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Protective activity of Guduchi and green tea extract on nicotine-induced toxicity in mice

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Abstract
This study was designed to observe the effects of nicotine on various hematological parameters in adult male mice. A total of 30 mice were divided into six groups; control (n=6) and test groups II, III, IV, V, VI (n=6 comprise in each group). After treatments blood was collected on 0th, 30th and 60th days. Hematological parameters were assessed by using bioanalyzer. Statistical analysis was performed for comparison between the groups by using Dunnett's test. The white blood cells (WBC) was found significantly higher in a nicotine treated group compared to control group, while the significant decrease was observed in hemoglobin and hemocrait and platelets in the nicotine treated group. In contrast the differential count *viz*. neutrophil, eosinophil, monocyte, lymphocyte, basophil were found significant ($p \leq 0.05$) compared to control group and test groups II, III, IV, V and V respectively. It is concluded that nicotine administration in mice resulted in toxic effects on various hematological parameters. Green tea and guduchi helps more to wakeup immune system and boost the body of person who regularly consume nicotine.

Keywords: Nicotine, hematological, dunnett's test

Introduction

Tobacco addiction has a considerable health and economic impact on society. It has become one of the largest health problems worldwide. Nicotine plays a key role in maintaining the smoking of tobacco and is the major component responsible for addiction [1]. It is well known that cigarette smoke is the most common oxidant stress in daily life, but it is still debatable whether nicotine is responsible for the effects, due to free radical generation, associated with tobacco use. Nicotine is a naturally occurring alkaloid found primarily in members of the *Solanaceae* plant family, which includes tobacco. It is responsible for some of the deleterious effects of smoking, such as cardiovascular diseases [2] and impairment of the immune system [3]. Nicotine contains nearly 6000chemical substance which causes pharmacological, mutagenic, cancerogenic, toxic, and inflammatory effects in humans [4]. Previously some studies reported the acute effects of nicotine consumption on hematological system by increases white blood cells (WBC), eosinophil, platelet counts and other imbalanced hematological parameters. Several studies reported that relatively higher concentration of WBC than normal peoples as well as increase levels of hematological parameters such as hemoglobin (Hb)%, neutrophil, eosinophil, monocyte and platelet countsin nicotine addicted peoples [5-15]. Green tea (*camellia sinensis*) has good potency of antioxidants nature in presence of polyphenols and catechins [16, 17]. Natural anti-oxidants like polyphenols of green tea extracts have made considerable attention for preventing oxidative stress related diseases *viz*. cancers, cardiovascular and degenerative diseases [18]. Chan *et al*., reported that use of green teagives health benefits and decreases risk of inflammation [19]. *In vitro* study of green tea polyphenols produced reduction in DNA strand breakage by nicotine based cigarette smoke in cultured human bronchial cells [20, 21].

Guduchi (*Tinospora cordifolia* Miers) is a medicinal plant and very useful in allergies, fever, arthritis, skin diseases, rheumatic disorder, hepatitis, jaundice, flu, cancer. This has a good potency to increase RBCs and control WBC [22]. However, any study investigating the effects of green tea and guduchi extract on nicotine toxicity has not been published yet. Thus, the present study was designed to evaluate the effects of green tea and guduchi extract on nicotine induced in mice.

Materials and Methods

Extraction

The prepared whole plant materials of guduchi and leaf part of green tea (30 g) were extracted

three times for 30 min with distilled hot water in separating funnel. The temperature was maintained at 37°C. Ratio of plant material and solvent was 1:10. The extracts were filtered through a paper filter (Whatman, No.1) and evaporated to dryness under reduced pressure by the rotary evaporator. The obtained crude extracts were stored in dark glass bottles for further processing.

Phytochemical analysis

Green tea and guduchi was subjected to phytochemical screening for the detection of various phyto-constituents [23].

Animals

Male albino Swiss mice (25–30 g) were used for the present study. The animals were maintained under standard environmental conditions and were fed with standard pellet diet and water *ad libitum*. The study was approved by Institutional Animal Ethics Committee. The guidelines of CPCSEA, India, were strictly followed during the maintenance and experiment.

