Journal of Advanced Zoology
ISSN: 0253-7214
Volume ***45*** *Issue* ***1 Year 2024*** *Page* ***903:913***

The Effects Of Hydro- Ethanolic Extract Of *Euphorbia Hirta* Leaves Extract On Hemato-Biochemical Changes In Cigarette Smoke-Exposed Rats

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Abstract

Cigarette smoke is a source of free radicals that cause health problems throughout the world. The study aimed to determine the effects of hydro- ethanolic extract of *Euphorbia hirta* leaves extract (EHLE) on hemato-biochemical changes in cigarette smoke exposed (CSE) rats. The study using 24 male Wistar rats consisted of Control (not exposed to cigarette smoke and given distilled water), group II Diseased group (cigarette smoke and given distilled water), group III treated group (Cigarette smoke and *Euphorbia hirta* leaves extract 200 mg/kg BW) and group IV plant alone (*Euphorbia hirta* leaves extract 200 mg/kg BW alone). Exposure to cigarette smoke and administration of extracts was carried out for 12 weeks. End of the experimental period, the rats were euthanized and dissected to collect the blood for hemato-biochemical studies. The results of the study showed that treatment with *Euphorbia hirta* leaves extract to CSE rats restored the hematological parameters, liver and kidney markers.

Keywords: Euphorbia hirta leaves extract, cigarette smoke exposed, hematological parameters, liver and kidney

INTRODUCTION

The smoking epidemic remains to be one of the biggest public health issues the world has ever faced (Goel *et al.,* 2017). Currently, the number of smokers reaches 1.2 billion people globally and most of them live in countries with low- and middle-income. Cigarette consumption has a serious impact on the increasing burden of smoking-related diseases and deaths (Kristina *et al.,* 2016). WHO estimates that direct tobacco use results in more than six million deaths while the non-smokers being exposed to second-hand smoke causes around 890.000 deaths (Messner and Bernhard, 2014). Disease burden attributed to cigarette smoke either mainstream or secondhand smoke (SHS) exposure is substantial, leading to 7 million deaths/annum and 6.3% of total disability adjusted life-years (WHO 2017), while these deaths are expected to exceed up to 10 million/annum by year 2030 (Singh and Kathiresan 2015). Cigarette smoke (CS) is a highly complex mixture

of more than 5000 chemicals including at least 250 toxic chemicals, 69 carcinogens (Vermehren *et al.*, 2020) and mutagenic ingredients, including nicotine, ammonia, acrolein, phenols, acetaldehyde, polycyclic aromatic hydrocarbons, polyphenols, carbon monoxide, nitrogen oxides, hydrogen cyanide, and trace metals. It has a broad spectrum of free radicals and non-radical oxidants (Kamceva *et al.,* 2016) Each CS puff contains 10^{15} free radicals that cause damage to macro- and micro-molecules of the body systems particularly the brain, lungs, liver, and kidneys. The major cigarette smoke free radicals are nitric oxide (NO^{-}) and O^{-2} that combine with each other for the formation of peroxynitrite (Hasan *et al.,* 2020).

Phytochemicals are the major group of naturally occurring secondary metabolites that exist in the plant kingdom. They are abundantly available in the various plant parts including fruits, flowers, and leaf of herbs and terrestrial plants. More than 8000 phenolic compounds of diverse structural arrangements have been reported from the plant kingdom (Tresserra-Rimbau *et al.,* 2018). These phytoconstituents are often found in the plants as a conjugate with one or more sugar moiety and are termed as glycosides. Chemically, they contain one or more phenolic rings with multiple hydroxyl groups on aromatic rings comprising a large number of substitution and structural diversity (Durazzo *et al.,* 2019). Because of the presence of multiple hydroxyl groups, most of these classes of compounds exhibit strong antioxidants and are well known as free radical scavengers. Polyphenols also exhibit wide ranges of biological activities, such as antioxidant, hepatoprotective, nephroprotective, antibacterial, anticancer, antidiabetic, antihypertensive, etc., depending on their structural features (Tsao *et al.,* 2010). Therefore, the present study was conducted to evaluate the effects of hydro- ethanolic extract of *Euphorbia hirta* leaves extract on hemato-biochemical changes in cigarette smoke-exposed rats.

