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Comparative anti anaemic activity of *Murraya koenigii* (Linn.) Spreng leaves and its combination with *Emblica officinalis* in aluminium chloride induced anaemia using rodents.

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ABSTRACT–*Murrraya koenigii (curry leaves) and its combination with Emblica officinalis (amla) were evaluated for their antianaemic activity in aluminium chloride induced anaemic animals. Rats were divided into 7 groups of 6 each. Group 1 was given normal saline and served as control and all other groups were given 0.5 mg/kg b.w of $AlCl_3$ for 30 days to induce anaemia. Group 2 served as positive control and Group 3 was treated with synthetic iron (40mg/kg) and served as standard .Group 4 and 5 were treated with different doses (200 and 400 mg/kg b.w.) of aqueous extracts of curry leaves respectively. Whereas Group 6 and 7 were treated with combination of aqueous extract of curry leaves and amla fruit (200 and 400 mg/kg b.w.) respectively. All the treatments were given orally and continued upto 30 days. On 0 and 31 day blood samples were collected by retroorbital puncture and haematological parameters such as haemoglobin (Hb) concentration, RBC count, Mean Haemoglobin Volume (MHV), serum iron, ferritin, and antioxidant parameters such as lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) were estimated. Results showed that both curry leaves and its combination with amla showed significant anti anaemic and antioxidant activity but compared to curry leaf extract alone, its combination with amla showed better activity. This may be due to synergistic action of herbs when used in combination and this combination may be an alternative to synthetic iron therapy in anaemia.*

Keywords: Aluminum chloride, anaemia, haematological parameters, antioxidant.

1. INTRODUCTION

Anaemia is defined as reduction of haemoglobin concentration, RBC count, or packed cell volume to below normal levels. As a result the oxygen carrying capacity of blood is reduced.

Anaemia is a common disease that affects people of all ages but elder population, young women of child bearing age and infants are at greater risk. There are many types of anaemia and in all types there is decrease in circulating RBC [1,2].

Aluminium is ubiquitous element present in every food product. When it is accumulated in humans it can cause bone diseases, CNS disorders, hepatotoxicity and anaemia. Aluminium leads to impaired erythropoiesis and can produce peroxidative damages in erythrocyte membrane leading to haemolysis. Iron is an essential nutrient and critical for haemoglobin synthesis [3]. Ferritin is major cellular iron storage protein, and by sequestering iron, it protects cells from iron induced oxidative damage. Several lines of evidence suggest that aluminium interferes in fundamental ways with iron and ferritin metabolism [4].

Murrraya koenigii is commonly called 'curry patta', belongs to family Rutaceae, is traditionally used in India as spice for its characteristic flavour and aroma. The aromatic leaves are considered as tonic, antihelminthic, analgesic, digestive and appetizer. The leaves are used for treatment of piles, inflammation, itching, fresh cuts, dysentery, vomiting and dropsy. *M.koenigii* leaves contains a range of active pharmacological agents including carbazole alkaloids, flavonoids, furanocoumarins, terpenoids and tannins. It has been reported that curry leaves are rich source of magnesium, zinc, iron and copper [5].

Emblica officinalis commonly called amla, belongs to Euphorbiaceae. Amla fruits are acrid, cooling, refrigerant, astringent, diuretic and laxative. The fermented liquor prepared from fruits is used in jaundice, dyspepsia and cough. *E.officinalis* fruits contain vitamin C, tannins, flavonoids, ellagic acid, gallic acid, quercetin and other constituents which are responsible for its immunomodulatory and anticancer activity [6].

In this context, we have under taken anti anaemic and antioxidant activity of curry leaves and its combination with amla in aluminium chloride induced anaemia using rats.

2. MATERIALS AND METHODS

2.1 Animals

Healthy male Wistar albino rats (200-230g) were procured from Venkateshwara enterprises, Bangalore and housed in polypropylene cages at KLE University's College of Pharmacy, Bangalore , animal house facilities and maintained under standard conditions (12 h light and dark cycle, 22±2^{o}C ,and 55±5% relative humidity) in accordance with CPSCEA guidelines. All animals were given standard rat pellet diet and water *ad libitum*. The study protocol was

approved by IAEC, KLE University's College of Pharmacy, Bangalore (Ref No: IAEC/07/PA/2011-12).

