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BIOCHEMICAL STUDY OF *TRIDAX PROCUMBENS* WEED ON VITAL ORGANS OF FEMALE SPRAGUE DAWLEY RATS

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ABSTRACT

Biochemistry and medicine are closely related. Health depends on the proper balance of biochemical reactions occurring in the body and diseases indicate abnormalities in biochemical reactions or processes. The perfect knowledge of biochemistry is most essential for the practice of medical and related health Sciences. India is endowed with a rich wealth of medicinal plants. Herbal drugs are emerging as a leading area for new drug discovery and development, and are being looked at seriously for health problem. Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. Medicinal plants and the active principles isolated from them are and will be of immense importance to humanity in their fight against diseases due to fewer side effects. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. The purpose of this study is to infer ethnobotanical value of weed i.e. *Tridax procumbens* on biochemical parameters in adult female albino rats of *Sprague dawley* strain. The experimental groups were received daily doses of ethanolic extract of plant at the concentration of 300mg/kg b.wt and 600mg/kg b.wt for the interval period of 7, 14 and 21 days. After the regimen period, the content of protein, glycogen, and cholesterol, activity of acid & alkaline phosphatase was estimated in liver, kidney, ovary and uterus of albino rats. And it was noted that there was significantly increased in content of protein and activity of acid & alkaline phosphatase ($p<0.05$) as compared to control rats while content of cholesterol was decreased significantly. But there were no significant changes occurred in wet- weight and glycogen content of albino rats. The activity was uniform in all the organs. This may all due to presence of active phytochemical constituents of plant.

Keywords: *Tridax procumbens*, protein, glycogen, cholesterol content, acid & alkaline phosphatase.

Introduction

Tridax procumbens are medicinally important plant. It is best known as a widespread weed and pest plant. It belongs to family 'Asteraceae' (Composite). It shows a number of pharmacological activities like hypotensive, hair growth promoting, wound healing, anti- inflammatory, hepatoprotective and immunomodulatory, anti-pyretic, anti-diabetic, antioxidant, analgesic, haemostasis etc. The leaves are reported to be employed in bronchial catarrh, malaria, headache, stomach-ache, dysentery and diarrhoea (Mundada *et al.*, 2010). The leaf juice also possess as antiseptic, insecticidal and parasiticidal properties that prevent various types of ailments and disorders. It is used to check haemorrhage from cuts bruises and wounds. Even alcoholic extract of plant is useful in Liver regeneration (Pathak *et al.*, 1991). The plant has been found to contain various types of chemical constituents such as flavonoids, alkaloids, sterols, carotenoids, saponin and tannin etc (Ikewunchi *et al.*, 2009). *Tridax procumbens* has been found to be a potential source to regulate various biological activities and due to its multiple virtues, it has been frequently used by rural natives. As biochemical constituents display equilibrium of all cytoplasmic inclusions, it is, therefore required to study the effect of *Tridax procumbens* on the biochemical constituents of various vital organs.

Materials and Methods

Drug Used: Whole plant of *Tridax procumbens* except root was collected from B.U. campus, BHEL campus and adjacent areas of Bundelkhand region. The plant taxonomically identified by Dr. H.B Singh, Head, RHMD, NISCAIR, New Delhi, NISCAIR/ RHMD/ 2010-11/1681/279.

Preparation of Extract: Collected material was shade-dried until total moisture was removed from the plant. Then the material is grinded in an electric mixer and further it was extracted with absolute alcohol in Soxhlet's apparatus. After extraction the total solvent from the extract was removed and the crude drug was made by keeping it on heating plate. Finally, it was preserved inside the desiccators. For experimentation the dose at a concentration of 300mg/kg b.wt and 600mg/kg b.wt was prepared.

Animal used: The study used female albino rats of *Sprague- dawley* strain weighing 200 ± 10gm. They were kept in polypropylene cages in centrally air condition room 12 hours light & 12 hours dark cycle. The animals had free access to water & food (rat pelleted- diet, Amrut Feeds, Pranav Agro Industries Ltd, Sangli) and were left to acclimatize at least one week before starting the experiment. All experiments were carried out in accordance with the guideline of the CPCSEA. Approval Number for IAEC/CPCSEA usage 716/02/9/CPCSEA and Proposal number BU/ Pharm/07/41.

