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HAEMATINIC AND IMMUNOMODULATORY EFFECTS OF LEAF EXTRACT OF *GONGRONEMA LATIFOLIUM* FOLLOWING THE ONSLAUGHT OF MUCOSA ULCERATION IN WISTAR RATS

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ABSTRACT

Helicobacter pylori (*H. pylori*) infection is a global public health problem; a higher burden of the infection was reported in developing countries including Nigeria and Cameroun. It has been associated with several gastrointestinal diseases, and recently implicated with some haematological abnormalities. This research was carried out to determine the effect of ethanol extract of *Gongronema latifolium* on haematological parameters of ulcerated Wistar rats.. The experiment was carried out on thirty-six (36) healthy male Wistar rats of weight ranges from (180-200) g. The rats were divide into six (6) groups of six (6) each. Group A served as normal control, Group B (standard control), C and D was treated with100 and 200mg/kg body weight of extract of G*ongronema latifolium* respectively, Group E and F, was induced with ulcer and treated with 200 and 400mg/kg body weight of extract of G*ongronema latifolium* respectively .The extract was administered orally for 14 days. After 14 days of administration all rats were fasted for 14 hours, the rats were sacrificed and the blood was collected by cardiac puncture. Blood samples collected in to EDTA bottles were analyzed for haematological profile. The extract showed a significant increase in ($p<0.05$) in LYM, RBC, PLT, PCV, MXD, Hb, of the ulcerated rats when compared with the normal and standard control. However, the extracts of *G. latifolium* showed a significant decrease in ($p>0.05$) in WBC and HCT when compared with the normal control. This present study suggests that the extract of *Gongronema latifolium* following the onslaught of mucosa might fight against foreign infection possibly by phagocytosis or by generating antibodies that might enhance immune response possibly by immunomodulatory or suppressant or adjuvant action or by generating antibodies that might enhance immune response and might be a panacea to anaemic condition following mucosa ulceration.

KEYWORDS: Adjuvant, Anaemia, Immunomodulators, Immunosuppressant, Mucosa Onslaught.

INTRODUCTION

Most indigenous plants are used in the management of ulcer and other diseases in developing countries of Africa and beyond due to their availability and more safety than the synthetic chemical drugs (Saravanan and Prakash, 2004).

There are wide varieties of trees and plants whose seeds, roots and leaves are widely used by all humans throughout the world because of their nutritional or medicinal values (Burkill, 2015). It has been proven that natural products serves as the major source of drug, providing about half of pharmaceutical ingredients in use today. Over five

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hundred (500) plants are known to be useful for medicinal purposes in Africa, but only a few have been described or studied (Taylor *et al*., 2011; Ayoka &Onakoya, 2013). One of the emerging plants of interest is *Gongronema latifolium.*

Gongronema latifolium (Benth) (*Asclepiadaceae)* is a climber with woody hollow glaborous stem below and characterized by a greenish -yellow flowers (Anaso and Onochie, 2016).It is widely spread in tropical Africa and can be found from Senegal to Chad, Congo, Cameroun and Nigeria. It can also be found in rain forest (Dasofunjo *et al.,* 2020).

There have been reports of various uses of *Gongronema latifolium* in folk medicine by different ethnic groups. In some West African communities to manage cough, intestinal worms, dysentery, dyspepsia and malaria. An infusion ordecoction of the stems with lime juice is taken to treat colic and stomach ache, asthma while the boiled fruits of this plant are eaten as a laxative (Dasofunjo *et al.*, 2020. Anaemia and mucosa ulceration are some of the global health challenges that are common among both developed and developing countries possibly due life style and other factors. This present research work was designed to investigate the effect of ethanol extract of *Gongronema latifolium* on haematological parameters of ulcerated Wistar rats.

MATERIALS AND METHODS

Plant material

Fresh leaf of G*ongronema latifolium* were collected from Obudu, ObuduL.G.A,Cross River State, Nigeria. The leaves were taken to the Department of Botany, University of Calabar for identification and authentication.

Preparation of plant material

Fresh leaf of G*ongronema latifolium* were air-dried at room temperature for twenty (20) days, macerated and pulverized into powdery form using the blender and then sieved.

Ethanol extraction

Three hundred (300) g of powdered G*ongronema latifolium*, leaves were dissolved 1200mls of ethanol water for 72 hours in a refrigerator. Thereafter, it was filtered with muslin cloth and filtered using Whatman filter No. 1.The filtrate was evaporated to dryness and the percentage yield was calculated reconstituted into dosage and administered into rats.

Experimental animal

Thirty- six (36) Wistar albino rats (120-200) g was obtained from the Animal Holding Unit of the Department of Medical Biochemistry, Cross River University of Technology, Cross River State, Nigeria. The animals were allowed to undergo acclimatization period for seven days before the commencement of the research.

