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PHCOG MAG.: Research Article
Protective Effect of Tefroli - a polyherbal mixture (Tonic) on cadmium chloride induced hepatotoxic rats

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ABSTRACT - Tefroli is a type of ayurvedic tonic composed of 6 herbal extract mixtures such as Phyllanthus niruri, Ocimum sanctum, Tephrosia purpurea, Andrographis paniculata, Eclipta alba and Terminalia chebula and the importance of the present work is to evaluate the protective effect of Tefroli tonic (a poly herbal mixture) against cadmium induced hepatotoxicity in experimental rats. Subcutaneous injection of cadmium chloride ($CdCl_2$) to rats caused liver damage and was observed by analysis of serum bilirubin and assay of marker enzymes such as transaminases and phosphatases of both serum and liver. Histopathological observation of hepatic tissue also showed marked changes in liver cells. Oral administration of Tefroli to $CdCl_2$ induced hepatotoxic rats reversed the above parameters to near normal levels. From this study, it is concluded that the administration of Tefroli tonic has maximum protective effect against cadmium-chloride induced hepatotoxicity in experimental rats.

KEYWORDS- Cadmium chloride, Histopathology, Marker enzymes, Tefroli, Serum Bilirubin.

INTRODUCTION

Cadmium (Cd) is a naturally occurring metallic element widely present in environmental sources and industrial wastes. Cadmium is absorbed in human body from the grown foodstuffs, especially grain, leafy vegetables and readily from the soil (1,2), also present in cigarette fumes, fumes from vehicles (3) and contamination of drinking or well water due to leaching of industrial wastes. Cadmium has no essential biological function and is extremely toxic to humans (4). In chronic exposure, it also accumulates in the body, particularly in the kidneys and the liver (2) and is known to be carcinogenic, causing cancer in the lung and prostate (1,2).

The toxic effects of cadmium are due to its inhibition of liver metabolic enzyme systems containing sulphydryl groups and uncoupling of oxidative phosphorylation in mitochondria(2) which results in increased lipid peroxidation, hepatic congestion, ischemia, and hypoxia(5). The resultant ischemic hypoxia leads to neutrophil infiltration, Kupffer cell activation, and inflammation, which could potentially contribute to the widespread hepatocellular apoptosis and necrosis observed with Cd (5,6,7). The various indigenous systems of medicine such as Siddha, Ayurveda, Unani and sometimes Allopathy use several plant species for the treatment of degenerative diseases (8). Presently, the medical fraternity and the patients have increasingly started, using plants to overcome various illnesses and sufferings mainly to obviate the profound side effects encountered in usage of modern drugs (9,10). Hepatotoxicity is also a type of degenerative disease due to viral attack (11), metal toxicity (12), metabolic abnormalities (13) and environmental pollution (14), which can be cured by herbal medicine extracts or its individual active constituents(15).

Tefroli is a type of ayurvedic tonic composed of 6 herbal extract mixture, having components possessing hepatoprotective activity (16,17,18). The 6 components of the mixture of Tefroli tonic are the alcoholic extract of the leaves of *Andrographis paniculata*(19), the root and alkali preparation of *Tephrosia purpurea(*20), the whole plant hexane extract of *Phyllanthus niruri(*21), the standardized extract of *Ocimum sanctum(*22), the ethanolic extract of the leaves of *Eclipta alba(*23) and the aqueous extract of the bark and fruits of *Terminalia chebula(*24,25) and in the present work the possibility of protective treatment of Tefroli in cadmium chloride induced hepatotoxicity in rats was studied by assaying the activities of serum and tissue marker enzymes, serum bilirubin level and histopathological analysis of liver cells.

MATERIALS AND METHODS

Tefroli - It was obtained commercially from TTK Pharmaceuticals Ltd., Chennai. The presence of the six herbs in the mixture active compositions was estimated by qualitative chemical tests, which proved the existence of alkaloids, tannins, polyphenols and other active ingredients (26).

Chemicals - Oxoglutarate, D-L-alanine, L-aspartate, glycine and bovine serum albumin were purchased from Sigma Chemical Co., Bangalore., and remaining chemicals were obtained from Loba Chemie Co., Bombay.