Experimental Design: The animals were distributed randomly, six animals in each group. Two group serves as experimental that received the doses of extract i.e. 300 mg/kg b.wt and 600 mg/kg b.wt respectively while other served as control which received vehicle only. All groups were maintained simultaneously to make the data valid. The dosages were given daily orally to rats through gastric feeding needle for the interval of 7, 14 and 21 days. The animals were sacrificed after 24 hours of last treatment. Organs from the animal body were excised out on the day of autopsy, properly cleared of all the adhering muscles on the tissue and their wet weight was taken. Tissue homogenate at the concentration of 25mg/ml was prepared in $NaHCO_3$ and the total glycogen, protein, cholesterol, wet-weight and activity of acid & alkaline phosphatase were estimated in Liver, Kidney, Uterus & Ovary of albino rats according to method of Seifter *et al.* (1950), Lowry *et al.* (1951), Zlatkis *et al.* (1953) and Hawk *et al.* (1954) respectively.

Statistical Analysis: The results were expressed as the Mean ± S.E. Significance of differences compared to the control groups was determined using 'student t-test'. The minimum level of significance was set at $p<0.05$.

Results and Discussion

Authors revealed that during daily administration of *Tridax procumbens* extract were significantly increased ($p<0.05$) the content of protein in liver, kidney, uterus & ovary of albino rats as compared to their control rats (Table 1). As the plant of *Tridax procumbens* is reported to be rich in protein content quantity which may be responsible for the increase in protein content in tissues (Vats *et al.*, 2003). The plant act as a good antioxidant; since glycosylation of proteins is an oxidation reaction, due to the antioxidant activity of the plant it should be able to prevent this reaction that means the plant having capacity to increase the protein content in the tested organs of the albino rats (Asgary *et al.*, 1999). Plant also showed the hepatoprotective activity due to which it may also alters the activity of SGOT, SGPT and ALP etc which involved in the transport of metabolites across the cell membrane protein synthesis and enzyme synthesis and total bilirubin, which may be responsible for the increase in content of protein in the tested organs (Vilwanathan *et al.*, 2005). This study suggested that the possible activity may be due to the presence of flavonoid and phenolic compounds in the ethanolic extract of *Tridax procumbens* (Habila *et al.*, 2010). Similar finding was reported by many workers which evidently suggests the protective effect of the extract. Also, this further signifies the curative nature of extract against paracetamol toxicity (Wagh *et al.*, 2010).

Table 1: Protein Content (mg/100mg) in given organs with chronically administration of doses

S. No.	Tested Organs	Control Group	Experimental Groups (Mean ± SE, p Vs respective control < 0.05)					
			7 Days		14 Days		21 Days	
			300mg/ kgb.wt	600mg/ kgb.wt	300mg/ kgb.wt	600mg/ kgb.wt	300mg/ kgb.wt	600mg/ kg b.wt
1.	**Liver**	15.8±0.80	16.1±0.82	17.0±0.87	18.0±0.91*	18.8±0.93*	19.0±0.93*	19.9±0.97*
2.	**Kidney**	10.8±0.55	11.0±0.60	12.5±0.65	13.2±0.60	14.1±0.70*	13.5±0.67	15.0±0.72*
3.	**Uterus**	9.1±0.56	10.5±0.49	11.5±0.57	11.0±0.51	12.4±0.57	12.5±0.58	13.5±0.60*
4.	**Ovary**	10.5±0.30	11.5±0.43	12.0±0.45	12.8±0.60	13.3±0.61	13.7±0.65*	14.5±0.68*

Table 2: Glycogen Content (mg/100mg) in given organs with chronically administration of doses

