STUDY OF SOME BIOCHEMICAL PARAMETERS OF LIVER AND STOMACH OF ALBINO RATS TREATED WITH NICKEL AND *CAMELLIA SINENSIS.*

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Abstract

During the present study protective effect of ***C. sinensis*** *against nickel toxicity was evaluated. For this purpose, liver and stomach were selected as target organs. The body weight and organ weight showed decrease in nickel treatment group but it was increased after treatment with both 1 mg and 2 mg doses of* ***C. sinensis*** *aqueous extract co-treated with nickel. Among the biochemical parameters, protein and sugar showed decrease and urea, ALT and AST showed increase in nickel control group but all these parameters showed improvement in* ***C. sinensis*** *and nickel co-treated groups. indicating protective role of* ***C. sinensis*** *against nickel toxicity.*

Key words: *Camellia sinensis, nickel chloride, liver, stomach.*

Introduction:

The common name of *Camellia sinensis* is green tea and it belongs to the family Theaceae. Tea is the plant's leaf or beverage originating from what is now considered a single species *Camellia sinensis* (L.) O. Kuntze with two major varieties recognized are *sinensis* and *assamica*. In recent years green tea has become popular throughout the world. The active constituents in green tea (*C. sinensis*) are powerful antioxidants called polyphenols (catechins) and flavonols. Many scientific researches have proved that *C. sinensis* has many beneficial properties.

According to many reports *C. sinensis* can reduce cholesterol and triglycerides, activate immune system, can cause weight loss and has anti-carcinogenic properties. It is believed that high antioxidant activity of *C. sinensis* helps in protecting the body against oxidative damage caused by free radicals. People who regularly drink green tea may have a lesser risk of

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mental decline as they grow older. (Bogdanski , 2012)

The heavy metal nickel is a wide spread industrial pollutant that is produced during the manufacture of batteries, paints, plastics and fertilizers. Nickel is also being continuously released in to the atmosphere by the burning of fossil fuels. Many human beings are being exposed to increased levels of nickel through inhalation either by direct smoking or indirectly. Nickel, a potent toxic metal is very harmful to the environment and to humans because of in vivo accumulation of nickel in liver, kidney and other tissues. (Costa 1984, Forti, *et al.*, 2011)

Material and Methods

Plant Material – The leaves of ***C. sinensis*** were procured, powdered and were used for the preparation of aqueous extract with the help of soxhlet apparatus. The extract was kept in air tight container to be used during the experiments.

Experimental Animals - Adult healthy male albino rats weighing between 120-160 gm were used as experimental animals.

Experimental Design- Experimental animals were randomly divided into four groups of 18 animals each. Out of these four groups, group 1 served as normal control group, group 2 served as nickel control group (1mg/100gm body weight of $NiCl_2$), group 3 received 2 mg/100 g body weight of *Camellia sinensis* aqueous extract and 1 mg $NiCl_2$ and animals of group 4 received *C. sinensis* extract at a dose of 4 mg/100 g body weight along with 1 mg $NiCl_2$. Each group was subdivided into three sub groups of 15 days duration, 30 days duration and one reversibility group in which after 30 days treatment animals were kept on normal diet for 15 more days with a total duration of 45 days.

All experimental animals were weighed before starting the experiments and kept in separate polyvinyl cages. After the completion of experiments, experimental animals were again weighed and then dissected. Liver and stomach were removed, weighed and processed for biochemical and histological studies.

Biochemical parameters - Tissue homogenates of liver and stomach were prepared and sugar(GOD/POD method), total protein (modified Biuret and Dumas method), Urea (U.V. Kinetic method), ALT / Glutamate Pyruvate Transaminase (U.V. Kinetic method) and AST / Glutamate Oxaloacetate Transaminase (U.V. Kinetic method) were studied.

Results &Discussion:

The results of body weight, organ weight and biochemical parameters are given in Table 1 and figures.