MATERIALS AND METHODS

Animals

Healthy male Swiss albino mice weighing about 30–35 gm were used throughout the study. The healthy animals were purchased from the Venkateswar Enterprises, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use. The experiment was carried out according to the guidelines of the Committee (Ethical No: XXV/VELS/PCO/L/14/2000/CPCSEA/IAEC/09.10.2021) for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Experimental design

Body weights of the animals were recorded and they were divided into 4 groups of 6 animals each as follows. Group I: Normal control rats fed with standard diet and served as a control, which received air. Group II: Rats were exposed to cigarette smoke for the period of 12 weeks (Ramesh *et al*., 2015). Group III: Rats were exposed to cigarette smoke and simultaneously administered plant extract (200 mg/kg body weight per day by oral gavage) for a period of 12 weeks. Group IV: Rats were administered plant extract alone (200mg/kg body weight per day by oral gavage) for a period of 12 weeks. Group II and Group III rats were exposed to cigarette smoke by the method of Ramesh *et al*. (2015) as follows. The rats were placed in a polypropylene cage with a lid made of polythene paper. A lighted cigarette was fitted in a dropper and connected to the cage. A 5.9-cm length of burned cigarette smoke was allowed to by compressing and releasing the dropper when it was placed on a polypropylene cage. Cigarette smoke was exposed thrice daily (from 11 AM to 5 PM), the duration of each exposure was 3 h with an interval of 10 min between each cigarette using 6–8 cigarettes per day/cage for a period of 12 weeks. The same brand of locally available cigarette was used throughout the experiment (Scissors Standard). Control rats were treated similarly, except they were exposed to air instead of smoke.

Collection of samples

At the end of the days, overnight-fasted rats were sacrificed by cervical dislocation and blood was removed to obtain plasma and serum for analysis of various biochemical parameters, blood samples were used for the analysis of hematological parameters.

Hematological and Biochemical parameters
Hemoglobin was estimated by CyanmetHemoglobin method (Dacie and Lewis, 1968) (Beacon Diagnostic Kit). RBC and WBC counted, PCV followed by the method of Ochei and Kolhatkar, (2000).

The Mean Corpuscular Hemoglobin (MCH)
This indicates the weight of Hemoglobin in a single red blood cell and is expressed in picogram (pg).

$$MCH = \frac{\text{Hemoglobin (g/100ml)}}{\text{RCB count million per cu. mm}} \times 10$$

Values were expressed in pictogram (pg)/ cell.

Mean Corpuscular Hemoglobin concentration (MCHC)
This denotes the Hemoglobin concentration per 100 ml of packed red blood cells and is related to the colour of the red cells. This is expressed as percentage of packed cells.

$$MCHC = \frac{\text{Hemoglobin (g/dl)}}{\text{PCV\%}} \times 100$$

MCH values were expressed in %

The Mean Corpuscular Volume (MCV)
Mean corpuscular volume test measures the average size of the fish red blood cells. This is expressed as the volume in cubic microns or femtoliters of an average red blood cell.

$$MCH = \frac{\text{PCV\%}}{\text{RCB count million per cu. mm}} \times 10$$

MCV values are expressed in femtoliters (fL)

Protein was estimated by the method of Lowry *et al.,* (1951). Albumin was estimated by the method of Rodkey (1965). The serum total bilirubin was estimated by the method of Malloy and Evenlyn (1937*)* The serum GOT and GPT were estimated by the method of Reitman and Frankel (1957). The serum alkaline phosphatase activity was estimated by the method of Kind and King's (1954). Urea was estimated by the method of Natelson (1957). Serum creatinine was carried out by alkaline picrate method of Boneses and Taussy (1954). Serum sodium and potassium were estimated by colorimetric method of Maruna and Trinders (1958).

Statistical Analysis
The results were analyzed by SPSS Software ver. 25. Values are expressed as Mean ± SD for six rats. Mean values within the row followed by different letters (Superscript) are statistically significant ($P<0.05$) from each other, and the same letter is non-significant ($P>0.05$). The analysis was an ANOVA followed by post-hoc Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Evidence are increasing with passage of time that CS exposure increases the production of free radicals in the body leading to initiation of several pathological conditions such as depletion of antioxidant reserves and hence increase in oxidative stress and inflammation cascade. The current experimental trail was designed to investigate the effect of *Euphorbia hirta* leaves extract on hemato-biochemical changes against the oxidative stress induced by one of the most common and deadly environmental pollutants "cigarette smoke" exposure in rats. Haematological and biochemical profiles of blood can provide important information about the internal environment of the organism (Li *et al.,* 2011).
The blood is a vital fluid, which contains the Red Blood Cell (RBC), White blood cells (WBC) and platelets suspended in the serum in homeostatic concentrations. The Blood is important for pulmonary and tissue respiration, as a medium of endocrine and neurohumoral transmissions, biotransformation and metabolic excretion (Adebayo *et al.,* 2005), nutritional and immunological processes, as well as homeostatic responses.