S. No.	Tested Organs	Control Group	Experimental Groups (Mean ± SE, p Vs respective control < 0.05)					
			7 Days		14 Days		21 Days	
			300mg/ kgb.wt	600mg/ kgb.wt	300mg/ kgb.wt	600mg/ kgb.wt	300mg/ kgb.wt	600mg/ kg b.wt
1.	**Liver**	46.6±2.5	45.3±4.3	43.1±4.1	42.5±3.4	39.9±2.0*	38.3±2.1*	35.3±1.8*
2.	**Kidney**	23.5±0.5	18.6±1.1	18.3±1.0	18.0±1.2	17.8±1.9*	17.5±2.0*	17.2±1.8*
3.	**Uterus**	18.6±2.6	18.3±1.2	18.1±1.1	17.6±1.1	17.4±1.2	17.1±1.4	17.0±1.4*
4.	**Ovary**	22.0±0.8	21.5±0.9	21.2±0.8	20.5±0.4	20.0±0.5	20.0±1.8	19.9±1.7*

Table 3: Cholesterol Content (mg/100mg) in given organs with chronically administration of doses

S. No.	Tested Organs	Control Group	Experimental Groups (Mean ± SE, p Vs respective control < 0.05)					
			7 Days		14 Days		21 Days	
			300mg/ kgb.wt	600mg/ kgb.wt	300mg/ kgb.wt	600mg/ kgb.wt	300mg/ kgb.wt	600mg/ kg b.wt
1.	**Liver**	0.138±0.006	0.134±0.005	0.132±0.004	0.130±0.005	0.127±0.003*	0.128±0.003	0.124±0.002*
2.	**Kidney**	0.128±0.009	0.125±0.006	0.123±0.005	0.120±0.009	0.118±0.008*	0.115±0.006*	0.110±0.005*
3.	**Uterus**	0.184±0.009	0.178±0.008	0.175±0.007	0.168±0.009*	0.165±0.008*	0.156±0.007*	0.154±0.006*
4.	**Ovary**	0.519±0.020	0.442±0.021*	0.440±0.020*	0.418±0.021*	0.415±0.019*	0.410±0.011*	0.406±0.009*

Table 4: Wet-Weight of tissues (mg/100mg) in given organs with chronically administration of doses

S. No.	Tested Organs	Control Group	Experimental Groups (Mean ± SE, p Vs respective control < 0.05)					
			7 Days		14 Days		21 Days	
			300mg/ kgb.wt	600mg/ kgb.wt	300mg/ kgb.wt	600mg/ kgb.wt	300mg/ kgb.wt	600mg/ kg b.wt
1.	**Liver**	7.08±0.24	8.04±0.15	9.02±0.18	8.07±0.16	9.05±0.19	8.81±1.13	9.58±1.18*
2.	**Kidney**	1.28±0.05	1.52±0.03	1.58±0.08	1.56 ±0.07	1.62±0.09	1.78±0.10*	1.80±0.11*
3.	**Uterus**	0.45±0.003	0.51±0.008	0.53±0.005	0.58±0.006	0.56±0.006	0.63±0.007*	0.62±0.007*
4.	**Ovary**	0.12±0.001	0.13±0.008	0.15±0.009	0.21±0.012	0.25±0.012	0.38±0.010*	0.40±0.011*

Table 5: Activity of Acid Phosphatase (mg p/100g/hr) in given organs

S. No.	Tested Organs	Control Group	Experimental Groups (Mean ± SE, p Vs respective control < 0.05)					
			7 Days		14 Days		21 Days	
			300mg/ kgb.wt	600mg/ kgb.wt	300mg/ kgb.wt	600mg/ kgb.wt	300mg/ kgb.wt	600mg/ kg b.wt
1.	**Liver**	233±11.6	422±29.5*	502±30.7*	442±30.9*	515±30.9*	480±33.8*	534±42.7*
2.	**Kidney**	345±17.6	367±17.4	415±20.2*	409±18.9*	450±31.5*	421±29.4*	466±28.7*
3.	**Uterus**	139±8.0	230±12.5*	237±12.9*	240±12.5*	248±13.3*	260±13.8*	265±13.9*
4.	**Ovary**	218±12.9	228±12.6	248±17.3	236±13.6	279±17.8*	248±17.3	286±20.1*