Experimental model

Experimental design and treatment protocol

The animals were divided into six groups of six animals each and as follows:

Group I: Normal control group mice received saline;

Group II: Nicotine treated group mice (NIC) received nicotine at a dose of 1 mg/kg/p.o.

Group III: received green tea at a dose of 200 mg/kg/p.o;

Groups IV: received guduchi at a dose of 200 mg/kg/p.o;

Groups V: received nicotine (1 mg/kg/p.o.) and green tea at a dose of 200 mg/kg/p.o;

Groups VI: received nicotine (1 mg/kg/p.o.) and guduchi at a dose of 200 mg/kg/p.o.

Hematological parameters like Haemoglobin (gm/dL); Hemocrait %; MCV/RBC (fL); WBC (x1000); Platelets (x 100000) and differential count *viz*. Neutrophil (x10%); Eosinophil (%); Monocyte (%); Lymphocyte (x10%); Basophill (%) were performed on 0th, 30th and 60thdays (24).

Sample Collection

Blood samples were collected in EDTA tubes by performing retro orbital plexus.

Statistical Analysis

All analysis was performed using graph pad prism for Windows. All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

Results

Phytochemical screening

Phytochemical screening revealed the presence of Saponins, Flavanoids, Terpenoids, Glycoside, Phenol, Carbohydrate, Tannins in green tea extract as well as Saponins, Flavanoids, Phenol, Carbohydrate, Tannins compounds in guduchi extract.

Hematological parameters

In this study mice were induced by nicotine and treated with guduchi and green tea. Hematological parameters on every 0th, 30th and 60th day were performed. All the animals, which were used in the experiment, were very healthy and physically active. The values of various hematological parameters including WBC, RBC, Hgb concentration, Hct, MCV and PLT were assessed in control and test groups - II, III, IV, V and VI respectively. In test group Dunnett's test showed significant increase ($p\leq0.05$) in WBCs count, as compared to the control group as shown in Figures. However significant decrease ($p\leq0.05$) in RBCs count and Hgb concentration was observed in experimental group as compared to control group. Similarly, test groups-II, III, IV, V and VI of differential count *viz*. neutrophil, eosinophil, monocyte, lymphocyte, basophill were found significant ($p\leq0.05$) compared to control group as shown in Figures.

