha* is also *Nitya Rasayana* (daily medicine) for any *Visha* (poison) *that will effect hridaya* (heart). The present study is being undertaken to evaluate the efficacy of *Amrutha Ghritha* against hepatotoxicity.

MATERIALS AND METHODS

TEST DRUG COLLECTION AND PREPARATION

All the ingredients were collected from the Alva's Pharmacy, Mijar. All the drugs *Apamarga* (botanical name: achyranthes aspera)*, sirisha* (botanical name: albizia lebbeck)*, Aparajitha* (botanical name: Clitoria ternatea)*, Shankhapushpi* (botanical name: Clitoria ternatea)*, Kakamachi* (botanical name: Solanum nigrum) *, gomutra* (Cow urine) were identified and authenticated as genuine sample by the department of *Dravya Guna* (Materia medica of Ayurveda) Alva's Ayurveda medical college, moodbidri. *Amrutha Ghritha* was prepared according to the methods explained in classics and standard guideline in Ayurvedic Fomulary of India part *Gritha* after subjecting the *Ghritha murcchana*[7]. The *Amrutha Ghritha* was subjected to visible analytical parameters like Colour, Odour, Solubility, Loss on drying, Rancidity were studied while preparing and other analytical parameters pH, refractive index, specific gravity, saponification value, iodine value, Acid value, viscosity, peroxides value were noted.

EXPERIMENTAL ANIMALS

Wistar strain albino rats of either sex of body weight ranging from 250±50 g were selected for the present study. They were obtained from well-established animal house attached to S.D.M Centre for Research in Ayurveda and Allied Sciences, Udupi. Animals were maintained at standards laboratory conditions such as temperature at 22±03ºC, humidity of 50-70% and natural day and night cycle. They were fed with Amrut brand rat pellet feed supplied by Sai Durga feeds Bangalore and tap water given ad libitum. The study protocol was approved from Institutional animal ethical committee (SDMCRA/IAEC/MB-AT-10) and principles of laboratory animal care were followed as per **SPCA** (Society for Prevention of Cruelty to Animals) guidelines throughout the experimentation.

ACUTE ORAL TOXICITY TEST

The acute oral toxicity study was carried out as per OECD guidelines 425 using AOT software. The prepared test sample was made into suspension in ghee with suitable concentration and dosed in the following order 175, 550, and 2000mg/kg body weight. After the dosing animals were observed for 14 days for any mortality. The LD 50 was determined by using AOT software.

CHEMICALS

Cypermethrin [Insectisides (India) limited. Plot no.CH21, G.I.D.C, Industrial Estate Dahej, Tavagra, Dist: Bharuch, Gujarat, Batch no- D0171]

METHODOLOGY

Wistar Albino rats were randomly divided into five different groups of six rats each. Group I served as control group- treated with normal diet and water. Group II administered with Cypermethrin (30mg/kg body weight p.o.)-served as toxic control group.

Group III, IV, V considered as test group co administered with Cypermethrin- treated with *Amrutha Ghritha* 2.16 ml/kg, 4.32 ml/kg & 8.64 ml/kg of body weight along with Cypermethrin 30mg/kg. The toxicant administered orally and then the test drug *Amrutha Ghritha* was administered 1 hour later orally, for 28 consecutive days, on 28th day 1 hour after the test drug administration, the blood was withdrawn from retro- orbital plexus and then collected in the tubes and sent for biochemistry laboratory for biochemical investigations. All the animals were sacrificed by cervical dislocation. Important organs like liver, kidney and heart were dissected out, cleaned to remove extraneous tissues, blotted to remove blood stain and weighed. A piece of liver tissue was reserved in 10% formalin for histopathological processing. A known amount of liver tissue was homogenized to estimate different biochemical parameters.

STATISTICAL ANALYSIS

The data obtained was analyzed by using analysis of variance (ANOVA) followed by Dunnett's't' test for determining the level of significance of the observed effects. A 'P' value of less than 0.05 was considered statistically significant.

RESULT

BIOCHEMICAL PARAMETERS

Repeated administration of Cypermethrin resulted in significant increase in the SGOT, ALP, serum Total protein, Albumin, Globulin & Uric acid level as compared to normal control. The test drug *Amrutha Ghritha* co-administered with Cypermethrin significantly reversed elevated SGOT, ALP, serum Total protein, Albumin, Globulin & Uric acid.