Table 6: Activity of Alkaline Phosphatase (mg p/100g/hr) in given organs

S. No.	Tested Organs	Control Group	Experimental Groups (Mean ± SE, p Vs respective control < 0.05)					
			7 Days		14 Days		21 Days	
			300mg/ kgb.wt	600mg/ kgb.wt	300mg/ kgb.wt	600mg/ kgb.wt	300mg/ kgb.wt	600mg/ kg b.wt
1.	**Liver**	70 ± 2.8	74 ± 4.0	75 ± 4.5	76 ± 3.8	78 ± 4.5*	85 ± 4.1*	88 ± 4.7*
2.	**Kidney**	228±1.37	241±1.37	248±1.38	251±1.28*	253±1.29*	261±1.24*	268±1.17*
3.	**Uterus**	347±17.8	365±16.8*	380±19.2*	385±18.8*	398±19.6*	395±20.2*	416±20.8*
4.	**Ovary**	541±26.5	550±27.6	566±27.6	570±26.3*	580±24.5*	590±27.0*	620±28.8*

Authors also revealed that during daily administration of ethanolic extract of *Tridax procumbens*, the content of glycogen were decreased in tested organs of albino rats at both the doses level as compared to their respective control groups (Table 2) but the values were not significant ($p>0.05$). This may be due to protective effect of plant's chemical constituents. The presence of certain phytochemicals like saponin is helpful to maintain the function of certain enzymes. These phytochemicals are known to perform several general and specific functions in plants, and may exhibit different biochemical and pharmacological actions in different species of animals when ingested. These actions range from cell toxicity to cell protective effects (Trease *et al.*, 1996). Glycogen is the primary intracellular storable form of glucose and its levels in various tissues especially hepatic and skeletal muscle are a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthatase and inhibiting glycogen phosphorylase. Hence, glycogen contents remained unaltered due to antidiabetic potential of plant. As already reported that glycogen contents were significantly decreased during diabetes but extract of *Tridax procumbens* recouped the disease and manage the content of glycogen in tissues (Mayura *et al.*, 2008). In diabetes, the glycogen content was markedly depleted (Grover *et al.*, 2002) which are due to inadequate insulin secretion, which results in the inactivation of glycogen synthesis (Sumana *et al*, 2001). Since destruction of beta- cells of islets of langerhans resulting in marked decrease in insulin levels, it is rational that glycogen levels in tissues decreased as they depend on insulin for influx of glucose. Therefore, normal level of glycogen reflects the normalization of insulin levels. Insulin is a potent activator of the enzyme glycogen synthatase while inhibiting the enzyme glycogen phosphorylase responsible for glycogenolysis in liver and muscles.

During daily administration of doses significantly decrease in the content of cholesterol (Table 3) in body tissues because the plant contains some phytochemical such as saponins and β-sitosterol. Saponin are known to reduce the uptake of certain nutrients like cholesterol and glucose, so may help in lessening the metabolic burden or oxidative stress that would have been placed on the liver i.e. saponin are also known to possess hypocholesterolemic effects (Prince *et al.*, 1987). Beta- sitosterol has been isolated from the flowers of *Tridax procumbens* (Saxena *et al.*, 2005) which may also be responsible for the lowering of cholesterol contents. Because daily consumption of the β-sitosterol ester at an amount between 0.2 and about 20gm/day has shown to reduce the absorption of biliary and endogenic cholesterol. Also, plant having a good source of fiber and fiber forms a mucus type of mass that binds the cholesterol in the intestinal tract and promotes its excretion. When the diet lacks sufficient fiber, up to 94% of the cholesterol is reabsorbed and recycled for use in the body. Similar findings were reported by Ikewuchi *et al.* (2010), the total triglyceride, LDL, HDL, VLDL and total cholesterol levels of the *Tridax* treated animals was significantly lowered as compared to that of the test control and control. The significant lowering of mean daily weight gain produced by the *Tridax procumbens* extract, in the test animals implies that it may be useful in the management of hypertension, obesity and dylipidemia (Ikewuchi *et al.*, 2009). Extract of *Tridax procumbens* plant may also, inhibit the oxidation of LDL as well as atherogenic effects of oxidized LDL by virtue of its antioxidant and anti- inflammatory property (Assmann *et al.*, 2004 & Ademugiwa *et al.*, 2005).

