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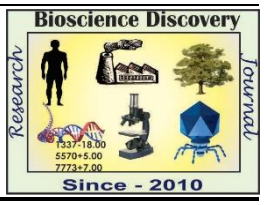

Effect of aqueous extract of *Ecbolium viride* on thioacetamide induced liver cirrhosis in albino Rat

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Abstract

The present experiment has been designed to check hepatoprotective activity of aqueous extract of *Ecbolium viride* in albino rat. The whole plant aqueous extract was used for treatment. The thioacetamide was used for induction of liver cirrhosis. For this work rats were divided into control group, thioacetamide induced group and *E. viride* treated group. The thioacetamide induced toxicity in albino rat was established by the significant increase in total bilirubin, direct bilirubin; SGOT and SGPT while significant decrease in liver protein. The treatment of *E. viride* resulted into remarkable decrease in total bilirubin, direct bilirubin; SGOT and SGPT while significant increase in liver proteins as compared to the thioacetamide induced group indicating its hepatoprotective activity.

INTRODUCTION

Liver disorders such as jaundice, cirrhosis and fatty liver disorders are common and worldwide public problem (Guillouzo *et al.*, 1989). Because of liver disorders there are about 20,000 deaths occur in every year (Akhtar *et al*., 2009). The liver diseases are caused by chemicals, ingestion of toxic foods or infectious agent overdose of drug etc. (Rangnekar and Fontana, 2011; Kiran *et al*., 2012; Sanghvi *et al*., 2013; Jaeschke *et al*., 2013).

The liver cirrhosis related with pathological processes. It shows progressive fibrosis, produce liver injury, portal hypertension and carcinoma (Friedman, 2003). Liver plays important role in metabolism, excretion and in detoxification of xenobiotics. Different toxins absorbed from intestine gain access first to the liver resulting into various diseases (Ramachandra *et al*., 2007). The foreign chemicals such as food additives, drugs and environmental pollutants to which human get exposed in their daily life. The drugs like CCl_4, alcohol, acetaminophen, lead, ibuprofen and contraceptives etc. cause liver damage. These drugs cause chemical changes in the human body. The liver has important role in this process (Akilavalli *et al*., 2011).

The drug thioacetamide is thiono-sulfur containing compound. It is used as a fungicide, organic solvent, stabilizer of motor oil and accelerator in the vulcanization of rubber (Lee *et al*., 2003). The effect of TAA is in bioactivation of oxidase system such as CYP2E1 and Flavin Adenine Dinucleotide (FAD), monooxygenases (Chilakapati *et al*., 2005). Metabolic activation of TAA forms reactive metabolites; it is represented by radicals which are derived from TAA-S-oxide and reactive oxygen species (ROS) formed as intermediates (Chilakapati *et al*., 2007). These reactive metabolites can be bind to cellular macro molecules covalently; it induces oxidative stress (Okuyama *et al*., 2003).

The use of herbs in a pharmacological treatment of disease is started from long years ago (Shulz *et al*., 2001). To provide proactive support of various physiological systems or in medical sense, for treatment, for cure or for prevention of disease in humans or in animals used herbal medicines and for their preparation plants, plant parts, solvent extract, essential oils, their water, gums, exudates, resins etc. are used (Weiss and Fintelmann, 2000). The plants are highly acceptable that serve as in expensive replacements to conventional medicine (Akah and Nwabie, 1994).

E. viride also known as 'Nilambari' is a member of plant family acanthaceae. It is a perennial woody undershrub occurs in plains of India and Arabia, Srilanka and tropical Africa.

Roots of the plants are used for jaundice, menorrhagia and rheumatism (Elumalai *et al*., 2011). In the present study such a medicinal plant, *E. viride* has been selected to investigate its hepatoprotective activity by using albino rat as an experimental animal.

MATERIALS AND METHODS

Animals

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

Preparation of injectable solution of Thioacetamide (TAA):

The injectable solution of TAA (Sigma Aldrich, Switzerland) was prepared freshly by dissolving its powder in sterile distilled water and it is 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 (Alshawsh *et al*., 2011).