Table 1: Effect on drug on biochemical parameter

	N.C	p.C	½ TED	TED	TED*2
SGPT(IU/L)	71.5±4.40	64.22 ±3.502	67±6.01	65.4±4.38	64.85±8.23
SGOT(IU/L)	136.4±5.02	115.42±4.05*	128.66±6.99	120.8±4.87	106.66±7.40
ALP(IU/L)	527.4±62.97	495.44±73.41	700.57±54.22	799.16±101.5*	677.87±85.52
T.P(g/dl)	6.22±0.10	7.20±0.17**	7.10±0.11	6.38±0.32*	0.67±7.20
Albumin(g/dl)	3.54±0.22	4.23±0.11**	4.20±0.09	3.88±0.13	4.15±0.14
Globulin(g/dl)	2.68±0.18	2.96±0.13	2.90±0.14	2.50±0.27	2.52±0.14
T.BIli(mg/dl)	0.11±0.01	0.1±0.01	0.10±0.01	0.11±0.01	0.07±0.01
D.Bili(mg/dl)	0.11±0.01	0.07±0.01	0.06±0.01	0.11±0.02	0.06±0.01
Urea(mg/dl)	36.8±0.97	30±1.57*	31.5±3.51	25.83±1.85	23.16±2.24
Creatinine(mg/dl)	0.36±0.09	0.67±0.06	0.56±0.07	0.72±0.22	0.75±0.08
Uric acid(mg/dl)	1.12±0.05	1.47±0.08**	0.98±0.13**	1.3±0.07	1.51±0.05

	N.C	p.C	½ TED	TED	TED*2
Cholesterol(mg/dl)	46.7±3.27	52±3.22	56.16±5.19	51.5±3.59	58.14±4.73
Triglycerides(mg/dl	68.3±6.75	86.11±12.74	77±8.29	70.83±7.13	88±2.30
HDL(mg/dl)	32±3.31	31±1.98	38.57±2.98	30.8±1.42	37.75±2.34
LDL(mg/dl)	8.83±0.81	11.07±1.19	13.13±1.51	12.73±0.94	9.58±1.59
VLDL(mg/dl)	13.66±1.35	17.22±2.54	17.28±2.39	14.16±1.42	18.35±0.85
Sugar(mg/dl)	125±3.63	132.44±2.84	135.8±6.30	136±6.17	139±6.11

Data expressed in Mean ± SEM, *p<0.05, **p<0.01,

ANTIOXIDANT STUDY

Antioxidant activity is not much significant when compared to hepatoprotective action of the drug. In Table (2) Cypermethrin control group (A1&2), ½ TED group (B1&2), TED group (C1&2), TED*2(D1&2). FC-Fatty degenerative change, CV-dil-dilatation of central vein, CD-cell depletion, CI-Cell infiltration

Table 2: Effect on drug on Antioxidant parameter

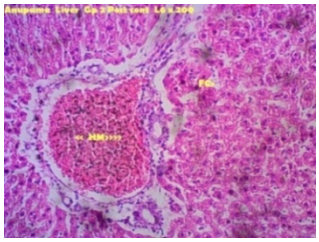
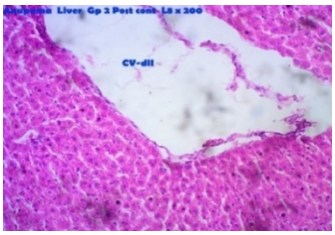
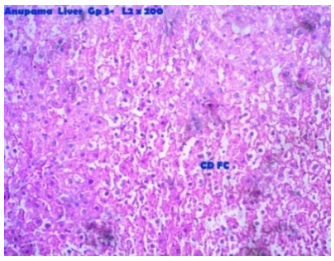
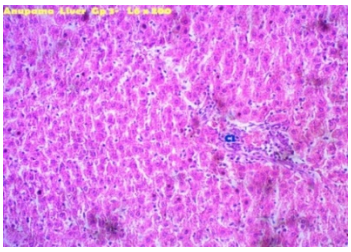
Group	Catalase activity (M moles/min/mg protein)	Glutathione peroxidation (M moles/min/mg protein)	Lipid peroxidation (Moles/min/mg protein)
Positive control	1.19±0.08	16.27±1.72	3.13±0.47
½ TED	1.20±0.05#	13.09±1.14#	4.23±0.94#
TED	1.24±0.05#	19.50±2.02#	5.48±2.07#
TED*2	1.19±0.11#	18.73±2.13#	4.68±1.76#