The laboratory determination of blood products and parameters for the purpose of disease diagnosis is highly accurate, sensitive and reliable; and remained the bed-rock of ethical and rational research, disease diagnosis; prevention and treatment (Nwaogwugwu *et. al.*, 2020).

Hematological Parameters are extensively used tools that help in monitoring animal health, reproductive status, and disease status and in differentiation of physiological processes. Hematological parameters illustrated in Table 1 showed that, there were a significant raise ($P<0.01$) of white blood cell (WBC), MCV and MCH while decreased red blood cell (RBC), hemoglobin (HBG), PCV and no significant change of MCHC in cigarette smoke exposed rats (Group II) compared to the control (Group I). In the other hand, treatment by *Euphorbia hirta* leaves extract was significantly ($P<0.05$) restored (Group III) hematological profile as compared with cigarette smoke exposed group. There is no significant changes were observed in *Euphorbia hirta* leaves extract treated alone as compared to group I (Figure 1).

Decreased in hemoglobin concentration is believed to be mediated by exposure of carbon monoxide and some scientists suggested that decrease in hemoglobin level in blood of CSE rats could be a compensatory mechanism. Carbon monoxide binds to Hb to form carboxy hemoglobin, an inactive form of hemoglobin having no oxygen carrying capacity. Carboxyhemoglobin also shifts the Hb dissociation curve in the left side, resulting in a reduction in ability of Hb to deliver oxygen to the tissue. To compensate the decreased oxygen delivering capacity, CSE rats maintain a low hemoglobin level than control rats (Verma and Patel, 2015). WBC count is perhaps the most useful, inexpensive and simple biomarker for endothelial damage. We found that smokers had significantly higher WBC count compared to control rats. The mechanism for smoking-induced increase in WBC count is not clear. It has been suggested that inflammatory stimulation of the bronchial tract induces an increase in inflammatory markers in the blood but it has also been suggested that nicotine may induce an increase in blood lymphocyte counts (Bain *et al.*, 1992). Decreased levels of hemoglobin are correlated with decreased numbers or sizes of RBCs. RBC values were significantly low in CSE exposed rats than those of control rat and are consistent with other investigation (Shakhanbeh, 2016).

MCV, MCH and MCHC are three main red blood cell indices that help in measuring the average size and hemoglobin composition of the red blood cells. We found increase in MCV, MCH and MCHC levels in CSE rats as compared to control rats. MCV indicates the size of a red blood cell and presence of red cells smaller or larger than normal size means anemia. The altered hematological parameters in CSE rats due to toxic effect of smoke chemicals results from disturbance in the hemoglobin synthesis and formation. On treatment with *Euphorbia hirta* leaves extract to CSE rats restored the hematological parameters. The improvement in hematological parameters caused by treatment with fenugreek may be due to the antioxidant activity of flavonoids present in *Euphorbia hirta* leaves, thereby elevating the antioxidant capacity of the blood (Sunil Kumar *et al.*, 2010).

Table 1: Effects of hydro- ethanolic extract of *Euphorbia hirta* leaves extract on Hematological profile in experimental rats

Parameters	Group I (Normal)	Group II (Negative)	Group III (Neg.+ PE)	Group IV (PE alone)
Hb (gm/dl)	13.97 ± 0.49^{a}	10.91 ± 0.45^{b}	13.89 ± 0.42^{a}	14.02 ± 0.43^{a}
RBC ($\times10^{6}$ /mm^{3})	4.52 ± 0.11^{a}	3.04 ± 0.21^{b}	4.50 ± 0.19^{a}	4.55 ± 0.11^{a}
WBC ($\times10^{3}$ /mm^{3})	7.30 ± 0.16^{a}	11.38 ± 0.20^{b}	7.38 ± 0.16^{a}	7.32 ± 0.19^{a}
PCV (%)	26.79 ± 0.94^{a}	20.01 ± 0.90^{b}	26.22 ± 1.06^{a}	26.91 ± 0.56^{a}
MCV (famato litre)	59.24 ± 1.87^{a}	66.03 ± 2.63^{b}	58.26 ± 3.41^{a}	59.10 ± 1.75^{a}
MCH (pico gram)	30.91 ± 1.19^{a}	36.04 ± 3.61^{b}	30.88 ± 1.67^{a}	30.82 ± 1.57^{a}
MCHC (%)	52.19 ± 1.80^{a}	54.58 ± 2.73^{a}	53.04 ± 1.90^{a}	52.15 ± 2.20^{a}

Values are expressed as Mean ± SD for six rats. Mean values within the row followed by different letters (Superscript) are statistically significant ($P<0.05$) from each other, and the same letter is non-significant ($P>0.05$). The analysis was an ANOVA followed by post-hoc Duncan's multiple range test (DMRT).