Animals - Adult male albino rats of Wistar strain weighing 150-200 g obtained from Tamil Nadu Veterinary and Animal Sciences University were fed with standard diet and water ad libitum and housed under standard environmental conditions. All the experiments were done according to animal institutional ethical committee (360/01/9/CPSEA/2001).

Experimental design -Animals were grouped into following 4 groups of six animals each.

Group 1 : Control rats. Group1 rats were referred as positive control rats.

Group 2 : Rats were given 7 subcutaneous injection (s.c.,) of $CdCl_2$ (3 mg of cadmium chloride/kg body weight) over a 2-week period. Group 2 rats were referred as $CdCl_2$ induced hepatotoxic rats.

Group 3: Cadmium chloride induced toxic rats, treated with Tefroli tonic (10ml/kg body weight) for 30 days from the last day of cadmium chloride administration. Group 3 rats were referred as $CdCl_2$ & Tefroli treated rats.

Group 4 : Control rats administered with Tefroli alone for the 30 days. Group 4 rats were referred as drug control rats.

At the end of the experimental period, (i.e. after 45 days) the animals were anaesthetized with pentobarbital sodium (35 mg/kg, i.p.,), blood was drawn from the external jugular vein of the rat and serum was separated by centrifugation. Serum was used for the assay of hepatic marker enzymes, serum protein and serum bilurubin. The liver tissues were dissected out immediately and washed in ice-cold saline. One portion of each fresh liver tissue was fixed in 10% formalin-saline for 24hrs for histopathological observation. Other portion of tissue (100 mg) was weighed accurately and homogenized with the help of Teflon homogeniser in 5 ml of 0.1M Tris-HCl buffer (pH 7.4) in ice-cold condition. The homogenate was centrifuged at 2500 g and the clear supernatant solution was used for the estimation of tissue marker enzymes and protein studies. Protein was estimated by the method of Lowry *et al* both in serum and liver homogenate (27). Serum and tissue alanine and aspartate transaminase were assayed according to the method of Bergemeyer and Bernt (28). Phosphatases were assayed by Tenniswood *et al* method (29). Serum bilurubin was estimated by Johnson micro method (30).

Histological studies - As mentioned earlier one portion of each fresh liver tissues were fixed in 10% formalin-saline for 24hrs for histopathological observation and histological evaluation was performed on whole portions of the liver tissue. The fixative was removed by washing through running tap water for overnight. After dehydration through a graded series of alcohols, the tissues were cleaned in methyl benzoate, embedded in paraffin wax. Sections were cut into 5µm thickness and stained with hematoxylin and eosin. Again after dehydration and cleaning, the sections were mounted and observed under light microscope with magnification of 100x for histological changes.

Statistical analysis- Results were presented as mean ± SD. The significance of difference among the groups was assessed using *students T test*. Significance was set at $P<0.05$, <0.01 and <0.001.

RESULTS - Table 1 shows the level of serum bilurubin and protein in serum and liver. Group 2 cadmium chloride induced hepatotoxic rats showed a significant reduction in the amount of protein ($P<0.001$) content both in serum and liver, with concomitant increase in serum bilurubin when compared to control rats. Group 3 drug treated rats showed near normal levels of serum bilurubin and protein ($P<0.001$) and tissue protein ($P<0.01$) when compared to Group 2 cadmium chloride induced toxic rats. Group 4 Tefroli alone- administered rats did not show any significant change when compared to Group 1 control rats (NS). Table 2 shows activity of alanine transaminase and aspartate transaminase enzymes both in serum and liver tissue. Group 2-cadmium chloride induced rats showed high levels of alanine transaminase and aspartate transaminase in serum ($P<0.001$) and liver ($P<0.01$). Group 3 drug treated animal showed near normal levels when compared to Group 2 rats ($P<0.001$). Group 4 Tefroli -alone administered rats did not show any significant change compared to control rats (NS).