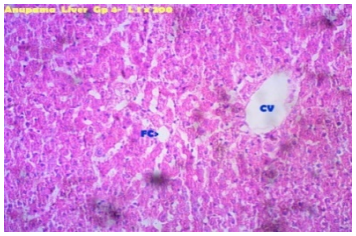
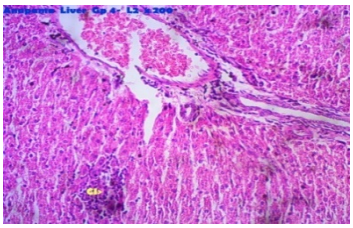
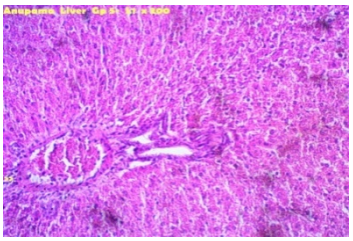
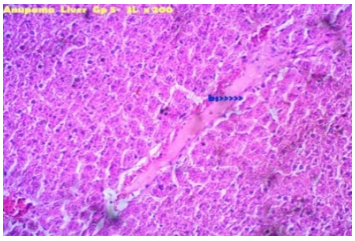
Data expressed in Mean ± SEM, *p<0.05, **p<0.01, # in comparison to Cypermethrin control group

HISTOPATHOLOGICAL EXAMINATION:

Scanning of the liver sections (on table 3) obtained from Cypermethrin administered control group showed extensive degenerative changes of moderate severity. The observed changes were cell infiltration at multiple sites, dilatation of the central vein, focal necrosis and cell depletion in the hepatocytes. ½ TED & TED dose administered group showed mild to moderate changes in the form cell infiltration at 3-4 sites much less in comparison to Cypermethrin administered control group. Other changes observed were cell depletion& fatty degenerative changes. In TED *2 group showed mild changes in the form cell infiltration at few sites, central vein dilatation. Majority of the sections from this group exhibited almost normal Cytoarchitecture.

Table 3: Photomicrograph representative of liver tissue

A1 	**A2** 
B1 	**B2** 

C1 	C2 
D1 	D2 

DISCUSSION

In the present study the Cypermethrin induced hepatotoxicity was evaluated by measuring SGPT, SGOT, ALP, Total protein, Albumin, Globulin, Total bilirubin, Direct bilirubin, Urea, Creatinine, Uric acid, Cholesterol, Triglycerides, HDL, LDL, VLDL, Sugar. Among these parameters significant elevation was observed in SGOT, ALP, serum Total protein, Albumin, Globulin & Uric acid level as compared to normal control. The test drug administered group shows reversal of important parameters like SGOT, ALP, Total protein, Albumin, Globulin, Uric acid.

Liver toxicity can be assessed by estimating the liver enzymes such as SGOT & SGPT activity. In Cypermethrin administered group has shown that there is significant decrease in all liver enzymes it may be due to the severe functional impairment of the livers ability to produce these enzymes. In case of SGPT Cypermethrin administrative group has shown that insignificant decrease. It may be due to the severe hepatocellular damage. It was reversed in test group administered group.

Proteins are the building blocks of the body are in state of dynamic equilibrium. If any changes in protein level are favored mechanism for long term adaptive changes. Here the very significant elevation of total protein level may be due to the increased tissue degeneration & release in to the serum or induction of liver activity. This was significantly reversed in TED test dose group. Cypermethrin administered group's shows there was significantly decrease in Urea level. Decreased levels of urea are found in liver failure. Here the decrease may be due to the impairment in the ability of liver to produce urea. It was reversed in half test dose group.

Albumin level was very significantly increased in Cypermethrin administered group. It may be due to the induction of the liver enzyme involved in its function; however it was reversed in the entire test group. Uric acid level was very significantly increased in Cypermethrin administered group & it was very significantly reversed in ½ TED & TED group.

Based on the data generated it can be inferred that the toxicant used in this study Cypermethrin induces a spectum of mild to moderate changes especially in the liver. These changes are reversed by simultaneous treatment with the test formulation. Antioxidant activity is not much significant when compared to hepatoprotective action of the drug.

Histopatholgy results in cypermethrin administered group shows there was an extensive degenerative changes of moderate severity. The observed changes were cell infiltration at multiple sites, dilatation of the central vein, focal necrosis and cell depletion in hepatocytes. ½ TED & TED dose administered group showed mild to moderate changes in the

form cell infiltration at 3-4 sites in ½ TED group & few sites in TED group much less in comparison to toxicant control group. Other changes observed were cell depletion & fatty degenerative changes. In TED* 2 group showed mild changes in the form cell infiltration at few sites, central vein dilatation, majority of the sections from this group exhibited almost normal Cytoarchitecture. Histopathological result indicate mild to moderate protection was observed in ½ TED group co-relating very well with the changes observed in the biochemical parameters. Thus the formulation can be used as adjuvant to other maintenance therapies for the toxicant poisoning it may also be used as low dose prophylactic agent.

CONCLUSION

The administration of Cypermethrin resulted in considerable changes in biochemical and Histopathological parameters which reveal the hepatotoxicity. Most of these changes were reversed by the treatment of the test drug especially at lower dose level (½TED group). Thus we can conclude the *Amrutha Ghritha* has significant effect in hepatotoxicity.

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