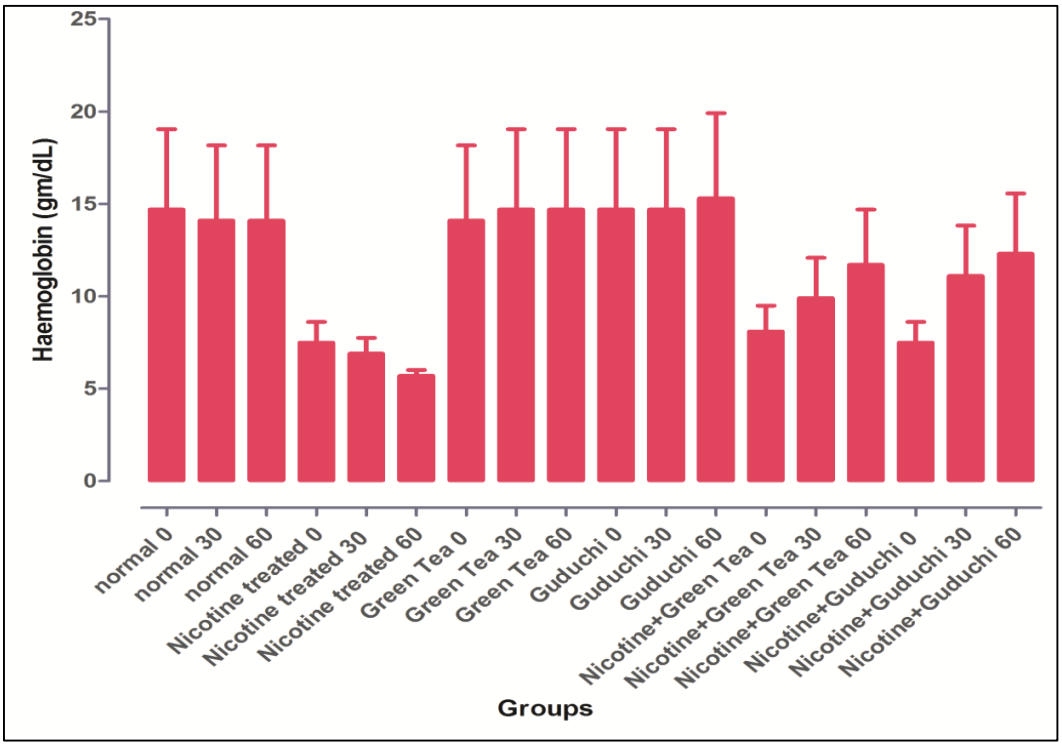

Fig 1: Haemoglobin concentration of control, nicotine induced, green tea, Guduchi, nicotine + green tea and nicotine + Guduchi treated mice

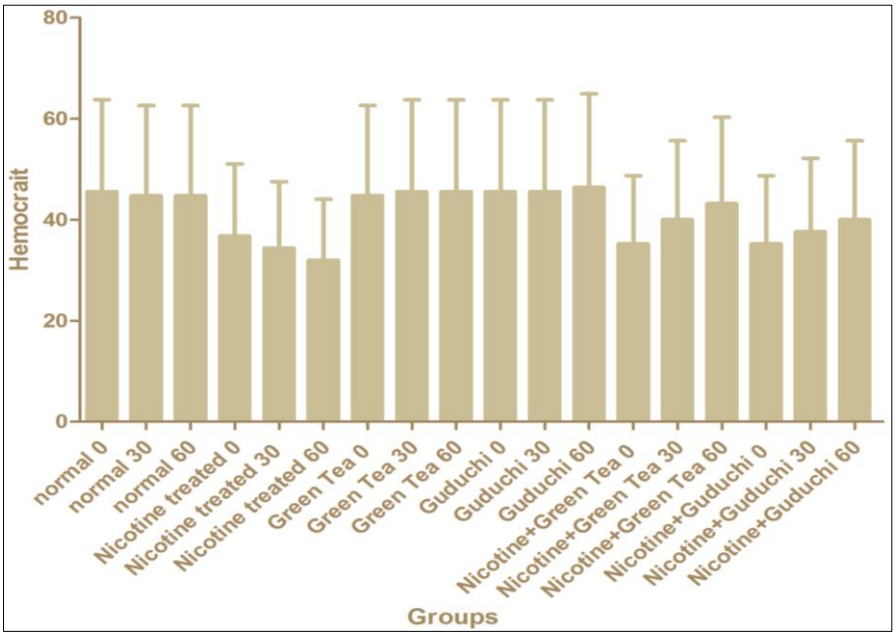

Fig 2: Hemocrait concentration of control, nicotine induced, green tea, Guduchi, nicotine + green tea and nicotine + Guduchi treated mice

