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HEPATOPROTECTIVE AND ANTIOXIDANT EFFECT OF *BALANITES AEGYPTIACA* (L.) DEL AGAINST CCL_4 INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The various parts of *Balanites aegyptiaca* (L.) Del (Balanitaceae) is used in Ayurvedic and other folk medicine for the treatment of various ailments such as syphilis, jaundice, liver and spleen problem, epilepsy and yellow fever. This study was designed to evaluate the hepatoprotective and antioxidant effect of ethanol extract of aerial part of *Balanites aegyptiaca* on CCl_4 induced hepatotoxicity in rats. Activities of liver marker enzymes, SGOT, SGPT and ALP, total protein, albumin, globulin, total, conjugated and unconjugated bilirubins at an oral dose of ethanol extract of *Balanites aegyptiaca* (100 and 200mg/kg) showed a significant hepatoprotective effect. Regarding antioxidant activity, ethanol extract of *Balanites aegyptiaca* exhibited a significant effect showing increasing levels of SOD, CAT, GPX and GRD by reducing malondialdehyde (MDA) levels.

INTRODUCTION: Liver diseases remain as one of the serious health problems. However, we do not have satisfactory liver protective drugs in allopathic medical practice for serious liver disorders. Herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver. A number of plants have been shown to possess hepatoprotective properties by improving the antioxidant status [1]. However, there is still, lack of scientific proofs to authenticate the hepatoprotective properties of some plants which are used traditionally to treat liver disorders.

Balanites aegyptiaca (L.) Del belongs to the family Balanitaceae. It is commonly known as nanjunda. It has been used in a variety of folk medicine in India and Asia. Various parts of the plant are used in Ayurvedic and other folk medicine for the treatment of various ailments such as syphilis, jaundice, liver and spleen problem, epilepsy and yellow fever and the plant also has insecticidal, antihelminthic, antifeedant, molluscicidal and contraceptive activities [2]. In view of the above medicinal properties, the present study was designed to investigate the hepatoprotective activity of ethanol extract of aerial part of *Balanites aegyptiaca* in CCl_4 intoxicated rats.

MATERIALS AND METHODS:

Plant material: The aerial part of *Balanites aegyptiaca* (L.) Del was collected from Vadavalli, Coimbatore, Tamil Nadu and identified by the Botanical Survey of India, Coimbatore. A voucher specimen was retained in Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin for further reference.

Preparation of plant extract for phytochemical Screening and Hepatoprotective Studies: The aerial part of the plant was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder, which was then subjected to successive extraction in a Soxhlet apparatus using ethanol. The extract was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures [3, 4, 5]. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antidiabetic studies.

Animals: Normal healthy male Wistar albino rats (180-240g) were used for the present investigation. Animals were housed under standard environmental conditions at room temperature (25±2°C) and light and dark (12:12h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum.*

Acute Toxicity Studies: Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study [6]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 and 2000 mg/kg body weight.

Experimental Design: In the investigation, a total of 25 rats (20 CCl_4 hepatic toxicity induced rats and 5 normal rats) were taken and divided into five groups of 5 rats each.

Group I: Rats received normal saline was served as a normal control.

Group II: CCl_4 hepatic toxicity induced control: Rats received 2.5ml/kg body weight of CCl_4 for 7 days.

Group III: Liver injured rats received ethanol extract of aerial part of *Balanites aegyptiaca* at the dose of 100mg/kg body weight for 7 days.
Group IV: Liver injured rats received ethanol extract of aerial part of *Balanites aegyptiaca* at the dose of 200mg/kg body weight for 7 days.
Group V: Liver injured rats received standard drug silymarin at the dose of 100mg/kg body weight for 7 days.

Biochemical Analysis: The animals were sacrificed at the end of experimental period of 7 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes. Serum protein [7] and serum albumins was determined quantitatively by colorimetric method using bromocresol green. The total protein minus the albumin gives the globulin. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by using the method of Reitman and Frankel [8]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong [9].