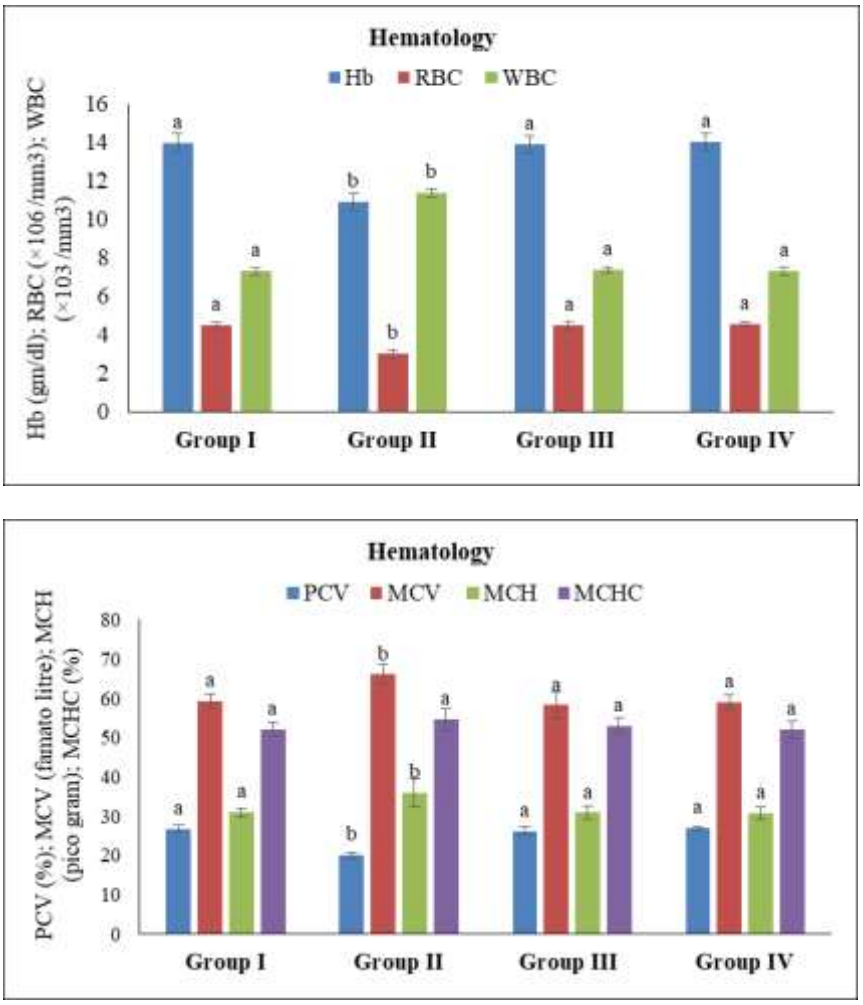


Figure 1: Effects of hydro- ethanolic extract of *Euphorbia hirta* leaves extract on Hematological profile in experimental rats

Liver and kidney are important organs of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites, and are especially vulnerable to damage. As the liver is an important target organ of detoxification mechanism and the kidney is an important organ to remove the waste (Epstein, 1997; Bunout, 1999). In the present study to assessed liver and kidney function. CS was determined as a major risk factor for diseases of many organs including liver, and kidney. The current study shows that oxidative stress induced by CS exposure adversely affected the functioning of liver and kidney and hence, caused leakage of hepatic enzymes from liver cytosol to blood stream and increased urea and creatinine in blood. These outcomes also reveal that *Euphorbia hirta* leaves extract is more potent in ameliorating oxidative stress induced adverse effects on liver and kidney functioning.