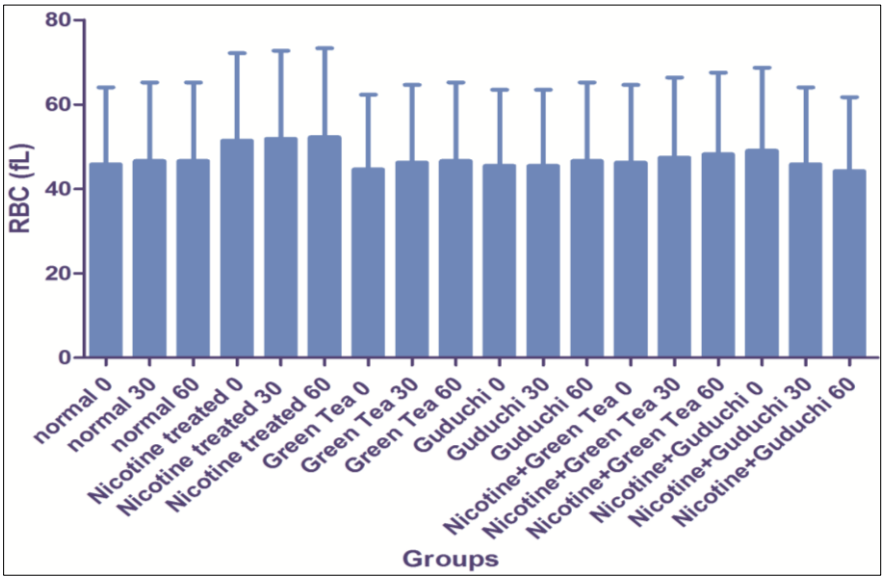

Fig 3: MCV/RBC counts of control, nicotine induced, green tea, Guduchi, nicotine + green tea and nicotine + Guduchi treated mice

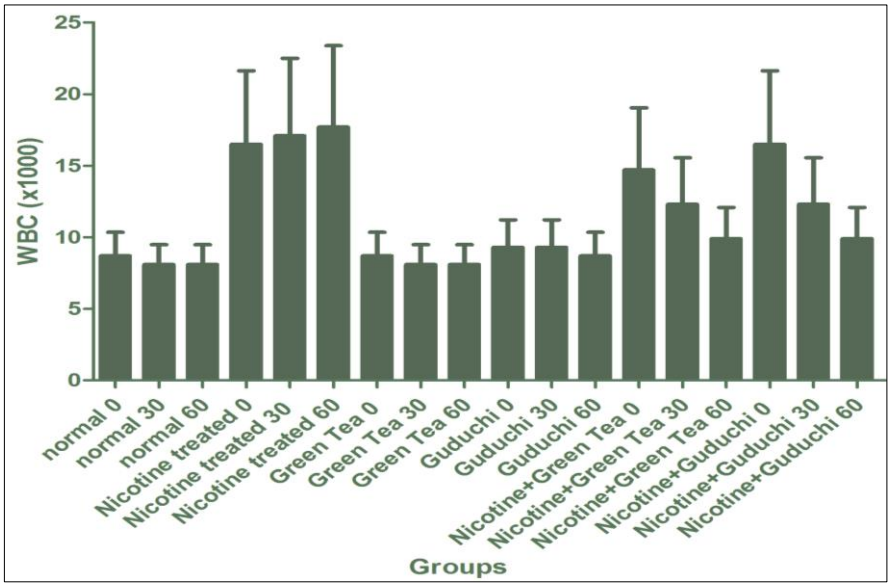

Fig 4: WBC counts of control, nicotine induced, green tea, Guduchi, nicotine + green tea and nicotine + Guduchi treated mice