Total bilirubin and conjugated bilirubin were determined as described by Balistrei and Shaw [10]. The unconjugated bilirubin concentrations were calculated as the difference between total and conjugated bilirubin concentrations. Liver homogenates (10%W/V) were prepared in ice cold 10mM tris buffer (pH7.4). Quantitative estimation of MDA formation was done by determining the concentration of thiobarbituric acid reactive substances (TBARS) in 10% liver homogenates by the method of Okhawa [11]. Enzymatic antioxidants, superoxide dismutase (SOD) [12] catalase [13, 14] and non enzymatic antioxidant glutathione peroxidase (GPx) [15] and glutathione reductase (GRD) [16] were also assayed in liver homogenates.

Statistical Analysis: The data were expressed as the mean ± S.E.M. The difference among the means has been analyzed by one-way ANOVA. $p<0.01$ and $p<0.01$ were considered as statistical significance using SPSS Software.

RESULTS: The ethanol extract of aerial part of *Balanites aegyptiaca* subjected for phytochemical study showed the presence of alkaloids, coumarin, glycosides, flavonoids, saponins, steroids, phenols, tannins and xanthoproteins. The ethanol extract did not show any sign and symptoms of toxicity and mortality upto 2000mg/kg dose. The effect of ethanol extract of *Balanites aegyptiaca* on serum total protein, albumin, globulin, A/G ratio, serum transaminases, alkaline phosphatases in CCl_4 intoxicated rats are summarized in **Table 1**.

There was a significant ($p< 0.01$) increase in serum GOT, GPT and ALP levels in CCl_4 intoxicated group (Group II) compared to the normal control group (Group I). The total protein and albumin levels were significantly ($p< 0.01$) decreased to 4.31 g/dl and 2.61g/dl in CCl_4 intoxicated rats from the levels of 9.38g/dl and 4.98g/dl respectively in normal group. Ethanol extract of *Balanites aegyptiaca* at the dose of 100 and 200mg/Kg orally significantly decreased the elevated serum marker enzymes and reversed the altered total protein and albumin to almost normal level.

The effect of ethanol extract of *Balanites aegyptiaca* on total, conjugated and unconjugated bilirubin is shown in **Table 2**. A significant elevation of total, conjugated and unconjugated bilirubin in the serum of CCl_4 intoxicated group (Group II) when compared to normal control (Group I). The ethanol extract of *Balanites aegyptiaca* at the dose 100 and 200mg/Kg reduced the levels of total, conjugated and unconjugated bilirubin (Group III and Group IV). The decreases in the concentration of total bilirubin, conjugated bilirubin and unconjugated bilirubin were found to be greater in standard silymarin (Group V) followed by Group IV and Group III (Table 2).

TABLE: 1 EFFECT OF ETHANOL EXTRACT OF *BALANITES AEGYPTIACA* ON THE PROTEIN, ALBUMIN, GLOBULIN CONCENTRATION AND ENZYME ACTIVITY OF SERUM GOT, GPT AND ALP IN THE NORMAL, LIVER INJURED AND DRUG TREATED RATS

Groups	Parameters						
	T .Protein (mg/Dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
I	9.58± 0.2	4.98 ± 0.04	4.40± 0.02	1.1:1	54.13 ± 1.6	39.51±2.4	173.6±2.1
II	4.31± 0.6**	2.61± 0.02**	2.01± 0.01*	1.2:1	151.61 ± 5.35*	103..42±4.9**	269.3±4.0**
III	8.56± 0.2[a]	4.91± 0.03[a]	2.65± 0.01	1.8:1	59.32±3.15	41.31±1.3	178.51±4.11[a]
IV	9.11± 0.3[a]	4.86± 0.03[a]	3.25± 0.03	1.5:1	51.11±2.32[a]	33.56±1.05[a]	171.32±3.34[a]
V	7.99± 0.1[a]	4.16± 0.03[a]	3.83± 0.03[a]	1.0:1	46.23±1.98[a]	36.51±1.68[a]	161.14±2.89[a]