2.2 Plant extracts

Murraya koenigii leaf aqueous extract and its combination with *Emblica officinalis* fruit aqueous extract were gift samples from Green Chem Herbal Extracts and Formulations, Bangalore.

2.3 Chemicals

All chemicals used were of analytical grade, obtained from commercial source and synthetic iron was a marketed product of Himalaya Drug House Ltd., Bangalore.

2.4 Experimental protocol

Animals were divided into 7 groups of six each. All animals, except normal group received 0.5 mg/ kg b.w of $AlCl_3$ for 30 days by oral route. Rats received single oral dose of selected treatment every day 15 minutes before administration of $AlCl_3$ and treatments were as follows:

Group 1: Normal (Normal saline)

Group 2: Positive control ($AlCl_3$alone)

Group3: Synthetic iron (40 mg/kg) + $AlCl_3$

Group 4: Curry leaves (200 mg/kg) + $AlCl_3$

Group 5: Curry leaves (400 mg/kg) + $AlCl_3$

Group 6: Curry leaves + amla (200 mg/kg) + $AlCl_3$

Group 7: Curry leaves + amla (400 mg/kg) + $AlCl_3$

Aluminium chloride and extracts were dissolved in distilled water before administration.

Synthetic iron was in the form of syrup and all were given p.o. using oral feeding needle.

2.5 Biochemical estimations

Blood samples were collected on day 0 and 31 through retroorbital puncture under mild ether anaesthesia. Fresh blood was immediately collected into heparinised tubes for routine

haemotological parameters such as RBC count, Hb concentration and MCV[7,8]. A second blood fraction was collected without anticoagulant and centrifuged at 5000 rpmfor 10 minutes and used for determining iron content and ferritin in serum. Serum analysis of iron was done by Atomic absorption spectrometer (Shimadzu AA 6300) and serum ferritin level estimation was done by immuno chemiluminescence commercial kit. Haemolysate was prepared from heparinized blood and used for determination of SOD [9], CAT [10] and LPO in terms of TBARS [11].

2.6 Statistical analysis

Statistical analysis was done by One-way ANOVA followed by Dunnet multiple comparision test. All values of $p<0.05$ were considered statistically significant.

3. RESULTS:

Oral $AlCl_3$ treatment resulted in significant increase in MCV ($p<0.001$) and decrease ($p<0.001$) in total erythrocytes count, blood hemoglobin (Hb), serum iron and ferritin levels compared to normal group (Table1).

Both the extract treated groups at different doses showed a significant decrease ($p<0.001$) in MCV and increase ($p<0.001$) in RBCs, haemoglobin content, serum iron and ferritin levels compared to positive control group and these are not significantly differ from synthetic iron treatment.(Table1). $AlCl_3$ treatment increased lipid peroxidation ($p<0.001$) and decreased SOD and CAT enzyme levels significantly ($p<0.001$) compared to normal group. Administration of extracts significantly reversed lipid peroxidation and normalized SOD and CAT levels and this did not significantly differ from synthetic iron treatment (Figure1,2,3).

Table 1: Haematological parameters in control rats and different treatment groups

S.N.	GROUPS	RBCs x 10^6	Hb(g/dl)	MCV(fl)	Serum iron µg/ml	Serum ferritin ng/ml
1.	NC	7.28 ± 0.160	15.33 ± 0.331	47.48 ± 0.44	2.56± 0.143	186±0.00
2.	PC	4.82 ± 0.295 a***	7.86 ± 0.166 a***	61.32 ± 0.396 a***	1.17±0.04 a***	150±0.0 a***

3.	**STD**	8.02 ±0.106^{b***}	16.02 ± 0.14^{b***}	49.93 ± 0.136^{b***}	3.42±0.08^{b***}	180±0.00^{b***}
4.	**CL 200**	6.83 ± 0.348^{b***,c**}	12.76 ± 0.23^{b***,c*}	46.27 ± 0.628^{b***}	2.274±0.015$^{(b,c)**}$	158±0.01$^{(b,c)***}$
5.	**CL 400**	7.53 ±0.20^{b***}	15.38 ±0.35^{b***}	48.95 ± 0.295^{b***}	2.665±2.57^{b***,c*}	165±0.00 $^{(b,c)***}$
6.	**CLA 200**	7.66 ± 0.145^{b***}	14.36 ± 0.211^{b***}	46.82 ± 0.192^{b***}	2.882±0.03 ^{b***,c*}	171.0±0.01$^{b***, c**}$
7.	**CLA 400**	8.03 ± 0.088^{b***}	16.64 ± 0.224^{b***}	48.25 ± 0.169^{b***}	3.301±0.104^{b***}	180.5±0.01^{b***}