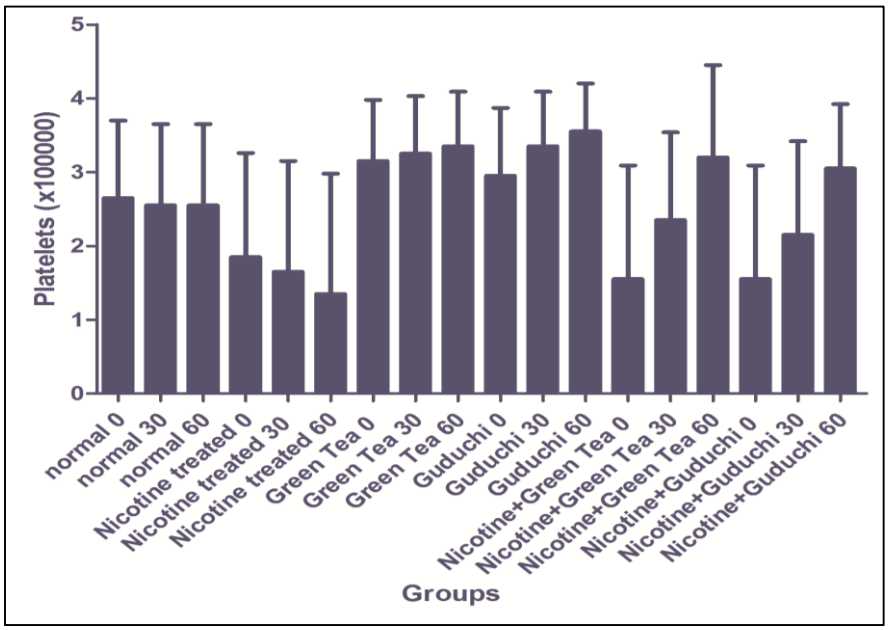

Fig 5: Platelets counts of control, nicotine induced, green tea, Guduchi, nicotine + green tea and nicotine + Guduchi treated mice

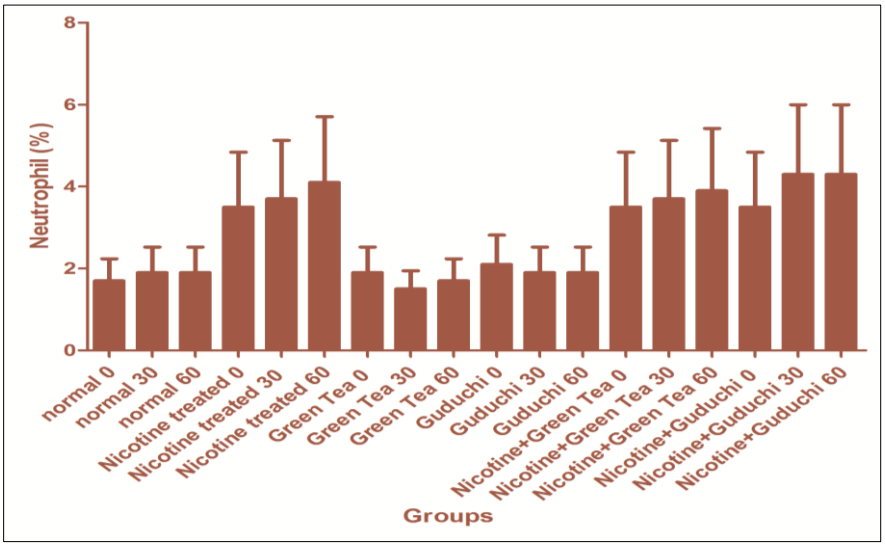

Fig 6: Different platelet counts *viz*. Neutrophil of control, nicotine induced green tea, Guduchi, nicotine + green tea and nicotine + Guduchi treated mice

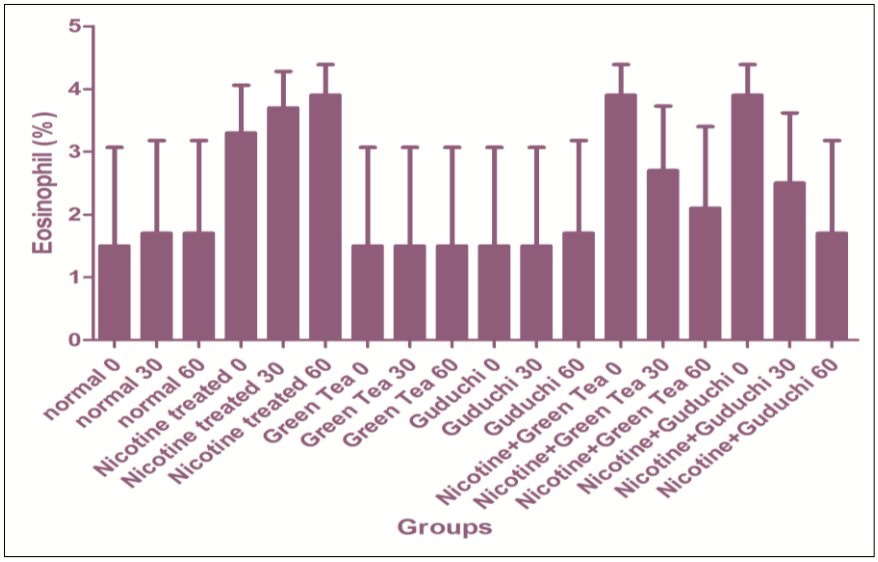

Fig 7: Different platelet counts *viz*. Eosinophil of control, nicotine induced green tea, Guduchi, nicotine + green tea and nicotine + Guduchi treated mice