The rats were housed in a plastic cage. The animal room was ventilated and kept at room temperature and relative humidity 29± 2^0 C and 70% relative humidity with 12 hours natural light dark cycle and were allowed free access to standard feed and water *ad libitum*, Good hygiene was maintained by constant cleaning and removal of faeces and spilled feeds from cages daily.

Experimental design

The experiment was carried using thirty six (36) male Wistar rats, Group A served as control, Group B was induced with ulcer and treated with 20mg/kgbwt omeprazole , C and D 100 and 200mg/kg body weight of extract of G*ongronema latifolium* respectively, Group E and F, was induced with ulcer and treated with 200(low dose) and 400 (high dose) mg/kg body weight of extract of G*ongronema latifolium* respectively .The extract was administered orally for 14days. After 14days of administration all rats were fasted for 14 hours, the rats were sacrificed and the blood was collected by cardiac puncture.

Determination of haematological parameters

Blood samples collected in EDTA bottles were analysed for haematological parameters u sing a haematology analyser (Sysmex, Kobe, Japan) following the manufacturer's instructions. The parameters analysed include white blood cell count (WBC) and the differentials, platelets, red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Procedures

Each blood sample was mixed well and then approximately 20 µl was aspirated by allowing the analyzer's sampling probe into the blood serum sample and pressing the start button. Results of the analysis were displayed after about 30 seconds, after which the analyzer generated a paper copy of the results on thermal printing paper.

Statistical analysis

Data obtained were analysed using one way analysis of variance (ANOVA) together with post hoc test at $p<0.05$. Statistical Package for Scientific Solutions (SPSS) Software version 20.0 was used for the analysis.

RESULT

The result below indicates the effect of *Gongronema latifolium*on haematological profile following the onslaught of mucosa ulceration in Wistar rats. Following the administration of *Gongronema latifolium*on both ulcerated and un-ulcerated rats, the extract produce a significant decrease ($p<0.05$) in WBC of rat administered with 100 and 200mg/body weight when compared with the normal control and standard control. The extract produced a significant increase ($p>0.05$) in WBC of ulcerated rat administered with 400 mg/kg body weight.

More so, the extract produced a significant increase ($p>0.05$) in LYM concentration of ulcerated and un-ulcerated rat when compared with the control. The extract also produced a significant increase ($p>0.05$) in serum platelets (PLT) and neutrophyl(NEU) concentration of both ulcerated and un-ulcerated rats when compared with the control (Table1).

Likewise, the extract produced a significant increased ($p>0.05$) in serum RBC, Hb of ulcerated

and un-ulcerated rat when compared with the control. Alternatively, the extract also produced a significant decrease (p<0.05) in serum HCT and PCV of both ulcerated and un-ulcerated rats when compared with both standard and normal control (Table 2).

The extract produced a significant decrease (p<0.05) in serum MPV of rat administered with 100mg/kg body weight but a significant increase (p>0.05) in rats treated with 200mg/kg when compared with both standard and normal control. The extract also produced a significant decrease (p<0.05) and a slight increase (p>0.05) in the ulcerated rats administered with 200 and 400mg/kg body weight when compared with the normal and standard control. The extract produced a significant (p<0.05) decrease in serum MCH and MCHC concentration of both ulcerated and un-ulcerated rats when compared with the normal and standard control (Table 3).

The extract produced no significant different in LYM of both ulcerated and un-ulcerated rats when compared with normal and standard control, similar trend was observed on the MXD and NEUT when compared with ulcerated and un-ulcerated rats when standard and normal control(Table 4).

Table 1:Effect of *Gongronema latifolium* ethanol extract on WBC, LYM, PLT and NEUT of experimental animals.

Groups	WBC(10^9/l)	LYM(10^9/l)	PLT(10^9/l)	NEUT(10^9/l)
NC	76.30±0.40	11.31±1.82	633.00±15.37	73.91±7.10
OMP	60.17±0.35*	54.93±45.04	596.67±53.65	63.99±4.23
GL100mg	61.97±1.21*	15.46±0.63	456.00±138.69	89.56±1.14^{*a}
GL200mg	57.17±0.96*	15.91±2.54	414.00±146.03	89.46±4.13^{*a}
GL200+Ulcer	73.03±3.52^{abc}	15.02±0.55	729.33±40.70^{bc}	91.91±1.83^{*a}
GL400+Ulcer	77.00±1.32^{abc}	13.53±0.93	756.00±3.22^{bc}	88.49±1.28^{*a}

Values are expressed as means ± SEM; (n = 5 rats per group). The same column; * = significantly different from NC ($P < 0.05$), a = significantly different from OMP(P< 0.05), b = significantly different from GL100(P< 0.05), c = significantly different from GL200(P< 0.05) and e = significantly different from GL400+Ulcer(P< 0.05). **Legend:** NC = normal control: received normal saline, OMP = standard control: received 20mg/kg body wt omeprazole, GL100 = Group which received 100mg/Kg b.w ethanol extract of *G. latifolium*, GL200 = Group which received 200mg/Kg b.w ethanol extract of *G. latifolium*, GL200+Ulcer = ulcer-induced group which received 200mg/Kg b.w ethanol extract of *G. latifolium* and GL400+Ulcer = ulcer-induced group which received 400mg/Kg b.w ethanol extract of *G. latifolium.*

Table 2: Effect of *Gongronema latifolium* ethanol extract on RBC, HB, HCT and PCT of experimental animals.

