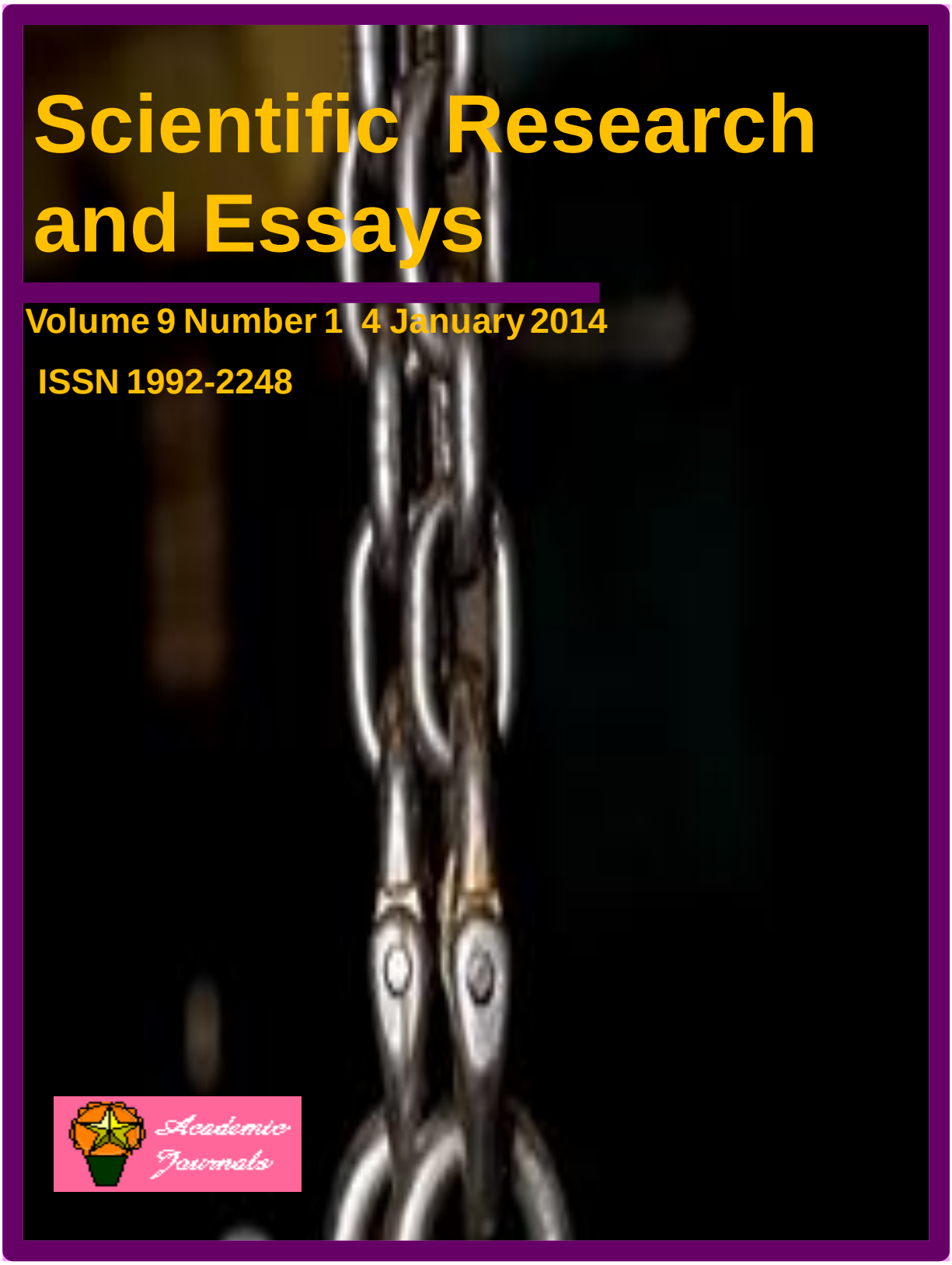

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Charnley AK (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In: Lomer CJ, Prior C (eds) Biological Controls of Locusts and Grasshoppers: Proceedings of an international workshop held at Cotonou, Benin. Oxford: CAB International, pp 181-190.