Table 1: Level of serum bilirubin and protein in the serum and liver of normal and experimental groups of rats. Values are expressed as mean ± SD for six rats in each group.

Parameters	Group 1 Control rats	Group 2 $CdCl_2$ induced toxic rats	Group 3 $CdCl_2$ induced and tefroli treated rats	Group 4 Tefroli treated rats
Serum bilirubin mg/dl	0.3 ±0.02	1.90 ±0.12	0.78 ±0.06***	0.28 ±0.02^{NS}
Serum protein g/dl	7.60 ± 0.19	6.75 ± 0.11***	7.24 ± 0.16***	7.68 ± 0.18^{NS}
Tissue protein mg/g tissue	300.09 ± 12.14	242.94±15.45***	275.13±14.79**	309.43±18.80^{NS}

Statistical significance comparison made by Student "T" test.

*Groups were compared as follows: Group 2 Vs Group 1; Group 3 Vs Group 2; Group 4 Vs Group 1; *** P<0.001, ** P <0.01, * P<0.05, NS = Non Significant*

Table 2: Activity of Alanine transaminase and Aspartate transaminase in the serum and liver of normal and experimental groups of rats. Values are expressed as mean ± SD for six rats in each group

Experimental group of rats	Alanine transaminase		Aspartate transaminase	
	Serum	Liver	Serum	Liver
	µg of pyruvate liberated/ml of serum	µg of pyruvate liberated/hr/mg protein	µg of pyruvate liberated/ml of serum	µg of pyruvate liberated/hr/mg protein
Group 1 (Control rats)	28.71 ± 2.03	128.10 ± 12.80	46.28 ± 6.22	130.95 ± 6.99
Group 2 (Cadmium chloride induced rats)	44.49 ± 4.31***	154.29 ± 7.91**	71.99 ± 7.80***	170.94 ± 6.52***
Group 3 (Cadmium chloride induced and tefroli treated rats)	32.43 ± 1.72***	132.64 ± 1.66***	53.93 ± 2.06***	138.38 ± 5.09***
Group 4 (Tefroli alone treated rats)	28.32 ± 1.80^{NS}	118.88 ± 6.59^{NS}	46.14 ± 0.68^{NS}	128.96 ± 2.19^{NS}

Statistical significance comparison made by Student "T" test.

*Groups were compared as follows: Group 2 Vs Group 1; Group 3 Vs Group 2; Group 4 Vs Group 1; *** P<0.001, ** P<0.01, * P<0.05, NS = Non Significant*

Table 3: Activity of alkaline and acid phosphatases in the liver of normal and experimental groups of rats. Values are expressed as mean ± SD for six rats in each group

Experimental group of rats	Acid phosphatase	Alkaline phosphtase
	n moles of phenol liberated /hr/mg protein	n moles of phenol liberated /hr/mg protein
Group 1 (Control rats)	4.98 ± 1.68	32.45 ± 5.68
Group 2 (Cadmium chloride induced toxic rats)	8.46 ± 2.32*	46.89 ± 2.34***
Group 3 (Cadmium chloride induced toxic and Tefroli treated rats)	5.05 ± 0.58**	41.94 ± 3.72*
Group 4 (Tefroli alone treated rats)	4.68 ± 0.33^{NS}	30.33 ± 2.25^{NS}

Statistical significance comparison made by Student "T" test.

*Groups were compared as follows: Group 2 Vs Group 1; Group 3 Vs Group 2; Group 4 Vs Group 1; ***P<0.001, ** P<0.01, * P<0.05, NS = Non Significance*