Groups	RBC(10^9/l)	HB (g/l)	HCT(%)	PCT(%)
NC	10.49±1.02	200.00±87.09	50.37±7.72	0.41±0.02
OMP	18.27±0.46*	324.67±13.48	25.12±10.72	0.39±0.02
GL100mg	18.78±0.14	216.10±106.40	43.53±1.47	0.30±0.01
GL200mg	18.65±0.91	300.33±34.09	43.67±6.33	0.29±0.09
GL200+Ulcer	18.74±0.32	320.67±10.84	43.65±1.29	0.48±0.11^{c}
GL400+Ulcer	19.03±0.03	329.33±2.33	46.49±0.04^{a}	0.45±0.04

Values are expressed as means ± SEM; (n = 5 rats per group). The same column; * = significantly different from NC ($P < 0.05$), a = significantly different from OMP(P< 0.05) and c = significantly different from GL200(P< 0.05). **Legend:** NC = normal control: received normal saline, OMP = standard control: received 20mg/kg body wt omeprazole, GL100 = Group which received 100mg/Kg b.w ethanol extract of *G. latifolium*, GL200 = Group which received 200mg/Kg b.w ethanol extract of *G. latifolium*, GL200+Ulcer =ulcer-induced group which received 200mg/Kg b.w ethanol extract of *G. latifolium* and GL400+Ulcer = ulcer-induced group which received 400mg/Kg b.w ethanol extract of *G. latifolium.*

Table 3: Effect of *Gongronemalatifolium* ethanol extract on MCV, MCH and MCHC of experimental animals.

Groups	MCV (f/l)	MCH(Pg)	MCHC(g/l)
NC	51.57±0.62	38.88±0.93	728.00±21.66
OMP	44.50±1.96*	32.83±1.05*	709.67±43.11
GL100mg	48.97±1.85a	34.11±1.20*	706.67±16.92
GL200mg	54.57±1.08a	34.83±1.02*	689.67±5.90
GL200+Ulcer	49.93±0.92a	35.63±0.68*	713.00±1.73
GL400+Ulcer	52.10±1.01a	36.78±0.47a	705.33±5.84

Values are expressed as means ± SEM; (n = 5 rats per group). The same column; * = significantly different from NC (*P < 0.05*) and a = significantly different from OMP(*P*< 0.05). **Legend:** NC = normal control: received normal saline, OMP = standard control: received mg/kg/kg body wt omeprazole, GL100 = Group which received 100mg/Kg bwt ethanol extract of *G. latifolium*, GL200 = Group which received 200mg/Kg b.w ethanol extract of *G. latifolium*, GL200+Ulcer = ulcer-induced group which received 200mg/Kg b.w ethanol extract of *G. latifolium* and GL400+Ulcer = ulcer-induced group which received 400mg/Kg b.w ethanol extract of *G. latifolium.*

Table 4: Effect of *Gongronema latifolium* ethanol extract on WBC, LYM and NEUT of experimental animals.

Groups	LYM%	MXD%	NEU%
NC	12.38±0.55	5.99±0.73	81.63±1.26
OMP	14.13±0.59	8.03±0.40*	65.81±3.19*
GL100mg	13.89±0.28	7.35±0.10	79.41±0.71a
GL200mg	14.03±1.43	6.03±0.58a	79.14±1.03a
GL200+Ulcer	13.09±0.29	6.81±0.67	81.69±1.07a
GL400+Ulcer	12.84±0.87	5.17±0.10abd	80.20±0.53a

Values are expressed as means ± SEM; (n = 5 rats per group). The same column; *= significantly different from NC (*P < 0.05*), a = significantly different from OMP(*P*< 0.05), b = significantly different from GL100(*P*< 0.05), d = significantly different from GL200+Ulcer(*P*< 0.05). **Legend:** NC = normal control: received normal saline, OMP = standard control: received 20mg/kg body wt omeprazole, GL100 = Group which received 100mg/Kg b.w ethanol extract of *G. latifolium*, GL200 = Group which received 200mg/Kg b.w ethanol extract of *G. latifolium*, GL200+Ulcer = ulcer-induced group which received 200mg/Kg b.w ethanol extract of *G. latifolium* and GL400+Ulcer = ulcer-induced group which received 400mg/Kg b.w ethanol extract of *G. latifolium.*

Table 5: Effect of *Gongronema latifolium* ethanol extract on RDWCV, RDWSD MPV PDW and of experimental animals.

