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Protective Effect of *Andrographis paniculata* against Thioacetamide Cytotoxicity in Liver and Kidney of Albino Rat

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Abstract: ***The present work has been designed to check hepatoprotective and renoprotective potency of aqueous extract of Andrographis paniculata. The whole plant extract of A. paniculata was used for treatment by using thioacetamide induced toxicity in liver and kidney of albino rat. Thioacetamide induces liver cirrhosis and nephrosclerosis. Thioacetamide induced toxicity in albino rat as manifested by the significant increase in total bilirubin, direct bilirubin, SGPT, SGOT and creatinine while significant decrease in liver protein and kidney protein. These results prove the potential hepatoprotective and renoprotective activity of A. paniculata extract.***

Keywords: A. paniculata, liver, and kidney

1. Introduction

Liver cirrhosis is the damage of cells and their gradual replacement with scar tissue that damages blood flow through the liver producing hepatocyte death and loss of liver function [1].

The liver is an important organ concerned with various states of metabolic and physiologic homeostasis of the body [2]. It is involved in all biochemical pathways to growth, fight against disease and nutrient supply. The carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamins are the major functions of liver. Thus to sustain a healthy liver is an important factor for overall health and well-being [3].

The kidney is an essential organ that plays a dominant role in homeostasis by excreting the metabolic waste products and keeps necessary products depending on the needs of the body [4]. Kidney damage by various toxicants causes morphological with tubular or interstitial changes to nephropathy [5].

Thioacetamide (TAA) a potent hepatotoxin is used to control the decay of citrus fruits especially oranges there it is used as fungicide. TAA acts as a sulfur donor and used for various industrial and pharmaceutical application. It is also a carcinogenic [6] [7] [8] [9]. Thioacetamide induces cirrhosis pathological features of which are similar in many aspects to those of human liver disease caused by alcohol. It is one of numerous agents that cause structural and functional changes not only in liver but also in other tissues such as kidney, thymus, spleen and lungs [10].

In present medicine due to lack of defensive drugs in the treatment of liver diseases, medicinal plants are highly popular [11]. The long times history of using herbs in medicinal practice [12] [13]. In recent decades herbal medicine has received great attention because low costs and greater compatibility [14]. A number of plants have been shown to possess hepatoprotective property by improving antioxidant status [15].

A. paniculata also known commonly as “King of Bitter” is a member of plant family Acanthaceae. It is an herbaceous plant and used in traditional medicine. *A. paniculata* commonly known as Kalmegh is used as a bitter component in 26 Ayurvedic formulations. Modern pharmacological studies have demonstrated its cardioprotective, antithrombolytic, immunostimulant, antihyperglycemic, antimalarial and anti-inflammatory properties and so on [16].

In the present study the medicinal plant *A. paniculata* have been selected for hepatoprotective and renoprotective activity.

2. Material

2.1 Animals

Healthy adult Wistar rats (130 – 150gm) were procured from Hindustan Antibiotic Ltd, Pune and were acclimatized in laboratory condition for two weeks. They were fed with Amrut rat feed obtained from Pranav Agro Industries, Pvt. Ltd, Sangli and water *ad libitum*.

3. Methods

3.1 Preparation of drugs

TAA (Sigma Aldrich, Switzerland) was prepared freshly by dissolving in sterile distilled water and stirred until all crystals were dissolved. The TAA 200 mg / kg body weight was administered intraperitoneally (i.p.) to rats thrice a week for 8 weeks [17].

3.2 Collection of plant material and extraction

Fresh *A. paniculata* plant was obtained from Botanical garden of Krishna Mahavidyalaya Rethare BK. The plant

was authenticated by Dr. C. B. Salunkhe, Department of Botany, Krishna Mahavidyalaya Rethare BK. A voucher specimen (Collection No. 1719) has been kept in our laboratory for future reference.

3.2.1. Preparation of extracts

In a conical flask two hundred (200g) of powdered sample was mixed with 2000 ml of distilled water. The conical flask shake severally covered overnight and stored at room temperature. The mixture was filtered by using Whatman filter paper number 1. The filtrate was evaporated at 40^0C upto complete dryness and forms chocolate coloured powder. This dried filtrate was scrapped, weighed and percentage yield was calculated. The dried filtrate was stored in a capped bottle and fresh solution was prepared at the time of experimentation [18].

3.3 Experimental design

The animals were divided into three groups each containing eight animals.
Group I: Control group of 3 to 5 months rat with either sex receiving intraperitoneal injection of distilled water for eight week.
Group II: Induced group were given i.p. injection of thioacetamide (TAA) 200 mg/Kg body weight three times a week for eight weeks [19].
Group III: Treated group were induced rats which were given *A. paniculata* extract orally at a dose 250 mg/kg doses three times a week for eight weeks.

3.4 Blood sample collection

At the end of experiment the animals were fasted for 12 hrs. weighed and sacrificed by cervical dislocation. Blood sample was directly collected from left ventricle and were allowed to clot at room temperature. After clotting the sample was centrifused and serum is obtained on top of tube. Serum was collected for further experiment.