Regarding the liver damage caused by CS exposure, CS caused rise in serum ALP, AST, ALT, and total bilirubin concentration and decreased protein content by propagating the lipid peroxidation which damages the hepatocytic cell membrane (Alsalhen and Abdalsalam 2014). This lipid peroxidation might occur due to the nitrosative and oxidative stress induction by CS and its components which leads to alteration in hepatic protein structures and their functionality. Our results obtained concerning the biochemical liver markers (Table 2 and Figure 2) indicated that there were a significant increase ($P<0.05$) of ALT, AST, ALP activities, and bilirubin content while decreased the protein content (Total protein, albumin and globulin) in cigarette smoke exposed rats (Group II) compared to the control (Group I). Comparison with cigarette smoke exposed group II, results revealed that *Euphorbia hirta* leaves extract treatment decline significantly $P<0.05$) in ALT, AST, ALP activities, bilirubin content and improve the decreased the protein content. There is no significant changes were observed in *Euphorbia hirta* leaves extract treated alone as compared to group I (Figure 2). Treatment with *Euphorbia hirta* leaves extract significantly restored the CS induced hepatotoxicity.

Albumin and globulin are mixtures of protein molecules that are used to assess the health status of the liver. Albumin, which is manufactured in the liver, is a major carrier protein that circulates in the bloodstream while globulins are larger proteins responsible for immunologic responses (Tietz, 1986). Low serum albumin

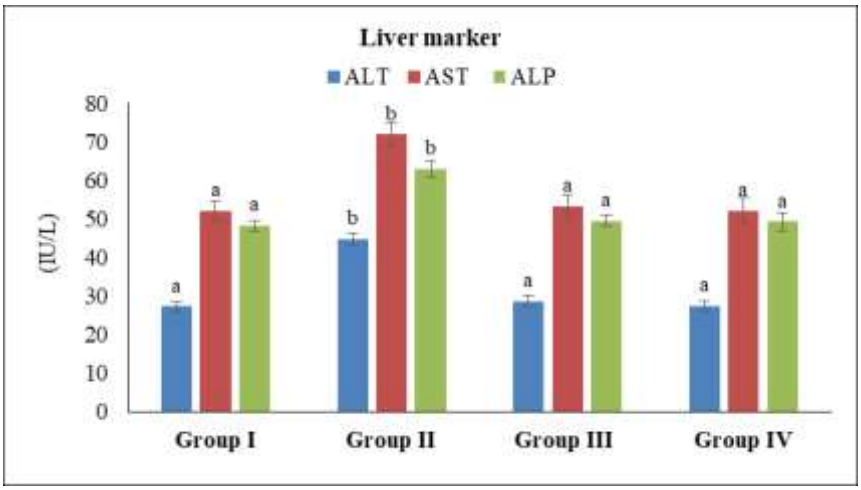
and globulin concentrations suggest chronic damage to the liver as a result of infection (Naganna, 1999). Therefore, the reduction in serum albumin and globulin levels in CSE rats is an indication of diminished synthetic function of the liver. Oral administration of *Euphorbia hirta* leaves extract, however, restored the albumin and globulin levels to normalcy.

Bilirubin is the major product that results from the breakdown and destruction of old red blood cells. It is an important metabolic breakdown product of blood with biological and diagnostic values (Tietz, 1986). It is removed from the body by the liver; hence, it is a good indication of the health status of the liver. Elevated serum level of bilirubin in CSE rats as observed in the present study may be a result of reduced uptake arising from liver disease. Treatment with *Euphorbia hirta* leaves extract was able to reverse this condition in diabetic rats, thereby lowering the bilirubin level to normalcy. All the data obtained with respect to liver function indices indicated absence of any significant liver damage as a result of treatment with *Euphorbia hirta* leaves extract alone in rats. The present study agreement with Prashant Tiwari *et al.*, (2011) who reported that Antihepatotoxic Activity of *Euphorbia hirta* and by using the combination of *Euphorbia hirta* and *Boerhaavia diffusa* extracts on some experimental models of liver injury in rats. Present results are also consistent with the outcomes of Dubey and Mehta (2014) who prepared and tested the ethanolic extract of *Euphorbia hirta* for its hepatoprotective effect against CCl_4- induced hepatitis in rats. Alteration in the levels of biochemical markers of hepatic damage like SGPT, SGOT, ALP, bilirubin, were tested in both treated and untreated groups. Carbontetra chloride (2 ml/kg) has enhanced the SGPT, SGOT, ALP, bilirubin. Treatment with Ethanolic extract of *Euphorbia hirta* Linn (100 mg/kg and 300 mg/kg) has brought back the altered levels of biochemical markers to the near normal levels in the dose dependent manner.