Groups	RDWCV(%)	RDWSD(f/l)	MPV(f/l)	PDW(f/l)
NC	20.83±0.44	60.26±0.20	6.73±0.08	20.96±3.75
OMP	42.87±23.63	62.30±1.52	7.93±0.65*	15.80±0.52
GL100mg	20.77±1.53	60.12±0.83	6.82±0.08a	19.42±4.13
GL200mg	19.60±1.21	59.76±0.65a	7.19±0.23	15.07±0.18
GL200+Ulcer	21.47±0.62	60.66±0.32	6.79±0.08a	14.53±0.27
GL400+Ulcer	20.29±0.02	59.20±0.48a	6.83±0.08a	14.90±0.06

Values are expressed as means ± SEM; (n = 5 rats per group). The same column; * = significantly different from NC (*P < 0.05*) and a = significantly different from OMP(*P*< 0.05). **Legend:** NC = normal control: received normal saline, OMP = standard control: received 20 mg/kg body wt omeprazole, GL100 = Group which received 100mg/Kg b.w ethanol extract of *G. latifolium*, GL200 = Group which received 200mg/Kg b.w ethanol extract of *G. latifolium*, GL200+Ulcer = ulcer-induced group which received 200mg/Kg b.w ethanol extract of *G. latifolium* and GL400+Ulcer = ulcer-induced group which received 400mg/Kg b.w ethanol extract of *G. latifolium*.

DISCUSSION

Ulcer is a sore on the lining of the gastrointestinal tract caused due to mucosal erosions (Wolfsthal, 2008). It can be classified mainly into four types viz gastric, duodenal. Oesophageal and

Meckel'sDiverticulum ulcer (Garoll*et al.,*2009*).*The predominant causes of ulcer are infection with the bacterium called *Helicobacter pylori* and the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as aspirin or ibuprofen.

The haematological analysis was carried out to provide useful information on the general; state of the blood chemistry after administration of extracts (Steven *et al*., 2013) following mucosal ulceration. Packed cell volume also known as haematocrit (Hb) or erythrocyte volume fraction (EVF) is the percentage (%) of red blood cells in blood. It measures the percentage volume of cells in the blood .Anaemic condition is associated with low production of red blood cell (Guenter& Lawrence, 2005). PCV is also involved in transportation of oxygen and absorbed nutrients. Increased PCV concentration may depicts a better transportation and thus may results in an increased primary and secondary polycythaemia (Isaac *et al*., 2013). The observed marked increase in PCV from this work suggest that the plant extract varying concentrations may positively interfere with osmoregulatory and haematopoietic system of the blood that can enhance anaemia management (Audu*et al.,* 2014).It also suggests that the ulcer induction may not alter the PCV chemistry.

In this study treatment with plant extract produced a significant increase in PLTs in rats administered with 100 and 200 mg/kg bwt of the ethanol extract according (McLellan*et al.,*2003), increase in PLT in experimental rat indicate good action on the blood oxygen transporting ability as well as thrombopoietin. The observed increase in PLT in this study therefore indicates that the extract may improve the blood oxygen transporting ability following mucosa onslaughts.

Increased RBC and Hb were also observed in this present study following the administration of the extracts. The observed increase in both RBC and Hb could indicate erythrocytic synthesis (Dede*et al*.,2002). Therefore, the observed increase in RBC count and Hb may connote that the extract enhances haematopoiesis and or erythropoiesis. Likewise, the oxygen transporting of the blood and the oxygen supplied to the tissues may be improved following the administration of the extract following the onslaught of mucosa ulceration.

The major role of the white blood cells and its differential are to fight against infection, defend the body by phagocytosis from invasion by foreign organism and to transport and distribute antibodies by immune response. Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high WBC counts are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to disease (Soetan*et al*., 2013) and enhance adaptability to local environmental and disease prevalent conditions (Nse-Abasi*et al*., 2014).From this present study the significant increase in WBC count following the onslaught of mucosa suggests that the extract may help against foreign infection possibly by phagocytosis or by generating antibodies that might enhance immune response possibly by immune modulatory or suppressant or adjuvant action.

CONCLUSION

This present study the suggests that the extract of *Gongronema latifolium* following the onslaught of mucosa suggests that the extract might fight against foreign infection possibly by phagocytosis or by generating antibodies that might enhance immune response possibly by immunomodulatory or suppressant or adjuvant action or by generating antibodies that might enhance immune response and might a panacea to anaemic condition following mucosa ulceration.

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