Research Article

Effect of *Tamarindus indica* extracts on haematological parameters in albino rats infected with *Entamoeba histolytica* parasite

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Abstract

Objective: The study aimed to investigate the effect of *Tamarindus indica* extracts on hematological parameters in rats infected with *Entamoeba histolytica* parasite which causes Amoebic dysentery disease. **Material and Methods:** A total of 12 albino rats were grouped into 4 groups of 3 rats each. Group 1 of rats serve as negative control group, while group 2, 3 and 4 were treated with metronidazole drug, ethanolic extract and aqueous extract respectively, at concentration 500mg/kg for 10 days, the blood parameters were evaluated hemoglobin (HG), hematocrit (HCT), red blood cell count (RBC), mean platelet volume (MPV), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), red cell distribution width (RDW), procalcitonin test (PCT), total white blood cell platelet distribution width (PDW), mean corpuscular haemoglobin concentration (MCHC), total white blood cell (TWBC), lymphocyte (LY), monocyte (MO) and mixed cells of neutrophile, eosinophile and basophile in the rats blood, using haematology auto-analyser system (CBC Mindray BC-3000Plus). **Results:** Show a significant increase at P≤0.05 in HG, HCT, RBC, MPV, MCV, MCH, RDW, PCT, TWBC, LY and mixed cells of neutrophile, eosinophile and basophile when compare with metronidazole drug and the control group of rats during treatment stages. The results also indicate a significant decrease at P≤0.05 in PDW, MCHC and MO compared to the control group of rats. **Conclusion:** Based on the results of this study, the present data suggest that the efficiency of *T. indica* extracts in improving blood standards through variations occurring in blood proportion.

***Keywords*:** *Tamarindus indica*, *Entamoeba histolytica,* haematological parameters

Introduction

Entamoeba histolytica is an intestinal protozoan parasite. It is the second leading cause of death in humans after malaria (Tachibana et al., 2004; Al-Warid et al., 2013). The World Health Organization (WHO) estimates that the *E. histolytica* parasite causes approximately 50 million cases of dysentery and 100,000 deaths per year (Ravdin et al., 2005). Amoebiasis is spreading worldwide and represents a serious health threat in tropical and subtropical developing regions and in the industrialized countries (Al-Warid et al., 2013). These high rates of mortality are due to several factors including ignorance, poverty, overcrowding, sewage contaminated drinking water and low maintenance of public health. These factors facilitate the transmission of infection from person to person (Allason-Jones et al., 1986).

Tamarindus indica is a plant belonging to the Fabaceae family (Wagh and Bhagure, 2012). This plant is cultivated in large quantities in different regions of India (Zohrameena et al., 2017). The extracts have received considerable attention for their potential therapeutic properties for many diseases. These include antiparasitic (Bhadoriya et al., 2011; Ahmed and Ayoub 2015), Antifungal (Satpute and Vanmare, 2017) antimicrobial (Abubakar, 2016). It has also been used as a folk remedy to treat various ailments such as abdominal pain, laxative, fever, wound healing, dysentery, and chronic diarrhoea exhaustion (Havinga et al., 2010). The study aimed to investigate the effect of *T. indica* extracts on haematological changes in rats infected by *E. histolytica* parasite.

Materials and methods

Plant sample

Leaves of *T. indica* were collected from the campus of Dr.

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Rafiq Zakaria College for Women-Aurangabad. The leaves were washed under tap water. Then they were dried in the laboratory in absence of sunlight for two weeks. The leaves were grinded with the mechanical grinder until they became soft powder. The powder was kept in an airtight container to protect the powder from air and light.

Preparation of the ethanolic extract

Accurately, 40g of leaf powder were weighed and 400 ml of ethanol were added. The plant was extracted with Soxhlet apparatus using ethanolic at concentration 70%. After the extraction, the extract was filtered using Whatman No.1 filter paper and was concentrated using rotary evaporator. After that, it was transferred to an incubator for 24 h at 50°C. The dry mass was weighed and stored in an airtight container before being refrigerated until used (Al-arabi et al., 2018).

Preparation of the aqueous extract

Total 40g of the powdered leaves were weighed into a beaker and 400ml of distilled water were added and stirred by magnetic stirrer for 24 h. It was then filtered using layers of gauze cloth through a buckner funnel, then centrifuged at 3500 rpm for 10 minutes and then filtered using Whatman filter. The supernatant was put in the oven at 60°C for drying. The dried mass was weighted, stored and refrigerated.