3.5 Biochemical method

i) Total protein content was estimated by Lowry method [20]
ii) Serum SGOT, SGPT, total and direct bilirubin was estimated by using commercial Kits.
iii) Blood creatinine was estimated by Jaffe's alkaline picrate method [21].

4. Result

The result obtained in the present investigation as shown in table No.1 and 2 and illustrated graphically in the fig.1, 2,3,4. The activities of transaminases SGOT, SGPT, bilirubin and creatinine were significantly elevated in induced group when compared to the control group. On the other hand the increase in these parameters was prevented by treatment of animals with *A. paniculata* 250 mg/kg which resulted in nearly normalized levels of these parameters (table1).

Increased level of SGOT was noticed in thioacetamide induction group (554.63 ± 1.33) as compared to control group (102.13 ± 1.23). In treated group SGOT level was found decreased (227.90 ± 1.15). The SGPT level in control rat was 40.23 ± 0.71, in induction group was 96.47 ± 0.84 and in a treated group was 56.77 ± 0.87 (Fig.1).

The total bilirubin in control, induced and treated rat was 0.3 ± 0.08, 0.8 ± 0.08 and 0.5 ± 0.08 respectively. The direct bilirubin was 0.13 ± 0.05, 0.5 ± 0.08 and 0.17 ± 0.05 mg/dl in control, induced and treated rat respectively (Fig. 2).

Table 1: Effect of aqueous extract of *A. paniculata* on SGOT, SGPT, bilirubin and creatinine

	Control	Induced	Treated
SGOT U/L	102.13±1.23	554.63±1.33	227.90±1.15
SGPT U/L	40.23 ± 0.71	96.47 ± 0.84	56.77 ± 0.87
Bilirubin (Total) mg/dl	0.3 ± 0.08	0.8 ± 0.08	0.5 ± 0.08
Bilirubin(Direct) mg/dl	0.13 ± 0.05	0.5 ± 0.08	0.17 ± 0.05
Creatinine Mg/lit	14.46 ± 0.69	36.38 ± 0.41	13.94 ± 0.81

Each value is the mean of 8 individual determinations ± indicates SD

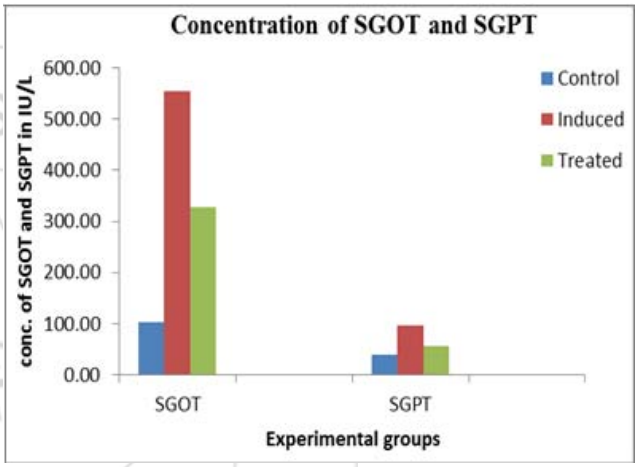

Figure 1: Variations in conc. of SGOT and SGPT of different group

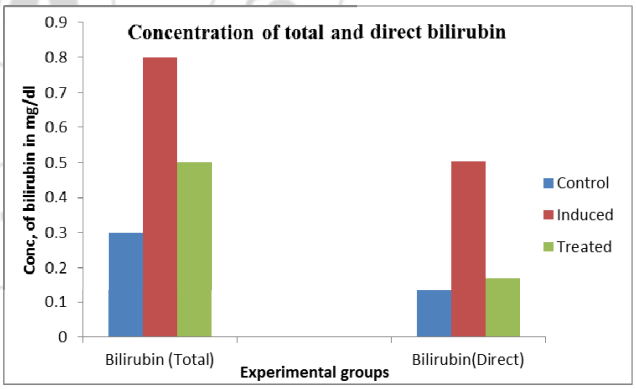

Figure 2: Change in conc. of total and direct bilirubin of different group of rats

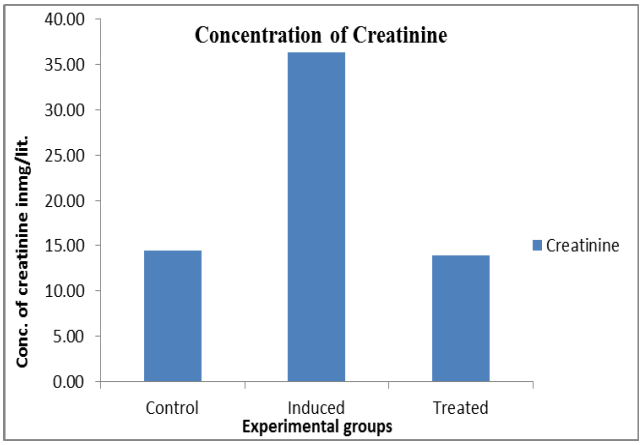

Figure 3: Conc. of creatinine in different group

Table 2: Effect of aqueous extract of *A. paniculata* on Protein in liver and kidney.