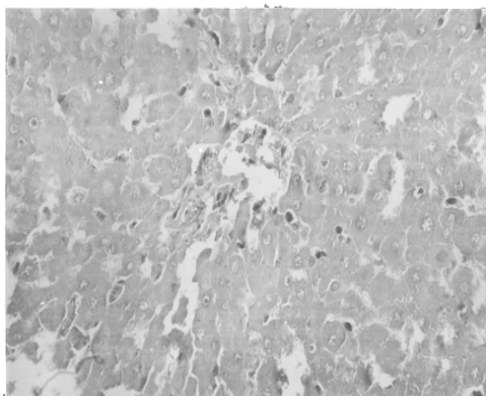

Figure 1a

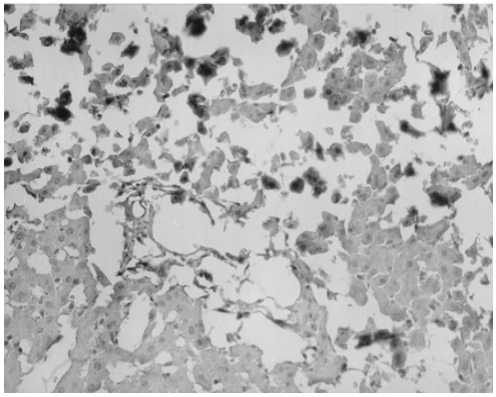

Figure 1b

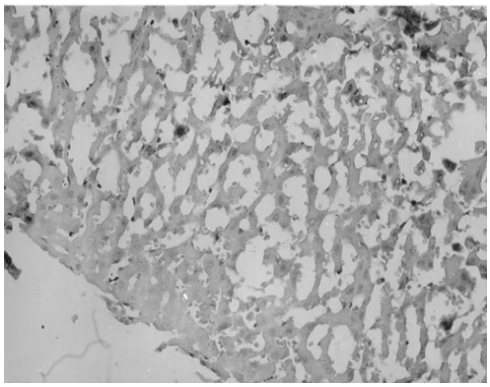

Figure 1c

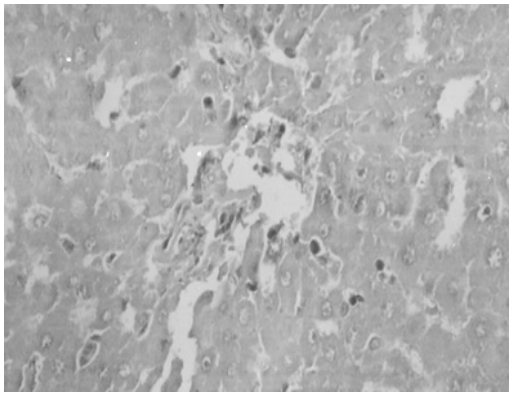

Figure 1d

Figure 1: Histological examination of liver tissue section in control and experimental animals (Hematoxylin and eosin 100x).

1a - Section of liver tissue from control rat showing normal architecture

1b - Section of liver tissue from cadmium chloride administered rat showing degenerative changes, hyalinization of muscle fibers and cellular infiltration.

1c- Section of liver tissue from cadmium chloride administered and Tefroli treated rat reveals less cellular infiltration, normal muscle fibers and the hepatoprotective effect are evident from reduced liver damage even on cadmium chloride administration.

1d- Section of liver tissue from Tefroli alone treated rat showing normal architecture similar to Group 1 rats.

Table 3 shows activity of acid phosphatase and alkaline phosphatase levels in liver tissues. Group 2-cadmium chloride induced rats showed increased level of acid phosphatase ($P<0.05$) and alkaline phosphatase ($P<0.01$) when compared to Group 1 control rats. Group 3 drug treated animals showed near normal levels of acid phosphatase ($P<0.01$) and alkaline phosphatase ($P<0.05$) when compared to Group 2 cadmium chloride induced rats ($P<0.01$). Group 4 Tefroli alone-administered rats did not show any significant change when compared to Group 1 control rats (NS).

Histopathological observation of hepatic tissue (Fig.1) showed an extensive necrosis and degenerative changes with loss of architecture in Group 2 cadmium chloride induced toxic rat liver compared to Group 1 control rats. Cadmium chloride induced and Tefroli treated Group 3 rat liver showed regeneration of hepatic sinusoids and hepatic cords as compared with Group 2 hepatotoxic rats. Group 4 Tefroli alone treated rats showed no morphological changes like Group 1 control rats.