Mundree SG, Farrant JM (2000). Some physiological and molecular insights into the mechanisms of desiccation tolerance in the resurrection plant Xerophyta viscasa Baker. In Cherry et al. (eds) Plant tolerance to abiotic stresses in Agriculture: Role of Genetic Engineering, Kluwer Academic Publishers, Netherlands, pp 201-222.

Babalola OO (2002). Interactions between Striga hermonthica (Del.) Benth. and fluorescent rhizosphere bacteria Of Zea mays, L. and Sorghum bicolor L. Moench for Striga suicidal germination In Vigna unguiculata . PhD dissertation, University of Ibadan, Ibadan, Nigeria.

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Table of Contents: Volume 9 Number 1 4 January, 2014

ARTICLES

Research Articles

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Scientific Research and Essays

Full Length Research Paper

Influence of season affecting flowering and physiological parameters in mango

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A field experiment was conducted at State Horticultural Farm, Kanyakumari District undertaken by the Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam during the year 2010 to 2012. In general, mango flowering is considered as a complex phenomenon. Besides, favorable climate conditions that favours off-season flowering, genetic potential of the varieties, physiological and biochemical variations and better management interventions could also play the vital role in promoting off season flowering. The environmental variables play a key and vital role in induction of mango flowering. The result was revealed by the Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam during the year 2010 to 2012. The number of inflorescence m^{-2} (32.10 and 26.40), hermaphrodite flower per cent (37.95 and 33.25), male flower per cent (47.97 and 52.60) and fruit set per cent (0.67 and 0.63) were higher in cv. Neelum during main season and off–season respectively. With regard to physiological parameters, the highest soluble protein (12.55 and 11.94 mg100 g^{-1}) and total phenols (3.510 and 3.250 mg100 g^{-1}) and the lowest of IAA oxidase activity (169.85 and 178.20 µg g^{-1}) and Gibberellic acid content (1.05 and 1.06 µg g^{-1}) were recorded in cv. Neelum during main season and off–season respectively.

Key words: Flowering, physiological parameters, mango cultivars, season.

INTRODUCTION

Mango (*Mangifera indica* L.) belonging to the family Anacardiaceae occupies a predominant place among the fruit crops grown in India and christened as the 'King of fruits' owing to its delicious flavour and taste. In India, mango is cultivated extensively in about 2.3 million hectares with the production of 15.27 million metric tonnes (Anonymous, 2011). The national average productivity of mango in India is 6.6 tonnes per hectare. In Tamil Nadu, mango is grown in an area of about 1,048,000 ha with the production of 823,000 MT of fruits and the productivity is about 5.60 MT per hectare (Anonymous, 2011). Normally mango flowering occurs during the month of December-January and fruiting takes place during April-May in Indian conditions. However, in certain pockets of Southern Tamil Nadu *viz.,* Tenkasi and Senkottai blocks of Thirunelveli district and Agasteeswaram block of Kanyakumari district, mango produces off-season, bearing and flowering occurs during July-August, and fruiting commences during November-December. This peculiar phenomenon of flowering and fruiting in mango is known as off-season bearing. The number of flushes varied greatly depending upon the variety, age of the tree, climatic conditions and the amount of crop borne in the previous season. They also reported that although flowering in mango trees generally took place during short days in the areas that fall nearer to the equator, the very fact that off-season cropping was possible at Kanyakumari Thirunelvelli district in South

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India suggested that flowering in mango is certainly under the environmental control, most probably photoperiod. They also reported that mango trees responded to temperature variations more critically than to photoperiods as evidenced by the different times of flowering at different places in India (Palanisamy et al., 2011). As a consequence of efforts to elucidate the mechanisms of this critical biological event in mango and other model plant systems, many of the important details are becoming clearer at the molecular, biochemical, and physiological levels resulting in a better understanding of how to manage flowering in the field. A conceptual flowering model has been described to explain the interaction of internal and external factors regulating vegetative and reproductive shoot initiation and induction in mango trees growing in tropical and subtropical environments (Davenport and Nunez-Elisea, 1997). The present study was undertaken to influence of season affecting flowering and physiological parameters in mango.