Table 2: Effects of hydro- ethanolic extract of *Euphorbia hirta* leaves extract on Liver markers in experimental rats

Parameters	Group I (Normal)	Group II (Negative)	Group III (Neg.+ PE)	Group IV (PE alone)
ALT (IU/L)	27.29 ± 1.23^a	44.63 ± 1.64^b	28.72 ± 1.36^a	27.37 ± 1.24^a
AST (IU/L)	52.09 ± 2.54^a	71.93 ± 3.04^b	53.18 ± 3.03^a	52.14 ± 3.18^a
ALP (IU/L)	48.30 ± 1.33^a	63.06 ± 2.19^b	49.47 ± 1.50^a	49.31 ± 2.26^a
Protein (mg/dl)	7.41 ± 0.18^a	5.11 ± 0.16^b	7.36 ± 0.16^a	7.42 ± 0.17^a
Albumin (mg/dl)	3.48 ± 0.12^a	2.34 ± 0.08^b	3.43 ± 0.10^a	3.46 ± 0.12^a
Globulin (mg/dl)	3.92 ± 0.22^a	2.76 ± 0.19^b	3.93 ± 0.20^a	3.95 ± 0.16^a
Bilirubin (mg/dl)	0.711 ± 0.04^a	0.998 ± 0.03^b	0.730 ± 0.04^a	0.721 ± 0.03^a

Values are expressed as Mean ± SD for six rats. Mean values within the row followed by different letters (Superscript) are statistically significant ($P<0.05$) from each other, and the same letter is non-significant ($P>0.05$). The analysis was an ANOVA followed by post-hoc Duncan's multiple range test (DMRT).