All values are mean ±SEM (n=6).*p<0.01 **p<0.05, ***p<0.0001. a= when PC was compared with NC, b= when groups compared with PC,c= when groups compared with STD.NC= Normal Control, PC= Positive Control (AlCl3 Treated), STD= standard (Synthetic Iron Treated), CL200= Curry leaves extract treated (200mg/kg),CL400= Curry leaves extract treated (400mg/kg), CLA400= Curry Leaves + Amla extract Treated (400mg/kg) and CLA400= Curry Leaves + Amla extract Treated (400mg/kg).

Fig 1: LPO measured as TBARS in RBC's of control and different treatment groups

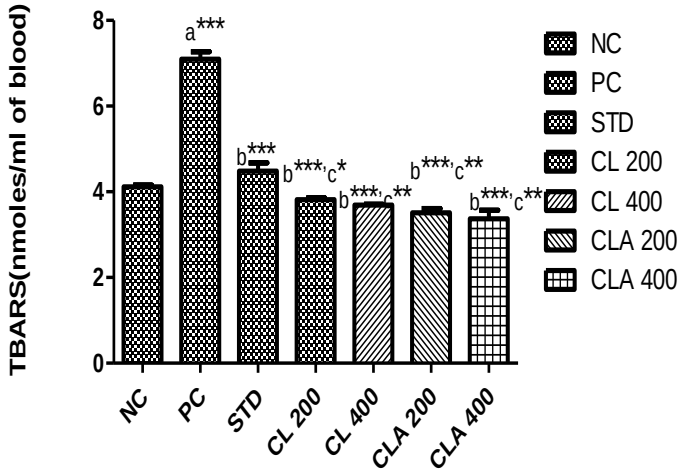

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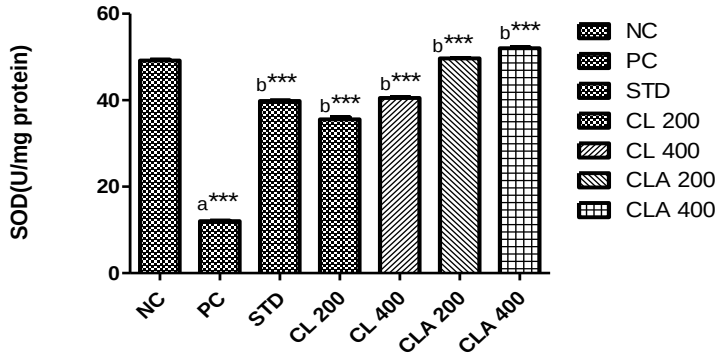
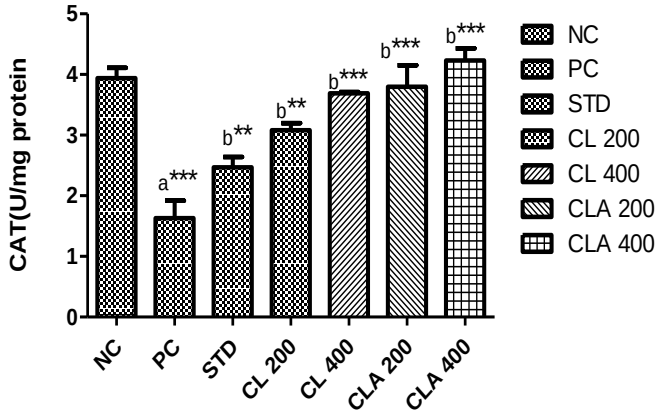
Fig 2: SOD activity in RBC of control and different treatment groups



All values are mean ±SEM (n=6).*p<0.01 **p<0.05, ***p<0.0001. a= when PC was compared with NC, b= when groups compared with PC,c= when groups compared with STD. NC= Normal Control, PC= Positive Control (AlCl3 Treated), STD= standard (Synthetic Iron Treated), CL200= Curry leaves extract treated (200mg/kg),CL400= Curry leaves extract treated (400mg/kg), CLA400= Curry Leaves + Amla extract Treated (400mg/kg) and CLA400= Curry Leaves + Amla extract Treated (400mg/kg).