Experimental animals

A total of twelve white albino rats (*Rattus norvegicus*) weighing between 200g and 220g (3-3.5 months old) were used for the study. Nine rats were infected by oral administration (17×10^3cell/ml) of *E. histolytica* obtained from the stool. After 7-10 days, the feces of each rat were examined. The infected rats were divided into three groups. Handling of rats was as approved by the University's ethics committee.

Collection of blood sample

Blood was collected from all rats in this study on three stages: pre-treatment stage, mid-treatment stage and post-treatmentstage in each group from the vein at region next to the eye using capillary tubes. Then the blood was put in sterile vials containing EDTA which were used as anticoagulant for blood. After that blood was tested for complete blood count (CBC).

Haematological analysis

Blood samples were analyzed for haematological parameters including Hemoglobin (Hb), Red blood cell count (RBC), Mean platelet volume (MPV), Platelet distribution width (PDW), Haematocrit (HCT), Mean cell volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Red cell distribution width (RDW), Procalcitonin test (PCT), total white blood cell (TWBC) and differential white blood cell count, using haematology auto-analyser system (CBC Mindray BC-3000Plus).

Statistical analysis

The results of the present study were analyzed by GenStat 5.2 using general treatment structure (no blocking), factorial experiment, with 3 replications. Least significant different test (LSD) was used to test the difference between means (groups) at $P\leq0.05$ and was considered significant.

Results

Data of table (1) revealed that Hb, MCV, MCHC, RDW, RBC and HCT decreased significantly ($P\leq 0.05$) in rats infected with *E. histolytica* parasite (Pre-treatment stage) in comparison with negative control group. The results also showed that PDW and MCHC increased significantly ($P\leq 0.05$) also in pre-treatment stage in comparison with negative control group, while the MPV and PCT were non-significant ($P>0.05$) in rats infected with *E. histolytica* parasite compared to the healthy control group (Table 1). After five days of treatment a significant increase was observed ($P\leq 0.05$) in Hb, MCV, RDW, RBC and HCT in groups which were treated by ethanolic and aqueous extracts of *T. indica* in comparison with pre-treatment stage and with negative control and metronidazole group. This increase in Hb, MCV, RDW, RBC and HCT continued during the treatment period until the end of treatment, where the results showed at end of treatment that MCHC and HCT had no significant differences ($P>0.05$) in groups which were treated by ethanolic and aqueous extracts of *T. indica* when compared with metronidazole group. However, the results showed that PDW decreased significantly ($P\leq0.05$) after five days of treatment in groups treated by *T. indica* extracts more than Metronidazole group. This decrease continued until the end of treatment. Also, the results showed that MCHC no significant differences ($P>0.05$) when compared with metronidazole group during the treatment period.

The results showed significant reduction in MPV values after five days of treatment by *T. indica* extracts more than metronidazole group. At the end of the treatment, the results showed a significant increase in the MPV values at $P\leq0.05$ compared with metronidazole group, but there is no significant difference between the groups treated by *T. indica* extracts and the negative control group.

The results showed a significant decrease in PCT values after five days of treatment in groups treated by *T. indica* extracts when compared with negative control. However, the reduction in PCT is not significant ($p>0.05$) in the group

Table 1. Effects of administration of *T. indica* extracts on some haematological parameters in albino rats infected with *E. histolytica*.