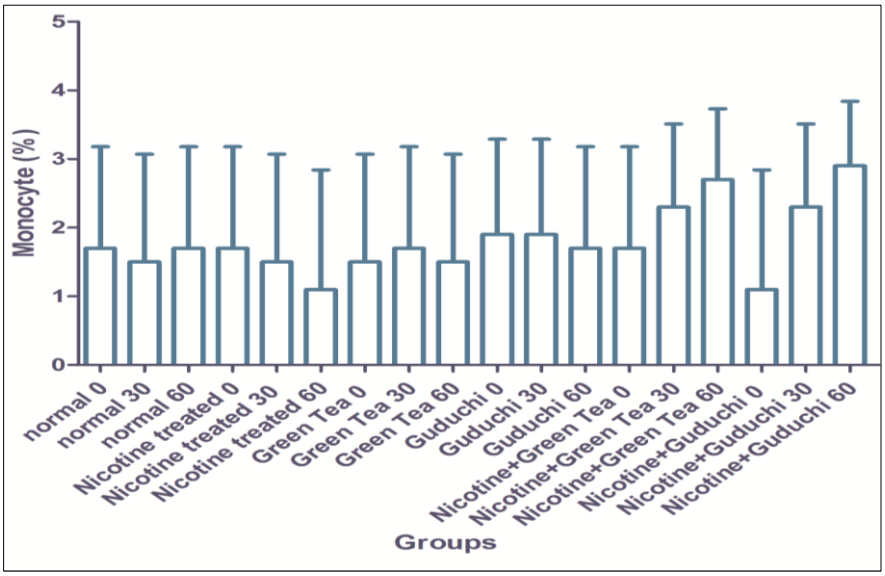

Fig 8: Different platelet counts *viz*. Monocyte of control, nicotine induced green tea, Guduchi, nicotine + green tea and nicotine + Guduchi treated mice

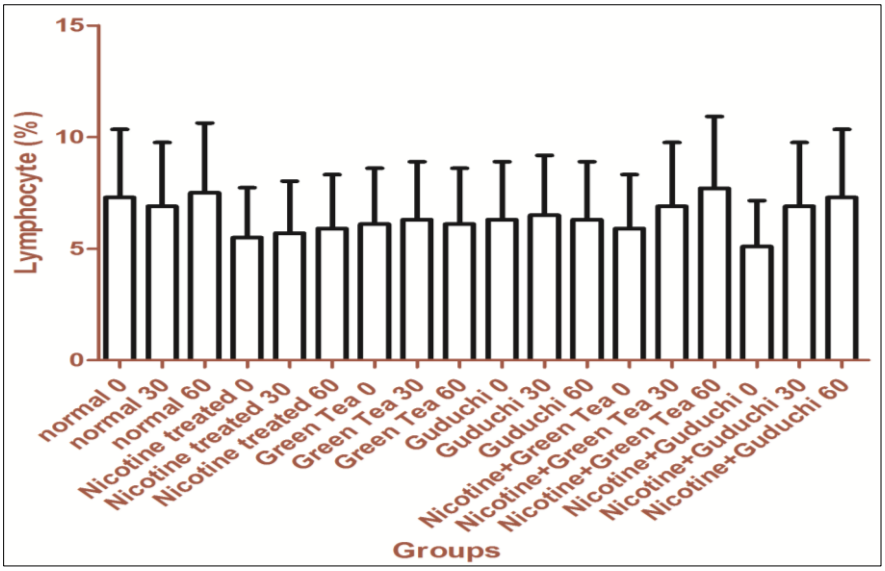

Fig 9: Different platelet counts *viz*. Lymphocyte of control, nicotine induced green tea, Guduchi, nicotine + green tea and nicotine + Guduchi treated mice