	Control	Induced	Treated
Protein (Liver) mg/gm	206.6 ±68.69	117.33±7.54	186.67±43.49
Protein (Kidney) mg/gm	20.67±52.67	125.33±15.08	149.33±19.96

Each value is the mean of 8 individual determinations ± indicates SD

The creatinine in control group is 14.46 ± 0.69 mg/lit. whereas in induced group it is increased to 36.38 ± 0.41 mg/lit. and in a treated group will be 13.94 ± 0.81(Fig. 3).

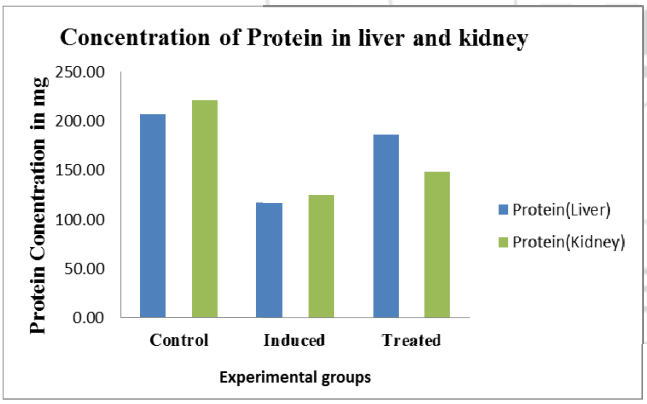

Figure 4: Change in conc. of total proteins in liver and kidney of different groups albino rat.

The total protein conc. is decreased in induced group i.e. in liver and kidney (117.33 ± 7.54 and 125.33 ± 15.08 mg/gm.) as compared to normal rat (206.67 ± 68.69 and 220.67 ± 52.67 mg/gm). The treated group shows increased protein conc. in liver and kidney i.e. 186.67 ± 43.49 and 149.33 ± 19.96 mg/gm as compared to induced group (Fig.4).

5. Discussion

Liver shows many important functions one of these is a detoxification of xenobiotics and toxin [22]. Some industrial and environmental toxicants and other drugs can cause renal damage due to activation of highly reactive free radicals [23]. Thioacetamide is a hepatotoxic and produce hepatic necrosis. On metabolism thioacetamide produce free radicals resulting in oxidative stress and induces apoptasis of hepatocytes [24]. On long term taken thioacetamide causes cirrhosis in rats. Thioacetamide induces centrolobular hepatic necrosis, hepatocellular carcinoma, liver cirrhosis and injury to the terminal portion of the proximal renal tubule [25].

In the present study thioacetamide administered 200 mg/Kg dose i.p. for 8 week resulted in increase in some liver biomarkers. These observations are similar to those of [26] [27] [28] [29] who have used thioacetamide to induce liver cirrhosis. The TAA is a potent hepatotoxin, it is processed by Cytochrome $_{450}$ enzyme in liver and converted into toxic substance TAAS- oxide and TAAS - dioxide by oxidative chain reaction [30]. In chronic condition TAA intoxication, considerable liver fibrosis and prominent regenerative nodule development are associated with portal hypertension and hyperdynamic circulation individuals of liver cirrhosis [31]. This is evidenced by variations in the level of ALT, AST, and Bilirubin in plasma.

The SGOT is a mitochondrial enzyme which is increased in liver damage. Some important liver biomarkers such as SGOT, SGPT, bilirubin, ALP, total proteins etc. which evaluate liver toxicity and its amounts of leak into blood stream indicate severity of hepatic damage [32]. Bilirubin is one of the most useful clinical markers to indicate to the severity of necrosis and its increase is a quantity of binding, conjugation and excretory capacity of hepatocytes. Creatinine, a non-protein waste product is freely filtered by the kidney. The serum creatinine concentration is the most commonly used parameter of the kidney function. The level of creatinine in the blood rises if kidney is not function properly. Current research has focused on alternative approaches to synthetic pharmacology, which rely on natural products. It target on medicinal plants which shows curative effect on diseases of the liver or other organ [33].

Whole parts of *A. paniculata* plant have many benefits by means of liver and kidney diseases such as it decrease in SGPT levels in different liver injury models such as ethanol [34], CCl_4 [35] [36] and acetaminophen induced liver injury [37] [38]. The elevated level of these hepatic marker enzymes such as SGPT, SGPT in the induced group which is reduced in treated group. The creatinine level which was increased in induced group was decreased or becoming nearly equal to the value of control group in a treated group.

The protein concentration in liver and kidney in a induced group is decreased and in a treated group protein concentration is increased [39] because induced group is treated by aqueous extract of *A. paniculata* 250 mg/kg. Collectively, from the above results suggest that *A. paniculata* at the dose of 250 mg/Kg body weight is recovering since it has improved activity on protein levels apart from other beneficial features. It contains andrographolides, alkaloids, flavonoids, phenolic compounds steroids and saponins.

6. Conclusion

The aqueous extracts of *Andrographis paniculata* have shown hepatoprotective and renoprotective effect against TAA induced hepatotoxicity and renotoxicity in rats in reducing serum bilirubin, SGOT, SGPT and creatinine levels. It also found that total proteins in liver and kidney are increased by *A. paniculata.*

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