Parameters	Type Treatment	Pre-Treatment	Mid- Treatment	Post- Treatment	Means	LSD 5%
Hb (g/dL)	Control	13.97	14.77	15.97	14.90	0.0758
	Metronidazole	12.17	15.23	15.60	14.33	
	Ethanolic extract	11.23	14.27	15.40	14.63	
	Aqueous extract	12.37	14.43	15.63	14.14	
Means		12.44	14.68	15.65	14.25	
RBC (10^6/mm^3)	Control	8.690	8.377	8.887	8.651	0.1075
	Metronidazole	6.987	8.040	8.070	7.699	
	Ethanolic extract	6.547	7.003	7.713	7.087	
	Aqueous extract	6.090	6.427	7.440	6.652	
Means		447	472	610	510	
MCV (mm^3)	Control	56.17	56.50	54.50	55.72	2.085
	Metronidazole	50.90	53.80	58.77	54.49	
	Ethanolic extract	53.20	55.53	55.00	54.58	
	Aqueous extract	54.37	54.90	57.77	55.68	
Means		53.66	55.183	56.51	55.12	
MCH (pg /cell)	Control	18.70	18.37	19.30	18.79	0.634
	Metronidazole	17.97	17.97	17.97	17.97	
	Ethanolic Extract	19.97	20.57	21.73	20.76	
	Aqueous Extract	21.10	22.80	23.03	22.31	
Means		19.44	19.93	20.51	19.96	
MCHC (g/dL)	Control	31.50	35.50	32.20	32.07	0.780
	Metronidazole	35.90	35.87	35.40	35.72	
	Ethanolic Extract	37.67	36.87	39.53	38.02	
	Aqueous Extract	39.00	39.03	39.73	39.26	
Means		36.02	36.82	36.72	36.02	
RDW (%)	Control	17.80	17.37	17.00	17.39	0.620
	Metronidazole	15.87	16.00	17.30	16.39	
	Ethanolic Extract	15.23	16.07	18.13	16.48	
	Aqueous Extract	15.23	16.70	17.33	16.42	
Means		16.03	16.54	17.44	16.67	
PDW (%)	Control	15.70	14.67	13.70	14.69	0.652
	Metronidazole	18.97	16.47	17.30	17.58	
	Ethanolic Extract	17.83	12.47	13.13	16.48	
	Aqueous Extract	17.33	13.00	13.33	16.56	
Means		17.46	14.15	14.37	15.33	
PCT (%)	Control	0.295	0.283	0.387	0.322	0.0779
	Metronidazole	0.367	0.677	0.318	0.454	
	Ethanolic Extract	0.293	0.534	0.262	0.363	
	Aqueous Extract	0.392	0.668	0.195	0.419	
Means		0.337	0.541	0.291	0.390	
MPV	Control	6.600	6.000	6.367	6.322	0.3234
	Metronidazole	6.600	6.167	6.000	6.256	
	Ethanolic Extract	6.533	5.767	6.367	5.856	
	Aqueous Extract	6.533	5.767	6.367	6.222	
Means		6.567	5.925	6.280	6.257	
HCT (%)	Control	41.20	45.40	47.67	44.76	0.879
	Metronidazole	35.40	43.37	46.97	41.91	
	Ethanolic Extract	34.93	42.80	46.20	41.31	
	Aqueous Extract	37.20	43.33	46.73	42.42	
Means		40.13	43.72	43.46	42.44	

LSD: Least significant differences, Hemoglobin (Hb), Red blood cell (RBC), Mean platelet volume (MPV), Platelet distribution width (PDW), Haematocrit (HCT), Mean cell volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin (MCHC), Red cell distribution width (RDW), Procalcitonin test (PCT).

treated by ethanolic extract at the end of treatment but it is significant at P≤ 0.05 in the group treated by aqueous extract when compared with metronidazole group.

The total white blood cells (TWBC) and differential

Table 2. Effects of administration of *T. indica* extracts on TWBC and differential in albino rats infected with *E. histolytica*.

Parameters	Type-Treatment	Pre-treatment	Mid-treatment	Post-treatment	Means	LSD 5%
TWBC	Control	8.07	7.27	9.20	8.18	1.78
(10^3)	Metronidazole	10.57	10.67	11.90	11.05	
	Ethanolic Extract	9.97	13.83	15.77	13.19	
	Aqueous Extract	11.9	14.33	12.93	13.05	
Means		10.13	11.53	12.45	11.37	
LY	Control	93.07	97.00	95.77	95.28	2.156
(%)	Metronidazole	88.97	89.93	92.00	90.30	
	Ethanolic Extract	89.53	84.20	89.90	87.88	
	Aqueous Extract	90.63	89.50	90.53	90.22	
Means		90.55	90.17	92.05	90.92	
MO	Control	3.97	2.00	3.30	3.09	1.478
(%)	Metronidazole	8.40	7.97	5.80	7.39	
	Ethanolic Extract	8.00	12.60	6.50	9.03	
	Aqueous Extract	7.23	8.27	6.50	7.33	
Means		6.90	7.71	5.53	6.71	
Mixed Neutro, Eosino and	Control	1.80	1.00	1.10	1.30	0.784
Baso (%)	Metronidazole	2.63	2.10	2.20	2.31	
	Ethanolic Extract	2.47	3.20	3.60	3.09	
	Aqueous Extract	2.13	2.23	3.00	2.46	
Means		2.26	2.13	2.48	2.29	

LSD: Least significant differences, total white blood cell (TWBC), monocyte (MO), lymphocyte (LY).

leucocytic counts values are presented in table 2. The results showed significant increase (P≤0.05) in TWBC, MO and mixed cells of neutrophile, eosinophile and basophile in rats infected with *E. histolytica* parasite (pre-treatment stage) in comparison with negative control group. On the other hand, the levels of LY decreased in rats infected with *E. histolytica* parasite.