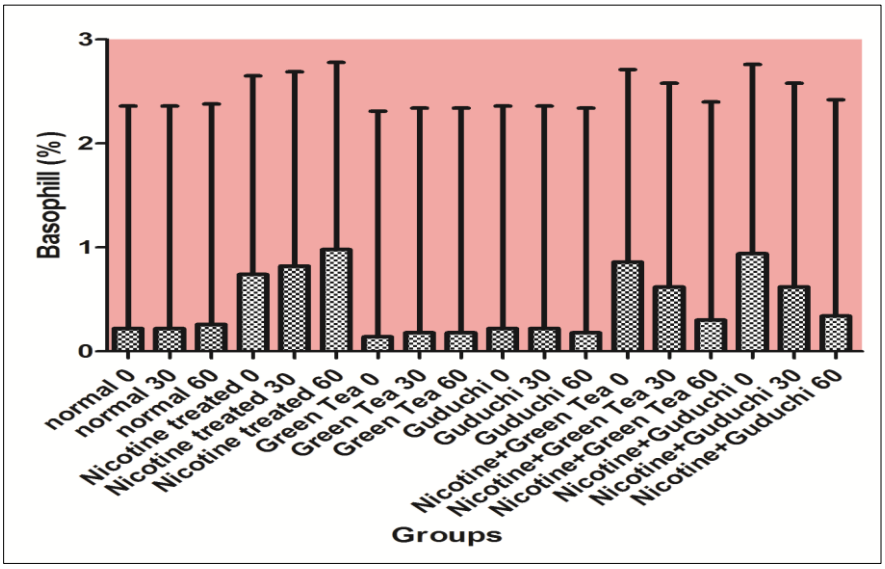

Fig 10: Different platelet counts *viz*. Basophill of control, nicotine induced green tea, Guduchi, nicotine + green tea and nicotine + Guduchi treated mice

Discussion

In the present study was shown that effect of green tea and guduchi extract in nicotine induced mice on various hematological parameters. Herxheimer *et al*. reported that nicotine produces the same hemodynamic changes as cigarette smoking [25]. Rausch *et al*. and Schwartz *et al*. reported that nicotine causes many changes in blood cells as it simply diffuses into the cells [26, 27]. This study confirmed that administration of nicotine to adult male mice significantly altered various hematological parameters including WBC, RBC, Hgb, HCT and PLT. A significant increase in WBCs count and slight fluctuation in RBCs count was observed. The elevated WBCs count in our study is in agreement with the findings of other investigators including [28-30]. One of the major effects of nicotine on the physiology of body is that it greatly suppresses the function of immune system and due to this reason the number of WBCs increased in the body to strengthen the immune system [31]. It is documented that nicotine inhibits the function of erythrocytes, fibroblasts and macrophages. The work of Sherwin & Gastwirth and Siana *et al*. clearly showed that the administration of nicotine causes the diminished proliferation of red blood cells and as a result the RBCs count decreases [32, 33]. Low erythrocytes count may lead to a number of physiological disorders that may affect the efficiency of various enzymes. In the present study it was observed that nicotine administration resulted in significantly ($p \leq 0.05$) decreased hemoglobin level. Similar results were also found by the work of Zafar *et al*. [34]. In our study the HCT level was found to be significantly ($p \leq 0.05$) lower in experimental animals as compared to control group. The PLT count in present study was found to be significantly lower in experimental group. Literature reports on the effects of nicotine on PLT count seem to be controversial. De–Gactano *et al*. showed that nicotine caused platelet and leukocyte activation, and this resulted in the stimulation of platelet function [36]. Moreover, the study revealed that immunological boost up potency hypothesis of green tea and guduchi extract in nicotine induced toxicity.

Conclusion

It is concluded that nicotine administration in mice resulted in toxic effects on various hematological parameters. As per results study shows that various changes in hematological parameters in take of nicotine. Green tea and guduchi helps more to improve the immune system and boost immune system even in the body. We plan to conduct further studies to better understand the mechanisms.

Conflict of Interest

There is no conflict of interest.

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