Nickel is a toxic transition metal with a variety of adverse effects on both humans and in animals (Mathur *et al.* 1978, Leonard *et al.* 1981). Chronic nickel exposure has been associated with profound effects in mammalian physiology, especially on the immune system (Haley *et al.*, 1987).

During the present study nickel treatment caused decrease in body weight with 1 mg dose after 15 and 30 days treatment. Camner (1985) reported that there were no significant differences observed in body weight at any weekly time point in the short term studies. Cempel (2002) reported that no significant dose related differences in body weight were seen in males during the first 100 days and in females at any point in the study, although no explanation is given for this gender difference.

Our observations are almost in agreement with the work of these scientists. The experimental animals treated with 2 mg and 4 mg doses of aqueous extract of *C. sinensis* showed slight increase in the body weight after 15 and 30 days treatment. Yung His-Kao and co-laborators (2000) have reported reduction in body weight with 2 to 7 days treatment with catechins of green tea. The effect of epigallocatechin (EGCG) on body weight is dose dependent.

Green tea is said to decrease body weight but we find some increase in body weight after feeding of green tea. But when we analyse these results carefully, it is evident that the gain percentage of body weight was increased in comparison to nickel control groups but decreased in comparison tc normal control groups.

Pum ing *et al.* (2001) reported no association between the final body weight or body weight gained and the action of green extract (30 gm/kg dose).

The weight of liver showed significant decrease after nickel treatment but improvement was seen in the both dose groups of *C. sinensis*. Not much significant differences were observed in the weight of stomach.

Obone *et al.* (1999) reported that in adult male Sprague-Dawley rats given $NiSO_4$ at 0, 0.02, 0.05 and 0.1 per cent or 0, 44.7, 111.75 and 223.5 mg/ 1 respectively (estimated doses of 0, 5, 12.5 and 25 mg/ kg/day) in their drinking water for 13 wk, both the absolute and relative liver weights in the 12.5 mg/kg and 25 mg/kg groups were significantly decreased. In rats, decreased liver weight was observed following exposure for 28 days to 2 yr to 0.97-75 mg/kg/day of nickel chloride or nickel sulphate. Recent studies on rats by Das *et al.* (2006) revealed a nickel sulphate-induced degenerative effect on hepatic tissue, hence loss of liver

weight. Venukumar and Latha (2004) have also reported that in carbon tetrachloride induced hepatopathy; the toxin is metabolically activated by liver enzymes to form a tricholoromethyl radical. All these events culminate in loss of integrity of cell membranes and damage of hepatic tissue resulting in reduction in liver weight. As treatment with *C. sinensis* appears to prevent all this damage, hence no loss in liver weight was observed during the present study.

Pang *et al.* (1996) has given nickel chloride in drinking water for 50 to 130 days to Sprague-Dawley rats and reported no change in the weight of stomach. Nielsen *et al.* (1999) had reported on absorption and retention of nickel from drinking water in relation to food intake and nickel sensitivity. The results during the present study are in agreement with all this work.

As liver is the chief site of metabolism, liver is the first organ affected with toxicity of any pollutant and toxicant hence it was thought worth while to include liver for biochemical analysis.

In the nickel control group decrease in the sugars and total proteins was observed both in liver and stomach and in the reversibility study also no alteration in the results was observed. Disruption in carbohydrate metabolism during acute and chronic liver injury may finally lead to glycogen disorder leading to decreased level of sugars. Sunderman and Kincaid (1959), observed loss of glycogen in the liver after prolonged exposure to nickel chloride.

Dubey *et. al.* (1994) reported that capacity of liver to synthesize albumin is adversely affected by hepatotoxins. The lowered level of total proteins in liver of nickel treated rats can be attributed to this fact only. No change in protein content after treatment with nickel was reported by Ramirez and Gimenez (2002) but other workers have reported decrease in protein level and present study is also in accordance with these findings.