Author's present findings revealed that the administration of ethanolic extract of *Tridax procumbens* either at single or in multiple doses slightly increased the wet weight of liver and kidney (Table 4). Wet weight of female reproductive organs like uterus and ovary fluctuates remarkably with reference to the different phases of estrous cycle (Yadav *et al.*, 1980 & Vaish *et al.*, 1981). Increase in the wet weight is associated with increased protein synthesis on one side and entry of electrolytes on another side. Formation of new proteins is usually added to the mass of an organ which causes an increase in the wet weight. If water is imbibed into the cells, their wet weight is increased remarkably (edematous condition). Therefore, it is only the dry weight of tissues which accounts for permanent increase in tissue weight via increased protein synthesis. Physiological or pathological oedema usually increases the wet weight of organs and number of chemicals and plant extracts are known to induce edematous changes causing significant increase in the wet weight.

On the contrary under chronic administration the constant loading of *Tridax procumbens* deteriorated the organs intensively with the result a constant release of acid phosphatase is maintained throughout the period of treatment (Table 5). The significant increase in the activity of acid phosphatase of the liver, may be due to induced enzyme synthesis triggered by certain components of the aqueous extract possibly by metal ions (Pinto *et al.*, 2007). Elevated acid phosphatase activities could result in indiscriminate hydrolysis of

phosphate esters which are potential energy sources for the cell (Butterworth *et al.*, 1968). The administration of plant products in crude or pure form are known to alter the activity of acid phosphatase. Some of them increase the activity but others cause a significant decrease. Increase in the activity therefore, indicates the disintegration of the tissue. In most of the cells the lysosomal enzymes are associated with the cytoplasmic bodies which revealed that the process of autolysis or phagocytosis is mediated through the disintegration of cytoplasmic organelle.

In the present findings author revealed that two different dose concentrations of ethanolic extract of *Tridax procumbens* when administered daily leads to significant increase in the activity of alkaline phosphatase in various tested organs (Table 6). It may due to alcoholic extract of plant, because Maruthappan *et al.* (2009) have also reported that significantly increased in the activity of alkaline phosphatase due to ethyl alcohol treated rats simultaneously with *Azadirachta indica* leaf powder and silymarin; standard drug which significantly suppressed the activity of alkaline phosphatase concentration. Under acute condition it recouped at longer duration due to hepatoprotective property of plant (Vilwanathan *et al.*, 2005). The efficacy of any hepatoprotective drug is essentially dependent on its capacity of either reducing the harmful effects or maintaining the normal physiologic function which has been disturbed by hepatotoxic agents. That is clear manifestation of anti-hepatotoxic effect. The results indicate that the herbal drug *Tridax procumbens* has very good hepatoprotective effect in liver injury. The increase in the activity of alkaline phosphatase may be attributed to the effects of some divalent ions such as Mg^{2+} and Zn^{2+} reported to be present in the leaves of plant (Pinto *et al*, 2007). These ions are known and established activators of alkaline phosphatase (Petitclerc & Fetau, 1977). Similarly, *Tridax procumbens* plant also contains Mg^{2+} ions that may also be responsible for enhancing the activity of alkaline phosphatase.

Conclusion

It is interesting to note that biochemical alterations, as observed in vital organs under the treatment of ethanolic extract of *Tridax procumbens* weed are not severe because administration of many plants extract to laboratory animals has been reported to cause severe metabolic disturbances in the vital organs both at cellular and molecular level. Therefore, it is assumed that there may be a common mechanism which increases the protein contents in all the organs. Probably the administration of *Tridax procumbens* may alter certain key enzymes which are needed for protein synthesis. Also, plant of *Tridax procumbens* are rich in protein concentration due to which new proteins are formed and increased the content of protein in various tested organs. Therefore, present study also supports a possible protective role of the extract against the development of atherosclerosis and coronary heart diseases, as well as the dyslipidemic conditions that characterize *Diabetes mellitus*, hypertension, metabolic syndrome and obesity.

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