DISCUSSION

The present experiment was concentrated mainly on serum bilurubin, serum and liver marker enzymes with protein and histopathological changes of liver cells. A significant decrease in protein level both in serum and liver of cadmium chloride induced toxic rats could be

due to the transfer of protein to other parts of the body (31) since liver plays an important role in synthesis of amino acids and protein. The incorporation of amino acid to synthesize protein might have been attacked by cadmium chloride and hence the decreased protein values were observed(18).

Treatment with Tefroli in Group 3 cadmium chloride induced rats showed protein values to near normal levels. The liver cell might have been protected from cadmium chloride poisoning and further degeneration of hepatic cells could have been avoided by the active constituents of the tonic possessing hepatoprotecive activity (16,17).

Serum bilurubin value is an index of severity of liver damage. The liver damage may have resulted due to obstruction induced by cadmium on liver. Novelli *et al* also have reported the cadmium-induced hepatotoxicity in the increased production of serum bilirubin (32). Tefroli is a polyherbal mixture which is composed of polyphenols, tannins and alkaloids derived from six herbs all of which possesses serum bilurubin reducing effect (33,34,35,36).

Levels of activities of marker enzymes namely alanine transaminases and aspartate transaminases in serum and liver were increased due to leakage of enzymes from the cytosol of liver that might have entered into the blood stream; hence high levels of activities of these enzymes were observed. The increased levels of these enzymes in hepatic damage could be due to malignant infiltration and cirrhosis of liver. Hence both the enzymes have been proved to be an excellent indicator of cadmium chloride induced hepatocellular necrosis in rats(37). In Group3 rats, the administration of Tefroli after induction of cadmium chloride hepatotoxicty showed a significant reduction in the activity of these enzymes towards normal which might be due to regeneration of hepatic cells by the active constituents in the tonic.

The increased enzyme activity of acid phosphatase and alkali phosphatase in liver of cadmium chloride treated rats could be due to the damage to the cell membrane of tissues, where these enzymes are firmly attached to the cell membrane and the damage releases these enzymes from the membrane joining the binary canalicules and the sinusoidal border of parenchyma cells(38). Treatment with the tonic showed a reversal of enzyme activity towards normal level, which could be due to flavanoids, triterpenes, steroids, lignans, polyphenols, volatile oils, saponins and glycosides as reported earlier(16,17,18).

Histopathological observation of liver tissue also confirmed the hepatotoxic levels of cadmium chloride which coincides well with the previous report(39). The study also confirms the curative effect of Tefroli tonic which causes regeneration of hepatic cells. This may be due to active principles such as flavanoids, triterpenes, steroids, lignans, polyphenols, volatile oils, saponins and glycosides (16,17,18, 40).

The novelty in this experimental study is that the statistically significant reversibility of the heavy metal induced toxicity to normal level. Tefroli is commonly used for treating viral hepatitis as a anti viral agent (16,17) and with the current knowledge no work yet is focused on the reversibility of the heavy metal induced hepatotoxicity by Tefroli drug(18). The present experimental study has proved Tefroli tonic as a good hepatoprotecive drug which acts by amelioration of histological changes and biochemical markers of the hepatic tissue without any adverse effect, which merits it to recommend as an excellent hepatoprotecive drug.

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MILESTONES AND ACHIEVEMENTS – PHCOG.NET - (2004 -2006)

Pharmacognosy Network Worldwide is a non-profit network dedicated to Natural Products Research in order to develop promising drugs.

Phcog.net was started on July 6, 2004

- Development and launch of Website – www.phcog.net
- Initiation of Discussion forum – http://groups.yahoo.com/group/phcog/
- Started a forum – www.phcog.net/forum.php
- Started a New Online peer reviewed magazine – **Pharmacognosy Magazine** (PHCOG MAG). Editorial team was finalized for the term of three years (2004-2007).
- Release of four issues in 2005.
- Project Phcog Refbase started in the month of May 2005.
- Release of 6th issue in April 2006.
- Print version of Pharmacognosy Magazine
- Knowledge base section – http://www.phcog.net/knowledge
- Online web based manuscript handling system – http://www.phcogmag.com
- First issue of Phcog E –news – http://www.phcog.net/bulletin
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