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Short Communication

Sub Chronic Toxic Effects of Ethanolic *Pongamia pinnata* and *Eucalyptus camaldulensis* Leaf Extracts on Hematological and Biochemical Parameters of Blood in the Male Albino Mice

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Authors' Contributions
FI designed and supervised the project. SI and MHZ performed the experiments. ZJ and QUAG performed the complete blood count and serum and data analyzed. All authors wrote the manuscript.

Key words
***Pongamia pinnata, Eucalyptus camaldulensis*, Ethanolic leaf extracts, Hematology, Albino mice.**

ABSTRACT

This study involves effects of ethanolic extracts of leaves of *Pongamia pinnata* (L.) Pierre and *Eucalyptus camaldulensis* Dehnh on hematological parameters and biochemical components of blood serum of male albino mice. The extracts were administrated orally (100 mg/ ml solvent/ Kg body weight) for 15 days and it was observed that the complete blood count (CBC) remained unaffected while cholesterol (P = 0.03) and triglyceride (P = 0.008) levels in serum were significantly elevated in *P. pinnata* leaf extract treated mice. Mice treated with *E. camaldulensis* leaf extract had significantly reduced white blood cells (P = 0.05), platelets (P = 0.05), alanine aminotransferase (P = 0.04) and significantly increased mean corpuscular hemoglobin levels (P = 0.04) as compared to control group. Our results indicated that both leaf extracts had tendency to disturb the blood biochemistry of healthy male albino mice. The effects were more pronounced in mice orally treated with *E. camaldulensis* leaf extract for 15 days.

Medicinal plants have been used for ages for the purposes of enhancing and maintaining health and organic resistance against body infection. *Pongamia pinnata* is ever green tree widely distributed in Pakistan and locally known as "Sukh Chain" (Sajid *et al*., 2012). Flowers of *P. pinnata* are reported to have anti-hyperglycemic and anti-lipid peroxidation properties (Punitha and Manoharan, 2006). Its bark is used for the treatment of piles; leaves are used to treat rheumatic pains while the seeds are used in treatment of hypertension, bronchitis, whooping cough and skin diseases (Ballal, 2011). *Eucalyptus camaldulensis* is regarded as one of the most cultivated *Eucalyptus* around the World. It is used in traditional medicine to reduce nasal congestion during common cold and for the treatment of fevers, diphtheria, whooping cough ulcers and wounds (Islam et al., 2014).

Despite extensive use of *P. pinnata* and *E. camaldulensis* as medicinal plants, little is known regarding their effects on blood and serum parameters of healthy albino mouse. The aim of this study was to determine the effect of *P. pinnata* and *E. camaldulensis* leaf extract on haematological and some biochemical parameters of male albino mice.

Material and methods

Pongamia pinnata (L.) Pierre is an ever green angiosperm that belongs to family Leguminosae (http://www.theplantlist.org/tpl1.1/record/tro-13051831). *Eucalyptus camaldulensis* Dehnh. is also an angiosperm that belongs to family Myrtaceae (http://www.theplantlist.org/tpl1.1/record/kew-72616). For the preparation of extract, *P. pinnata* and *E. camaldulensis* leaves were collected from Bio Park of Bahauddin Zakariya University, Multan (GPS coordinates 30°15′49″N 71°30′35″E) during

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March, 2019. They were identified by a professional plant taxonomist (zafarbzu@yahoo.com). Voucher specimen of *P. pinnata* (BZBOT0005342) and *E. camaldulensis* (BZBOT0001545) were deposited in the herbarium of Institute of Pure and Applied Biology, Botany Division of Bahauddin Zakariya University, Multan.