The levels of urea, glutamate pyruvate transaminase (GPT or AST) and glutamate oxaloacetate transaminase (GOT or ALT) were increased significantly in the liver and non-significantly in the stomach after 15 and 30 days treatment with 1 mg dose of nickel. Not much improvement was observed even after discontinuation of the nickel treatment for 15 days. ALT and AST activity is considered good indicator of liver function. Increased levels of ALT and AST are indicative of liver damage.

In some rats 2 mg dose of *C. sinensis* also caused decrease in elevated ALT level but in others it was slightly high. But 4 mg dose of *C. sinensis* was able to bring back the elevated level of ALT in liver even after

15 days treatment. On the other hand, both doses of *C. sinensis* were able to bring back the AST level to normal. ALT is a marker enzyme of liver damage and its increased level indicated that chronic nickel exposure affects liver function but normal level of ALT and AST indicates that *C. sinensis* is protecting liver damage by nickel.

Venukumar and Latha (2004) had reported decline in the level of ALT and AST (that was increased in CCl_4 control groups) in CCl_4+*Coscinium fenestratum* treated rats is indicative of hepatoprotective effect of *C. fenestratum*.

Most probably protective effects of *C. sinensis* are by inhibiting nickel uptake and its accumulation in various organs. Several other antioxidants of plant origin such as *Coscinum fenestratum, Curcuigo orchioides, Cassia fistula, Eclipta alba, Andrographis paniculata, Ganoderma lucidum, Ganoderma formosanum, Ganoderma neo-japonicum,* soyabeans and others such as glutathione, Vitamin-E, Vitamin-C, selenium etc. are also reported to protect tissue damage caused by various mechanisms.

This hypothesis is supported by the fact that when *C. sinensis* treatment was discontinued, all parameters i.e. sugar, total protein, urea, glutamate pyruvate transaminase (ALT), glutamate oxaloacetate transaminase (AST) remained normal. This fact indicates that some how nickel uptake was inhibited by catechins and polyphenols of green tea in the cells so toxic manifestations of nickel were not produced.

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Table 1:- Mean body, liver and stomach weight of normal, Ni control, and *C. sinensis* co-treated with Ni (15 days)

Parameter/ Duration	Initial body wt gm	Final body wt gm	%gain or loss in body wt.	LIVER		STOMACH	
				Wt. gm	%	Wt gm	%
Normal control	127.33	131.33	3.14	6.73	5.12	1.15	0.88
	±4.13	±3.78		±0.70		±0.19	
1mg/100g.$NiCl_2$ treatment	123.67	121.50	-1.75	4.66	3.84	1.15	0.95
	±6.77	±6.63		±0.33		±0.21	
1mg/100g.$NiCl_2$ treatment+ 2.0mg/ 100g. *C.sinensis*	129.33	131.17	1.42	5.98	4.49	1.16	0.87
	±11.79	±11.81		±0.49		±0.16	
1mg/100g.$NiCl_2$ treatment+ 4.0mg/ 100g. C.sinensis	131.17	133.17	1.52	6.50	4.96	1.20	0.91
	±3.71	±3.71		±0.54		±0.12	

Table 2:- Mean body weight and organ weight of normal, Ni treated and effect of different doses of *C.sinensis* for different durations on albino rats.

30 Days Duration

Parameter/ Duration	Initial body wt. gm	Final body wt gm	% gain or loss in body wt.	LIVER		STOMACH	
				Wt. gm	%	Wt gm	%
Normal control	129.50	131.83	1.80	6.14	4.66	1.02	0.77
	±2.17	±2.4		±0.24		±0.07	
1mg/100g.$NiCl_2$ treatment	126.83	125.33	-1.18	4.58	3.65	1.34	1.07
	±5.64	±5.5		±0.3		±0.09	
1mg/100g.$NiCl_2$ treatment+ 2.0mg/ 100g. *C.sinensis*	116.83	118.33	1.28	5.49	4.64	1.25	1.06
	±7.31	±7.12		±0.98		±0.31	
1mg/100g.$NiCl_2$ treatment+ 4.0mg/ 100g. C.sinensis	124.33	125.83	1.21	5.50	4.37	1.28	1.02
	±3.78	±3.82		±0.56		±0.07	