Each Value is SEM ± 5 individual observations * P < 0.05; ** P<0.01 Compared normal control vs liver injured rats; [a] P < 0.05; aa P<0.01 Compared liver injured rats vs. drug treated

TABLE: 2 EFFECT OF ETHANOL EXTRACT OF *BALANITES AEGYPTIACA* ON THE SERUM TOTAL, CONJUGATED AND UNCONJUGATED BILIRUBIN LEVELS IN THE NORMAL, LIVER INJURED AND DRUG TREATED RATS

Group	Total Bilirubin (µmol/L)	Conjugated (µmol/L)	Unconjugated (µmol/L)
I	0.89±0.01	0.23±0.02	0.66±0.01
II	3.98±0.04**	1.35±0.03*	2.63±0.06**
III	1.12±0.01	0.22±0.03	0.90±0.04
IV	0.93±0.02[a]	0.20±0.02[a]	0.73±0.02[a]
V	0.83±0.01[a]	0.23±0.01[a]	0.60±0.3[aa]

Each Value is SEM ± 5 individual observations * P < 0.05; ** P<0.01 Compared normal control vs. liver injured rats; [a] P < 0.05; aa P<0.01 Compared liver injured rats vs. drug treated

The effects of ethanol extract of *Balanites aegyptiaca* on lipid peroxidation (LPO), Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and Catalase (CAT) activity is shown in **Table 3**. Lipid peroxidation level was significantly (p<0.01) increased and glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase activity were significantly (p< 0.01) decreased in CCl_4 intoxicated rats when compared with those of the animals in normal control group. Rats treated with ethanol extract of *Balanites aegyptiaca* at the doses of 100 and 200 mg/kg significantly decreased the elevated lipid peroxidation levels and restored the altered glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase levels towards the normal levels in a dose dependent manner. The results are well comparable with silymarin (standard drug) treated group.

TABLE 3: EFFECTS OF ETHANOL EXTRACTS OF *BALANITES AEGYPTIACA* ON LIVER LPO, GPX, GRD, SOD AND CAT IN THE NORMAL CONTROL, LIVER INJURED AND DRUG TREATED RATS

Groups	LPO (n mole of MDA/mg protien)	GPX (µ/mg Protein)	GRD (µ/mg)	SOD (µ/mg)	CAT (µ/mg)
I	0.83±0.05	9.53±0.15	8.62±0.03	7.31±0.05	9.21±2.14
II	4.99±0.02**	2.94±0.21*	3.11±0.05*	2.96±0.04*	2.93±1.98*
III	0.93±0.01	8.91±0.18	7.32±0.03	6.34±0.01	7.46±3.96
IV	0.76±0.02[a]	8.89±0.15[aa]	8.09±0.02[a]	7.82±0.03[a]	8.24±2.59[a]
V	0.88±0.04[a]	8.99±0.13[a]	8.13±0.01[a]	7.98±0.01[a]	8.61±3.11[a]

Each Value is SEM ± 5 individual observations * P < 0.05; ** P<0.01 Compared normal control vs. liver injured rats [a] P < 0.05; aa P<0.01 Compared liver injured rats vs. drug treated

DISCUSSION: Liver diseases, especially viral hepatitis occurs predominantly in the developing world, an enormous impact on public health and economy. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play an important role in the management of various liver disorders. A number of plants show hepatoprotective activity [17]. Based on the promising results shown by the plant extracts in the *in vitro* studies, *in vivo* hepatoprotective studies were carried out in experimental rats using CCl_4 induced hepatotoxicity. CCl_4 is one of the most commonly used hepatotoxin. CCl_4 produces an experimental damage that histological resembles viral hepatitis.

Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures [18]. The toxic metabolite, CCl_3 radical, is produced and further reacts with oxygen to give trichloromethyl peroxy radical. Cytochrome P_{450} is the enzyme responsible for this conversion. Both the radicals can bind covalently to the macromolecules and induce peroxidative degradation of the membrane lipid of endoplasmic reticulum rich in polyunsaturated fatty acids [18]. This leads to the formation of lipid peroxidases followed by pathological changes such as depression of protein synthesis, elevation levels of serum marker enzymes such as SGOT, SGPT and ALP, depletion of GPX, GRD, SOD and CAT and increase in lipid peroxidation.

In the present study, it was obtained that, the rats treated with CCl_4 resulted in significant hepatic damage as shown by the elevated levels of serum markers. These changes in the marker levels will reflect in hepatic structural integrity. The rise in the SGOT is usually accompanied by an elevation in the levels of SGPT, which play a vital role in the conversion of amino acids to keto acids [19]. Ethanol extract of *Balanites aegyptiaca* at the doses of 100mg/kg and 200mg/kg significantly attenuated the elevated levels of the serum markers. The normalization of serum markers by ethanol extract of *Balanites aegyptiaca* suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against CCl_4 induced leakage of marker enzymes into the circulation. The above changes can be considered as an expression of the functional improvement of hepatocytes.

The alkaline phosphatase is the prototype of these enzymes that reflects the pathological alteration in biliary flow [20]. The CCl_4 induced elevation of this enzymatic acivity in the serum is in line with high level of serum bilirubins content. The ethanol extract of *Balanites aegyptiaca* induced suppression of the increased ALP activity with the concurrent depletion of raised bilirubins suggests the possibility of the extract to have ability to stabilize biliary dysfunction in rat liver during hepatic injury by CCl_4. Thus administration of ethanol extract of aerial part of *Balanites aegyptiaca* is against the toxic effect of CCl_4.

Lipid peroxidation has been postulated to the destructive process of liver injury due to CCl_4 administration. In the present study, the elevations in the levels of end products of lipid peroxidation in the liver of rat treated with CCl_4 were observed. The increase in malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatment with ethanol extract of *Balanites aegyptiaca* significantly reversed these changes. Hence, it may be possible that the mechanism of hepatoprotection by ethanol extraction of *Balanites aegyptiaca* is due to its antioxidant effect.

In the present investigations, CCl_4 intoxicated rats decreased the content of GPX and GRD in liver, whereas, treatment with ethanol extract of *Balanites aegyptiaca* (100 and 200mg/kg) able to reverse such effects. Superoxide dismutase

(SOD) is a key defense enzyme and catalyses the dismutation of superoxide anions. Catalase (CAT) is a haemeprotein that catalyses the reduction of H_2O_2 and able to prevent the tissue from reactive free oxygen and hydroxyl radicals. Decrease in SOD activity can result in the removal of superoxide anions that may inactivate SOD thereby causing an inactivation of H_2O_2 scavenging enzymes. Administration of ethanol extract of *Balanites aegyptiaca* treated rats able to prevent effectively the decrease in SOD and CAT activities, which may be directly correlated to scavenging or neutralizing of radicals by ethanol extract of *Balanites aegyptiaca* resulting in protection of these important defense enzymes.

The preliminary phytochemical analysis of the extracts has shown the presence of flavonoids and phenolics compounds which have been known for its antioxidant and hepatoprotective studies. It is suggested that, saponins in ethanol extract of *Balanites aegyptiaca* play an important role as antioxidant for prevention of oxidative hepatic damage. Furthermore, the flavonoids and saponins of ethanol extract of *Balanites aegyptiaca* may able to stabilize reactive oxygen species by reacting with them and oxidizes subsequently to more stable and less reactive radicals. Our findings support the reported therapeutic use of this herb in tribal medicine for liver ailments and jaundice. Further investigation is in progress to determine the exact phytoconstituent(s) responsible for hepatoprotective effect.

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