Collection of plant material and extraction:

The Fresh *E. viride* 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. KMR 8318) has been kept in our laboratory for future reference. The whole plant was dried at room temperature and was ground to obtain a dry powder.

Preparation of aqueous extract

In a conical flask two hundred (200g) of powdered sample of *E. viride* was mixed with 2000 ml of distilled water. The conical flask was shaked 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 (Nasir *et al*., 2011).

Experimental design

The animals were divided into three groups each containing eight animals.

Group I: Control group included 3 to 5 months rats with either sex receiving intraperitoneal injection (i. p.) of distilled water for eight weeks.

Group II: Induced group included the rats of either sex to which given i.p. injection of thioacetamide (TAA) 200 mg/Kg body weight three times a week for eight weeks (Salama *et al.*, 2013).

Group III: Treated group included induced rats which were given aqueous extract of *E. viride* orally at a dose 250 mg/kg body weight three times a week for eight weeks.

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 was allowed to clot at room temperature. After clotting the sample was centrifused and the serum obtained on top of tube, was collected and stored for further experimentation.

Preparation of Homogenate

The homogenates of liver were prepared for estimation of proteins. Homogenization of the tissue was carried out using refrigerated glass mortar for instantaneous freezing and gradual thawing with chilled distilled water. The perfectly uniform homogenate was centrifuged at 5000 rpm for 10 minutes. The supernatant was used for estimation of protein.

Parameters

i) Total protein content was estimated by Lowry method (Lowary *et al*., 1951).

ii) Serum SGOT, SGPT, total and direct bilirubin was estimated by using commercial Kits (Kumar *et al.*, 2010) (Shanmukha and Setty, 2013).

RESULTS AND DISCUSSION

The results obtained in the present investigation are illustrated graphically in fig.1, 2, and 3. A significant increase in the level of SGOT, SGPT, total bilirubin and direct bilirubin was noticed in animal from an induced group as compared to the animals from the control group. The level of SGOT, SGPT, total bilirubin and direct bilirubin was 554.63 ± 1.33 U/L, 96.47 ± 0.84 U/L, 0.8 ± 0.08 mg/dl and 0.5 ± 0.08 mg/dl respectively as compared to animals from control group where the level of the same was 102.13 ± 1.23 U/L, 40.23 ± 0.71 U/L, 0.3 ± 0.08 mg/dl and 0.13 ± 0.05 mg/dl respectively.

A Significant decrease in the level of SGOT, SGPT, total bilirubin and direct bilirubin was noticed in the animal from the induced group which were treated with aq. extract of *E. viride* where the values were 319.97±4.07 U/L, 60.7± 2.76 U/L, 0.33± 0.05 mg/dl and 0.23± 0.05 mg/dl respectively as compared to induced group where these values were 554.63 ± 1.33 U/L, 96.47 ± 0.84 U/L, 0.8 ± 0.08 U/L and 0.5 ± 0.08 U/L.