Table 3:- Mean body weight and organ weight of normal, Ni treated and effect of different doses of *C.sinensis* for different durations on albino rats

30+15 Days Duration							
Parameter/ Duration	Initial body wt. gm	Final body wt gm	% gain or loss in body wt.	LIVER		STOMACH	
				Wt. gm	%	Wt gm	%
Normal control	128.00	132.33	3.38	6.59	4.98	1.22	0.92
	±3.06	±4.18		±0.69		±0.24	
1mg/100g.$NiCl_2$ treatment	127.17	128.33	0.91	6.09	4.75	1.26	0.98
	±1.83	±2.07		±0.21		±0.1	
1mg/100g.$NiCl_2$ treatment+ 2.0mg/ 100g. *C.sinensis*	128.00	130.00	1.56	6.05	4.65	1.40	1.08
	±4.77	±3.45		±0.53		±0.31	
1mg/100g.$NiCl_2$ treatment+ 4.0mg/ 100g. C.sinensis	124.83	126.67	1.47	6.61	5.22	1.10	0.87
	±3.66	±3.44		±0.8		±0.09	

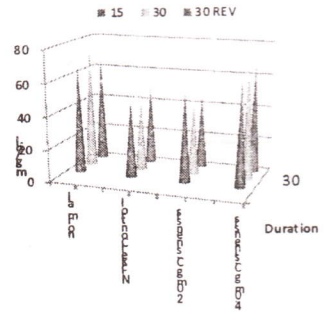


Fig:1 Histogram showing sugar in liver of 1 mg nickel chloride treated and C. sinensis aqueous extract treated groups

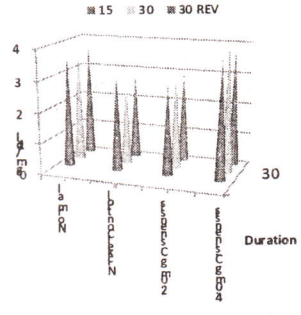


Fig: 2 Histogram showing total protein in liver of 1 mg nickel chloride treated and C. sinensis aqueous extract treated groups

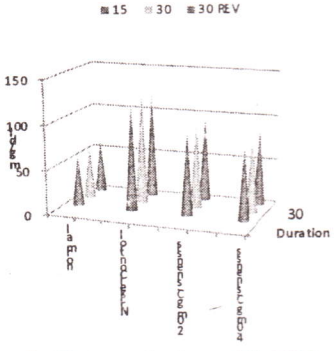


Fig:3 Histogram showing urea in liver of 1 mg nickel chloride treated and C. sinensis aqueous extract treated groups

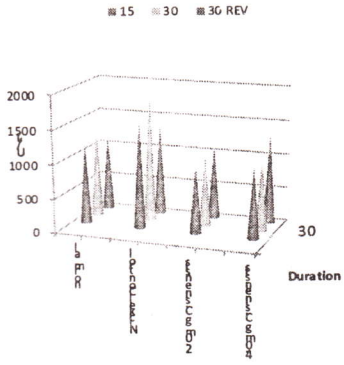


Fig:4 Histogram showing change in ALT in liver of 1 mg nickel chloride treated and C. sinensis aqueous extract treated groups