In the treatment period a significant increase was observed in TWBC in all groups treated when compared with negative control group. The increase continued until the post-treatment period. A significant increase was also observed in MO and mixed cells count in groups which were treated by extracts when compared with metronidazole group after five days of treatment. In the post-treatment, the results showed significant decrease (P≤ 0.05) in MO in all groups treated compared with negative control group but the mixed cells (neutrophile, eosinophile and basophile) were showing no significant increase (P>0.05) compared with metronidazole group but increasingly significant when compared with negative control group.

Discussion

Amoebiasis is considered a major health problem in many areas of the world, especially in developing countries (Braga et al., 2001). Recently the use of medicinal plants in different treatments has increased because they are widely available and easy to use (Lawal et al., 2015). However, the examination of haematological parameters are useful indices that can be employed to assess the treatment potentials of plant extracts for treatment of many diseases (Sunmonu and Oloyede, 2010), where that investigation of hamatological parameters to be highly accurate, sensitive, and reliable in disease diagnosis, prevention and treatment (Okonkwo et al., 2004).

In this study some haematological changes caused was determined which were by effect of *T. indica* in rats infected with *E. histolytica* compared with metronidazole drug. The present results have revealed a significant decrease in Hb, MCV, RDW, RBC and HCT in rats infected with *E. histolytica* parasite. This can be due to the ingestion of red blood cells by *E. histolytica* parasite this agree with report Paniker (2002) who stated that *E. histolytica* causes damage of intestinal mucosa which is facilitated by tissue lytic substances released by the amoebae. This may be due to the fact that this parasite causes digestive disturbance, which affects hemoglobin level.

significant changes in the MCV and MCH values could be an indication of the presence of macrocytic anaemia. This

type of microcytic anemia can be caused by lack of iron inability of your body to absorb iron, which can be caused by digestive disturbance because of *E. histolytica* parasite. This result agrees to that found by Darlan et al., (2018) who stated that parasitic intestinal infection generally and protozoa infection effect on Hb, MCV and MCH values, which leads to infection anemia. In addition, the MCHC values were higher in rats infected with *E. histolytica* when compared with negative control. It can occur this increase when red blood cells are fragile or destroyed, also, maybe the reason why the rate of RBC production leads to the release of immature RBC into blood circulation, which may cause an increase in MCHC values (Kotepui et al., 2015).

During the treatment period, an increase was observed in Hb, RBC, MCV, RDW and HCT. This suggests that *T. indica* extracts have the potential to reduce the numbers of *E. histolytica in vivo*, which leads to reducing the effect of this parasite on the body. Also, *T. indica* extracts contain on some vitamins such as B12, B6 and C (Uzukwu et al., 2016) which are an important factors in the production of RBC (Mahmood, 2014; Tu et al., 2015). This suggests that *T. indica* extracts have the potential to stimulate erythropoietin releases from the kidneys in the treated rats which leads to of RBC production, where the values of RBC and associated parameters are lower than normal ranges which are indicative of anaemic conditions while higher values are suggestive of polycythemia (American Diabetes Association, 2000).

In the present study, the TWBC, MO and mixed cells (neutrophile, eosinophile and basophile) in rats infected with E. histolytica parasite were showed significant increase in rats infected with *E. histolytica* parasite as compared with control group in pretreatment stage. The increase in TWBC might be due to the increase in mixed cells (neutrophile, eosinophile and basophile) because they reform first immune defense against the infection (Raddam and Hasson 2008). This result agrees with the result found by Hussein and Shaker (2016). While observing decrease in LY in rats infected with *E. histolytica*, this may be explained that LY are non-specific cells against *E. histolyticea* infection. This result agrees to that found by Vinayak (1987).

In rats treated by *T. indica* extracts a significant increase was observed in TWBC and mixed cells (neutrophile, eosinophile and basophile) compared with control group. This increase continued during the treatment stages. This increased the in TWBC count caused by an extract of *T. indica* maybe lead possible immunomodulatory effects of the extract which augmented the production of more TWBC (Bashir et al., 2015). This will increase the body capability of generating antibodies in the process of phagocytocis and have high degree of resistance to diseases (Okunlola et al., 2012). This result agrees with that found by Mishra and Tandon (2012) who stated that aqueous extract of Ornamental plants increases TWBC count in male Swiss albino mice. This result also agrees to that found by Sunmonu et al., (2014) who stated that aqueous extract of *Picralima nitida* seed increases the differential white blood cell count.

Conclusion

This study has shown that *T. indica* extracts could have some beneficial potentials in improving blood standards through variations occurring in blood proportion in albino rats infected with *E. histolytica* parasite.

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Ethics approval

Institutional guidelines for the care and use of animals were followed. All procedures performed in the study involving animals were in accordance with the ethical standards of the institution or practice at which the study was conducted date 16/08/2018.

Conflict of Interest

The authors declare that there is no conflict of interest.

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