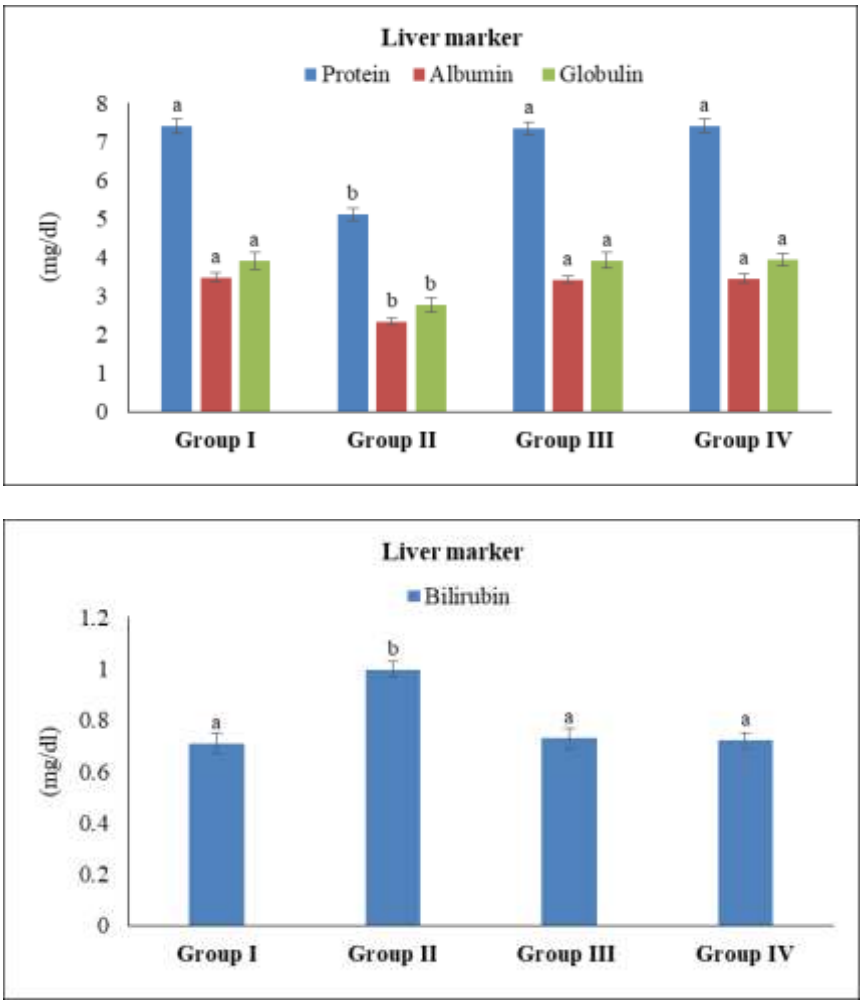


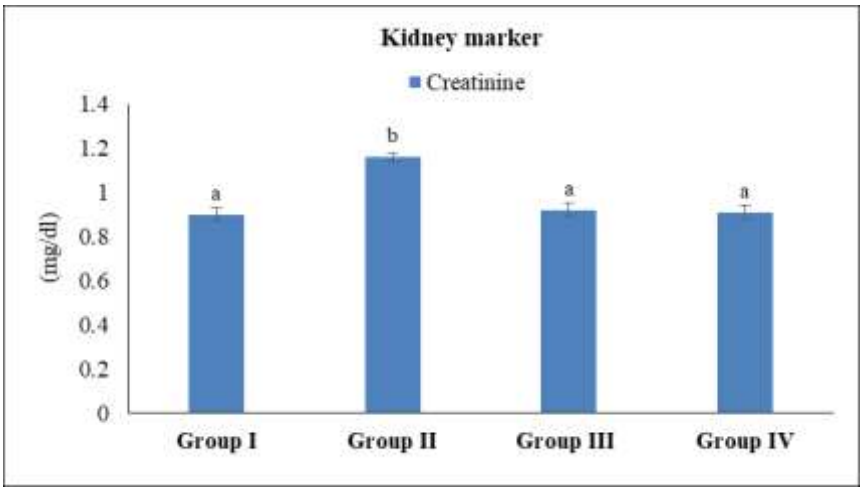
Figure 2: Effects of hydro- ethanolic extract of *Euphorbia hirta* leaves extract on Liver markers in experimental rats

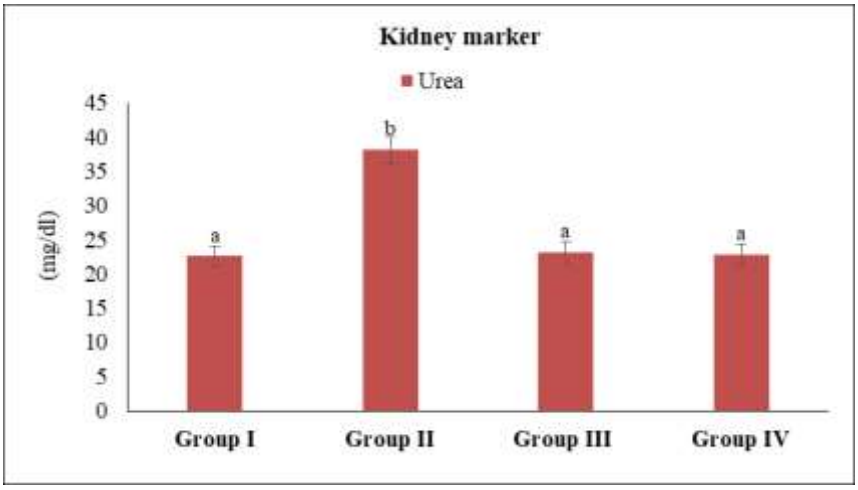
Intervention of *Euphorbia hirta* leaves extract showed significant diminution in renal stress markers in comparison to the rats receiving CS exposure with treatment. In comparison to control group, the results demonstrated that there was a significant increase of urea, creatinine and sodium level while decreased potassium level in cigarette smoke exposed rats (Group II) compared to the control (Group I). Comparison with cigarette smoke exposed group II, results revealed that *Euphorbia hirta* leaves extract restored the kidney markers. Our findings are in corroboration with previous studies (Insaf *et al.*, 2019). A group of peers in their experiment testified that significantly raised serum urea, creatinine, sodium levels and decreased potassium levels in comparison to control rats (Saxena and Shahani 2017). High serum level of urea and creatinine indicates the improper functioning of the kidney and this might occur due to interference of CS chemicals with creatinine metabolism in rats. Cigarette smoke contains several nephrotoxic components and its exposure for 4 h/day caused histological alteration in kidney tissues and disrupted their regular functioning (Alizadeh *et al.*, 2020). Our findings are in concordance with Suganya *et al.*, (2011) who reported that Amelioration of nitrobenzene-induced nephrotoxicity by the ethanol extract of the herb *Euphorbia hirta*.