Leaves of *P. pinnata* and *E. camaldulensis* were weighed and dried in shade for seven days. Leaf extract solution was prepared following Zahra *et al*. (2015) and 100 mg of each extract was dissolved in 1ml of distilled water to prepare their 100 mg/ml solvent/Kg body weight dose. The above mentioned extracts were administrated to four weeks old, male albino mice (C57BL/6 strain) (N = 24) orally for 15 days. The blood samples (about 1.5 ml) were collected through direct cardiac puncture. About 250 µl of this blood was sampled in tube containing 0.5M EDTA as anticoagulant and was used for complete blood count analysis in hematological analyzer (CBC Analyzer, Sysmex 21, Japan). A second blood aliquot from each subject was centrifuged at 14000 rpm for 10 min to separate the serum from the blood cells. Cholesterol, alanine transaminase, aspartate transaminase, total protein, creatinine and triglycerides were determined in each serum samples through the diagnostic kits (Labtest, France) as per the instructions of their manufacturers.

All the data is expressed as mean ± standard error of mean. Statistical package Minitab (version 16, Pennsylvania) was used for the statistical analysis of the results. Two sample *t*-test was applied to compare all studied parameters of complete blood count and serum biochemistry between a specific leaf extract treated and untreated male albino mice.

Results

Table I shows the effect of leaf extracts of *P. pinnata* and *E. camaldulensis* on the haematological and biochemical parameters of mice blood. *P. pinnata* leaf extracts administered as 100 mg/kg body weight causes 49% decrease in WBC, 20% decrease in platelets, whereas, *E. camaldulensis* leaf extracts causes 61% and 39.5% reduction in WBCs and platelets, respectively. Some other haematological parameters are upregulated *i.e.* MCH 22%, MCV 16%, red cell distributions width 30% and platelet distribution width 37.6%.

Table I. Effect of ethanolic extract of *Pongamia pinnata* and *Eucalyptus calmedulensis* at 100 mg/ ml solvent/ Kg body weight on hematological parameters and biochemical components of male albino mice.

	Control (n = 6)	***Pongamia pinnata* treated mice (n = 6)**	***Eucalyptus calmedulensis* treated mice (n = 6)**
Hematological parameters			
White blood cell (x 10^3 μL^{-1})	17.9 ± 10.2	9.14 ± 4.45	7.0 ± 1.52 *
Red blood cells (x 10^6 μL^{-1})	4.3 ± 0.5	4.99 ± 1.12	4.31 ± 1.34
Hemoglobin (gdL^{-1})	10.32 ± 2.25	11.1 ± 0.91	12.74 ± 4.33
Mean corpuscular hemoglobin concentration (gdL^{-1})	31.78 ± 1.86	31.4 ± 3.36	33.62 ± 3.79
Mean corpuscular hemoglobin (pg)	23.93 ± 4.68	23.8 ± 6.06	29.22 ± 2.28*
Mean corpuscular volume (f L)	75.3 ± 13.4	75.7 ± 11.6	87.22 ± 4.32
Hematocrit (%)	32.42 ± 6.73	36.87 ± 4.13	37.3 ± 11
Red cell distribution width (f L)	39.4 ± 13.5	32.5 ± 14.8	48.12 ± 6.63
Platelets (x10^3 μL^{-1})	297.8 ± 94.9	238.0 ± 92.3	180 ± 74.4 *
Platelet distribution width (fL)	7.92 ± 4.32	10.6 ± 3.34	10.9 ± 1.85
Large platelet concentration ratio (%)	25.3 ± 37.7	13.3 ± 11.9	15.98 ± 5.74
Biochemical parameters of blood			
Cholesterol (mg/dL)	129.83 ± 15.6	185.5 ± 8.92 *	150.5 ± 30.93
Triglyceride (mg/dL)	177 ± 16.3	276.67 ± 21.41**	190.5 ± 14.48
Alanine transaminase (IU/L)	242 ± 32.47	232.5 ± 47.69	143.33 ± 17.91*
Aspartate transaminase (IU/L)	250.17 ± 32.26	252.83 ± 46.92	198 ± 47.46
Creatinine (mg/dL)	0.9 ± .11	0.93 ± 0.1	0.78 ± 0.09
Total protein (mg/dL)	5.7 ± 0.38	5.82 ± 0.21	5.65 ± 0.5

P > 0.05, Non significant; P ≤ 0.05, Least significant (*); P < 0.01, Significant (**)

Table I shows that cholesterol (P = 0.03) and triglyceride (P = 0.008) levels were significantly elevated (43% and 56%, respectively) in treated male mice orally supplemented with 100 mg/Kg body weight of *P. pinnata* leaf extract as compared with control mice. All other studied serum parameters varied non-significantly (P > 0.05) when compared between two experimental treatments.