Fig 3: .CAT activity in RBC of control and different treatment groups



All values as mean ±SEM (n=6).*p<0.01 **p<0.05, ***p<0.0001. a= when PC was compared with NC, b= when groups compared with PC,c= when groups compared with STD. NC= Normal Control, PC= Positive Control (AlCl3 Treated), STD= standard (Synthetic Iron Treated), CL200= Curry leaves extract treated (200mg/kg),CL400= Curry leaves extract treated (400mg/kg), CLA400= Curry Leaves + Amla extract Treated (400mg/kg) and CLA400= Curry Leaves + Amla extract Treated (400mg/kg).

4. DISCUSSION

The present results showed haematological modifications and decreased serum iron levels associated with orally administered aluminum chloride in rats and the reversible effect of curry leaves and its combination with amla on these modifications.

There are reports on the reduction in erythrocyte count, haemoglobin levels and hematocrit values on oral administration of aluminum chloride in rats [12,13]. The present work supports this statement as evidenced by results and our treatment with curry leaves and its combination with amla reversed the above mentioned effects with $AlCl_3$.

The ferritin levels measured usually have a direct correlation with the total amount of iron stored in the body [14]. Ferritin levels indicate stores of iron and in this work there is a substantial increase in ferritin levels more than synthetic iron by the samples tested which is an added advantage in anaemic conditions.

Since curry leaves are a rich source of iron [5] and amla [6] contains ascorbic acid which facilitates iron absorption, the synergistic effect seen with the combination in this work is convincing.

The UNICEF report states, the possibility that certain individuals may at some time develop iron overload is a risk-benefit question. Direct absorption of synthetic iron can upset the gastrointestinal tract. Most commonly, the side effects viz., nausea or, more rarely, diarrhoea and

constipation are untoward effects to who consumes direct iron tablets, which may be greatly reduced by organic supplement of curry leaves with amla especially in the condition of pregnancy. The present findings support the treatment in the conditions like insufficient dietary intake and absorption of iron, and/or iron loss from intestinal bleeding, parasitic infection, menstruation, etc. with curry leaves or its combination with amla.

Moreover the most significant cause of iron-deficiency anemia is parasitic worms: hookworms, whipworms, and roundworms. The worms cause intestinal bleeding which isn't always noticeable in faeces and is especially damaging to growing children and develops anaemia of chronic disease (ACD) is also referred to as anemia of inflammatory response. This can nullify by herbal supplements powerful antioxidant and anti-inflammatory activity.

$AlCl_3$ caused damage was corrected by oral treatment with these herbs as they normalised the levels of enzymatic components of antioxidants such as LPO, SOD and CAT in RBC's of the treated groups. The protective effect of curry leaves and its combination with amla against $AlCl_3$ induced anaemia and increased levels of oxidative stress can be attributed to their antioxidant property.

Thus, the present work reveals the fact that curry leaves or its combination with amla will protect from the hazards of $AlCl_3$ in rodents and can be thought as a supplement to synthetic iron preparations. Since combination of curry leaves with amla enhanced the absorption of iron due to its the ascorbic content which by itself is an antioxidant agent, this might be an added advantage to use this combination in place of synthetic iron.

5. CONCLUSION

In conclusion**,** our findings support the fact that $AlCl_3$ is capable of causing marked alteration in some haematological parameters, inducing anemia and inhibiting the antioxidant components in the RBC, curry leaves and its combination with amla will minimize these hazards. The increase in iron and ferritin level**s** observed with curry leaves as well as curry leaves with amla is comparable to synthetic iron and can be thought as an alternative to marketed synthetic iron preparations. Further, of the two herbs tested, combination proved to be a better alternative than curry leaves alone in alleviating the symptoms of anaemia produced by $AlCl_3$.

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BIOGRAPHY

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Awards and achievements:

- Dr.P.S.Lalitha award for UG student (guide).
- Best teacher award for the year 2011 from KLE University, Belgaum.
- Presented a poster at Bangkok, Thailand-2006.
- Co-chairman for Indian Pharmaceutical Congress, Varanasi - 2007.
- Loc member - 46th annual conference of Indian Pharmacological Society,2013, Bangalore.
- **PATENT -** Extraction of phytochemicals from *Trichilia connaroides.*