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

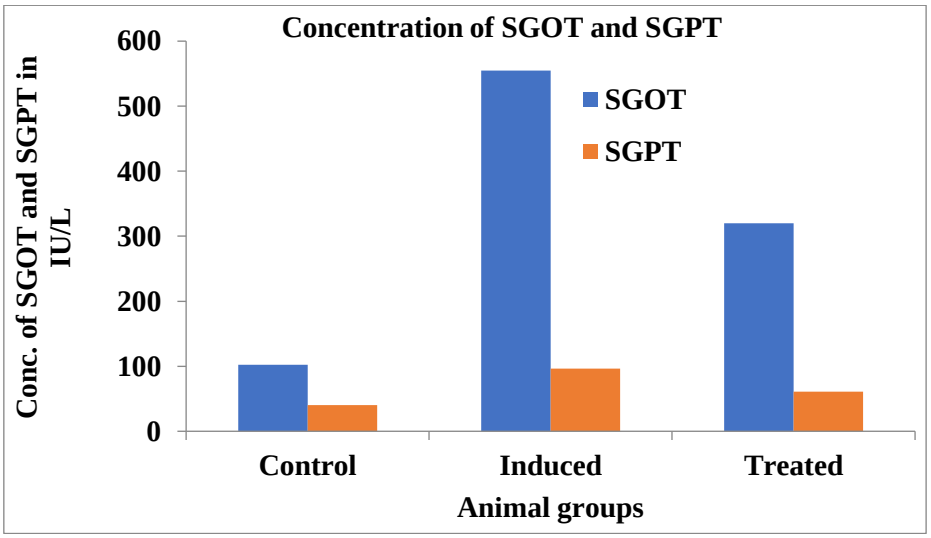
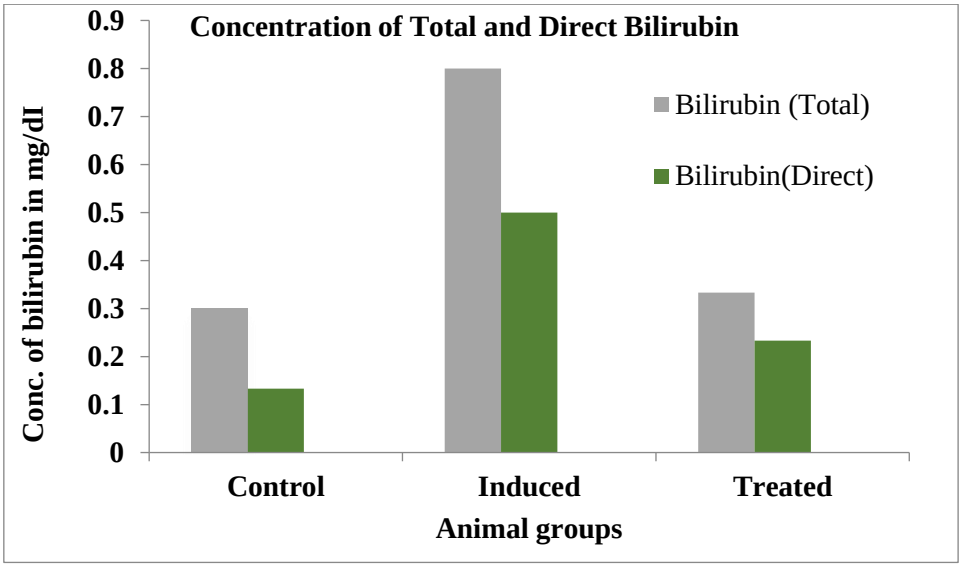
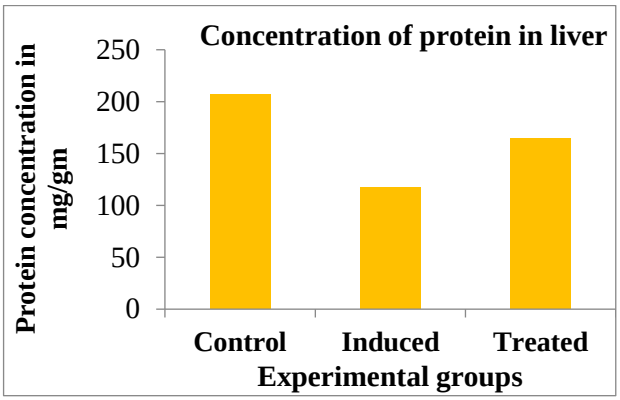


Figure 2: Change in conc. of total and direct bilirubin in rats of different group.



A significant decrease in the concentration of protein was noticed in rat from an induced group as compared to the animals from the control group. The concentration of protein was 117.33±7.4 mg/gm as compared to rats from controlled group were the concentration was 206.66±68.7 mg/gm. A Significant increase in the conc. of protein was noticed in the rats from the induced group which was treated with aq. extract of *E. viride* where the value was 164.33±11.3 mg/gm as compared to the rat from induced group.