Analysis of serum parameters indicated that *E. camaldulensis* leaf extract treated mice had significantly decreased alanine transaminase (41%, P = 0.04) concentration than control group. All other studied complete blood count and serum parameters varied non significantly (P > 0.05) when compared between leaf extract treated and untreated male mice.

Discussion

Recently, Akhtar *et al*. (2020) have shown that oral supplementation with ethanolic leaf extract of *Ficus religiosa* had significantly reduced the exploratory behavior, neuromuscular coordination and object recognition capacity of healthy albino mice. This has influenced us to investigate the sub chronic toxicological effects of ethanolic leaf extracts of *P. pinnata* and *E. camaldulensis* in male albino mice.

It has been reported that *P. pinnata* has rich chemical composition that includes alkaloids like demethoxy-kanugin, gamatay, glabrin, glabrosaponin, kanjone, kaempferol, karangin, kanugin, quercitin, pinnatin, neoglabrin, pongamol, pongapin, b-sitosterol, saponin and tanni with a large range of biochemical activities (Usharani *et al*., 2019). Manurial values of leaves are: nitrogen 1.16, phosphorus 0.14, potash 0.49 and lime (CaO) 1.54% (Sangwan *et al*., 2010). During present study, CBC remained unaffected in male mice treated with ethanolic leaf extract of *P. pinnata*. Our results are in agreement with Baki *et al*. (2007) as they did not observe any significant change in CBC of rats treated with 300 µg/day of pongamol (seed extract of *P. pinnata*) for consecutive 14 days. They also reported no effect of this dose on studied serum parameters which is contradictory to our observations as we had observed elevated cholesterol and triglyceride levels in mice exposed to leaf extract of *P. pinnata.* The higher levels of serum cholesterol are in line with the increased triglyceride synthesis in male albino mice during present study as triglycerides are among the building blocks of cholesterol. Elevated triglyceride and cholesterol levels are directly associated with cardiovascular diseases and are valuable indicators of hypertension and atherosclerosis (Sarwar *et al*., 2007). These higher cholesterol and triglyceride concentrations may have an overall negative impact on the health of subjects and need to be explores further. The differences in serum results are probably the different nature of the applied extracts in two studies as it has been reported that the extracts of various parts of same plant may differ in their chemical composition bring different biochemical response in experimental subjects (Zahra *et al*., 2015).

Chemical composition of *E. camaldulensis* revealed that it contains several vital compounds including p-cymene, 1,8-cineole, β-phellandrene, spathulenol, cryptone aldehydes, cuminal, uncommon, phellandral, α-phellandrene, β-phellandrene 5-Hydroxy-7, 40-dimethoxyflavone and 5- hydroxy-7,40-dimethoxy-8-methylflavone have a variety of biochemical reactions in living systems (Barra *et al*., 2010). Siramon *et al*. (2013) had reported that essential oil yields of *E. camaldulensis* leaves ranged from 1.07% to 2.23% based on dry leaves. γ-terpinene were the highest content in the components of oil sample, followed by 1,8-cineole and p-cymene (Siramon *et al*., 2013). During present study, we observed a significant reduction in white blood cells, platelets and serum ALT concentrations while an increase in the mean corpuscular hemoglobin and in male mice treated with *E. camaldulensis* leaf extract. Our results are in agreement with Kabiru *et al*. (2013) who had reported significant decrease in % eosinophils, % basophils, % neutrophils and serum ALT levels in Wistar rats treated with 200mg/ Kg Bodyweight of *E. camaldulensis* leaf extract for three weeks as compared to control group.

Conclusion

In conclusion, we are reporting that despite of known medical importance of both *P. pinnata* and *E. camaldulensis,* the applied dose of 100 mg/ ml solvent/ Kg of both leaf extracts may have long term effects on complete blood count and serum parameters of male albino mice.

Statement of conflict of interest

The authors have declared no conflict of interests.

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