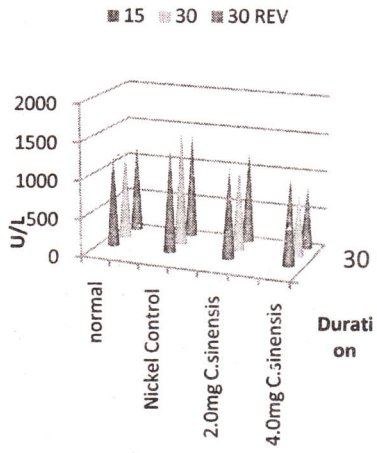


Fig:5 Histogram showing change in AST in liver of 1 mg nickel chloride treated and C. sinensis aqueous extract treated groups

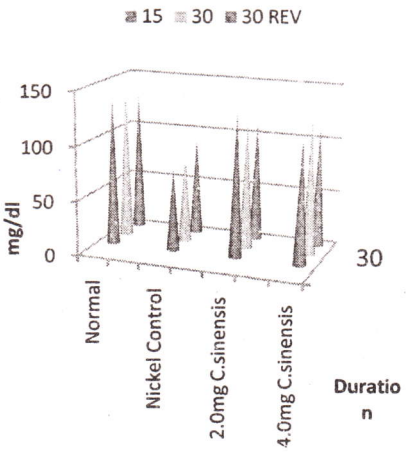


Fig:6 Histogram showing change in sugar in stomach of 1 mg nickel chloride treated and C. sinensis aqueous extract treated groups

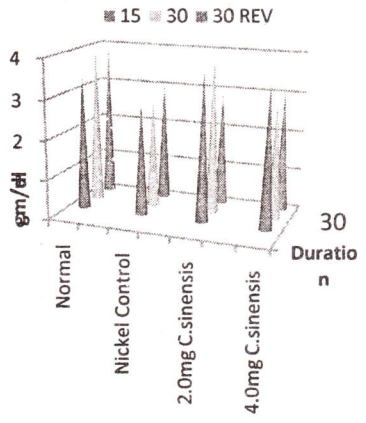


Fig:7 Histogram showing change in total protein in stomach of 1 mg nickel chloride treated and C. sinensis aqueous extract treated groups

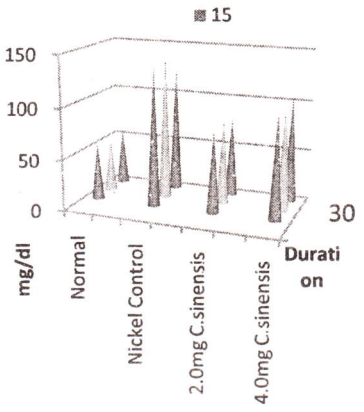
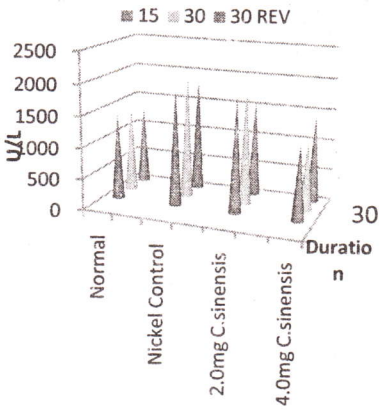


Fig:8 Histogram showing change in urea in stomach of 1 mg nickel chloride treated and C. sinensis aqueous extract treated groups



Fig·9 Histogram showing change in ALT in stomach of 1 mg nickel chloride treated and C. sinensis aqueous extract treated groups

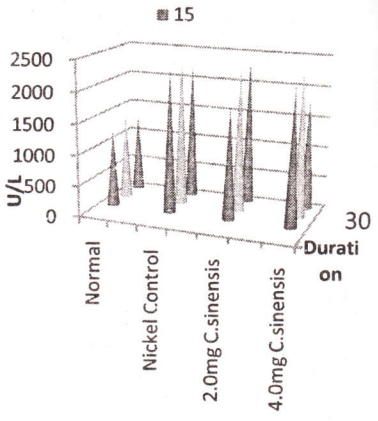


Fig:10 Histogram showing change in AST in stomach of 1 mg nickel chloride treated and C. sinensis aqueous extract treated groups