Figure 3: Change in Conc. of total proteins in liver in rats of different group



One of the important functions of liver is detoxification of xenobiotics and toxins (Mitra, 1998). The reactive oxygen species are formed in many cases during detoxification (Jeong *et al*., 1999). Liver cirrhosis is a main important disorder, it showes various pathological processes such as progressive fibrosis, portal hypertension and carcinoma (Friedman, 2003). In liver cirrhosis disorder free radical generation, mitochondrial dysfunction, depletion of antioxidant, progression of fibrosis and lastly cirrhosis (Nataranjan *et al*., 2006).

Thioacetamide produces liver cirrhosis in rats and histologically it is similar to human cirrhosis (Li *et al*., 2002). Thioacetamide is a sulfur containing and necrogenic (Landon *et al.*, 1986), carcinogenic (Kizer *et al.*, 1985) and producing fulminant hepatic failure (Bruck *et al.*, 1999).

In the present study typical liver cirrhosis was noticed in rats after administration of thioacetamide (200 mg/kg body weight) intraperitoneally for 8 weeks. These results are identical to the result obtained earlier (Ahmed *et al*., 2000; Kadir *et al*., 2011; Salama *et al.*, 2012; Zahra *et al*., 2012).

The SGOT and SGPT are important indicators of hepatic injury. These enzymes catalyze transfer of alpha- amino group asparate alanine to the alpha ketoglutaric acid. The SGPT is primarily localized to liver but SGOT present in heart, skeletal, kidney, brain and liver tissues. SGOT is present in mitochondria and cytosol of liver cells but SGPT is found only in cytosol. The elevation in level of SGOT and SGPT is a diagnostic clue (Rosen and Keeffe, 1998).

Bilirubin is the accepted indicator of liver diseases (Achliya *et al*., 2004). The TAA damages erythrocyte plasma membrane stability forms labile membranes which break easily. There is a high bilirubin concentration is detected in hepatotoxic rats it means increased erythrocyte degeneration rate. The liver expels collapsed products of Hb such as bilirubin whose level is used to calculate hepatic injury and necrotic condition of hepatocytes (Gressner *et al*., 2007). Liver cirrhosis in the present investigation was evident by increased level of SGOT, SGPT, total and direct bilirubin concentration and decreased level of protein in the liver of rats after the administration of TAA.

The numerous phytochemicals formed from herbs, vegetables and fruits have involved much attention because of their potent and diversified biological functions. Liver marker enzymes are present in the cytosol of hepatocytes and thus are extruded into the serum when cells are injured. The whole plant *E. viride* showes many benefits by means of liver. It increased in SGPT levels in different liver injury models paracetamol (Cheedella *et al*., 2013) (Preethi Priyadharshni, 2011), CCl_4 (Ashoka Babu, 2012) and acetaminophen in liver injury (Malarvizhi, 2012). Increase in level of hepatic enzymes such as SGOT and SGPT was noticed in induced group whereas treated group these enzymes level was found reduced. The total and direct bilirubin was also increased in different liver injury models such as CCl_4 (Akram, 2012), ethanol (Sharma *et al*., 2012). The increased value of bilirubin was decreased in rats treated with plant models such as *Mimosa pudica* (Rajendran *et al*., 2009), *Phyllanthus amarus* (Pramyothin *et al*., 2007), *Moringa oleifera* (Omotoso1., 2015), *Acacia modesta* (Rahaman and Chaudhry, 2015).

In the present study also there was an increase in the level of bilirubin in TAA induced rats induced with TAA whereas in the induced rats those were treated with extract of *E. viride* the bilirubin level was found decreased.

The protein concentration of liver in an induced group is decreased and in treated group protein concentration is increased (Nehar *et al*., 2012) when thioacetamide induced group was treated with *E. viride* 250 mg/kg body weight, same results were obtained *E. viride* might contains flavonoids, phenolic compounds, terpenoids, steroid and alkaloids that might be resulted into change in level of SGOT, SGPT, bilirubin and protein level.

The result obtained in the present study clearly indicated that the aqueous extract of *E. viride* may contain some chemical constituent which had significant hepatoprotective effect.

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