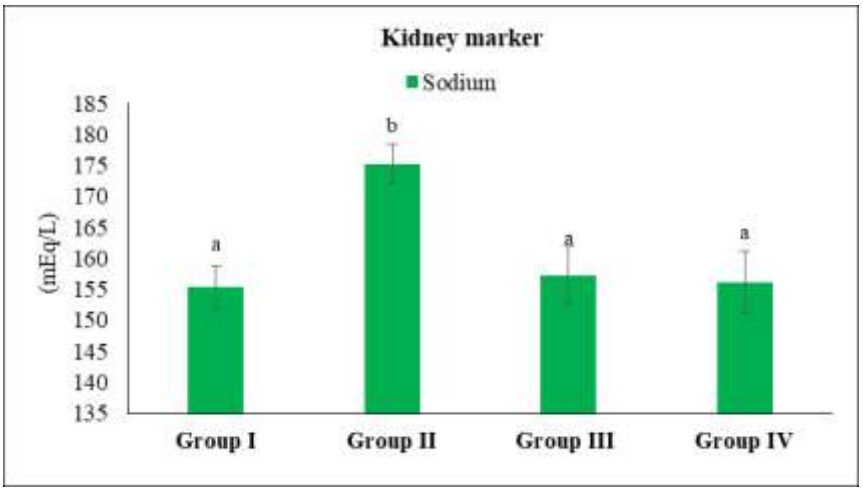
Table 3: Effects of hydro- ethanolic extract of *Euphorbia hirta* leaves extract on Kidney markers in experimental rats

Parameters	Group I (Normal)	Group II (Negative)	Group III (Neg.+ PE)	Group IV (PE alone)
Creatinine (mg/dl)	0.90±0.03^{a}	1.16±0.02^{b}	0.92±0.03^{a}	0.91±0.03^{a}
Urea (mg/dl)	22.55±1.48^{a}	38.08±1.98^{b}	23.10±1.65^{a}	22.83±1.51^{a}
Sodium (mEq/L)	155.46±3.42^{a}	175.30±3.15^{b}	157.22±4.68^{a}	156.17±4.93^{a}
Potassium (mEq/L)	4.63±0.10^{a}	3.18±0.11^{b}	4.59±0.10^{a}	4.62±0.09^{a}

Values are expressed as Mean ± SD for six rats. Mean values within the row followed by different letters (Superscript) are statistically significant (P<0.05) from each other, and the same letter is non-significant (P>0.05). The analysis was an ANOVA followed by post-hoc Duncan's multiple range test (DMRT).







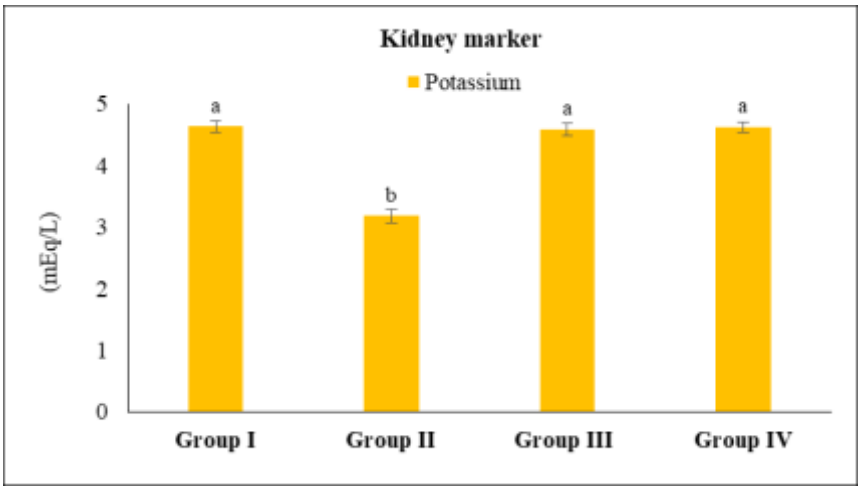


Figure 3: Effects of hydro- ethanolic extract of *Euphorbia hirta* leaves extract on Kidney markers in experimental rats

CONCLUSION

The cigarette smoke exposed rats has severe adverse effects on hematological (Hemoglobin, white blood cells count, mean corpuscular volume, mean corpuscular hemoglobin concentration, red blood cells count, PCV) and biochemical parameters (SGOT,SGPT, ALP, Protein, Urea, Creatinine, sodium and potassium). On treatment with *Euphorbia hirta* leaves extract restored the hematological and biochemical parameters in cigarette smoke exposed rats. The data of the study showed that the hepato-renoprotective effect of *Euphorbia hirta* leaves extract was observed. The protective effect of *Euphorbia hirta* leaves extract due to the phytochemicals present in it. The findings may help to raise awareness of tobacco smokers about the potential toxicities likewise, the results can be used by physicians and public health officials in tobacco prevention programs.

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