MATERIALS AND METHODS

The present investigation was conducted at State Horticultural Farm, Kanyakumari District and undertaken by the Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam during the year 2010 to 2012. The experimental design was laid out in a Factorial Randomized Block Design (FRBD), with two seasons and ten varieties and replicated twice. Ten year old trees of mango cultivars were selected for this study. Mango cultivars selected for this study are Alphonso, Bangalora, Kalepad, Himayuddin, Sendura, Mulgoa, Neelum, Rumani, Banganapalli and Swarnarekha and seasons are main and off-season. The weather parameters *viz.*, maximum and minimum temperature, relative humidity, average rainfall and rainy days in Kanyakumari, were recorded in experimental location.

Number of inflorescence per metre square

Number of inflorescence m^{-2} was counted in a square metre area of four different places in a tree with the help of wooden frame of 1 m × 1 m dimension and the mean was obtained.

Male flowers percent

The percentage of male flowers was calculated from the randomly selected ten panicles $tree^{-1}$ employing the following formula and expressed in percentage.

$$Percentage\ of\ male\ flower = \frac{number\ of\ male\ flowers}{total\ number\ of\ flowers}\ X\ 100$$

Hermaphrodite flowers per cent

The percentage of hermaphrodite flowers was calculated from the randomly selected ten panicles $tree^{-1}$ using the following formula and expressed in percentage.

$$\text{Percentage of Hermaphrodite flower} = \frac{number\ of\ hermaphrodite\ flowers}{total\ number\ of\ flowers}\ X\ 100$$

Fruit set (pepper stage) per cent

The fruit set was recorded at pepper stage in twenty tagged panicles in all the selected trees and the mean values were expressed in percentage (Sharma and Singh, 1969).

$$\text{Fruit set percentage} = \frac{number\ of\ fruits}{total\ number\ of\ flowers}\ X\ 100$$

Total phenol content

The total phenol content of the leaves was estimated by adopting the method of Bray and Thorpe (1954) and the mean values were expressed in mg 100 g^{-1}.

Soluble protein content

The soluble protein content was extracted with phosphate buffer and estimated as per the method described by Lowry et al. (1951) and the mean values were expressed in mg g^{-1} of fresh weight.

Chlorophyll content

The leaf chlorophyll content was estimated through Simple Portable Diagnostic (Minolta SPAD – 502) and expressed as simple portable diagnostic value.

IAA oxidase

The estimation of indole acetic acid oxidase was done as per the method suggested by Parthasarathy et al. (1970) and values were expressed as µg g^{-1}.

Gibberellic acid bio assay

The gibberellic acid content of leaf samples was estimated as per the method of Holbrook et al. (1961) method and the mean values were expressed in µg g^{-1}.

RESULTS AND DISCUSSION

The present study revealed that the environmental factors played very effective role to induce flowering and fruiting. With regard to flowering characters, the highest values in number of inflorescence per metre square, hermaphrodite flower percentage and fruit set percentage; and the lowest male flower percentage were registered by Neelum during main season and followed by cv. Kalepad during main season (Table.1). This is in agreement with the findings of Kulkarni (1988), and Robbertsen and Stassen (2004). Similar results were also reported in different mango cultivars of Australia (Winston, 1992), Indonesia (Voon et al., 1991; Tongumpai et al., 1991). This might be attributed due to

Table 1. Influence of season on flowering characters in mango cultivars.

Varieties	Number of inflorescences per metre square		Hermaphrodite flower (%)		Male flower (%)		Fruit set (%)	
Seasons	**Main season**	**Off season**	**Main season**	**Off season**	**Main season**	**Off season**	**Main season**	**Off season**
Alphonso	17.32	14.55	19.60	16.05	70.76	74.77	0.32	0.26
Bangalora	18.90	13.40	22.85	19.10	66.80	74.59	0.50	0.40
Kalepad	23.40	17.25	31.30	26.25	53.10	57.55	0.58	0.54
Himayuddin	14.75	10.80	17.67	15.65	78.95	78.06	0.28	0.23
Sendura	18.45	14.05	20.47	17.45	71.92	76.39	0.31	0.26
Mulgoa	12.05	9.00	16.27	13.45	83.05	85.87	0.28	0.24
Neelum	32.10	26.40	37.95	33.25	47.97	52.60	0.67	0.63
Rumani	15.65	12.75	13.22	9.55	84.91	86.06	0.27	0.24
Banganapalli	20.15	16.20	19.90	15.85	78.10	77.23	0.38	0.30
Swarnarekha	11.60	8.45	16.12	13.05	78.95	77.94	0.23	0.20
SEd	0.04305		0.05035		0.07256		0.00138	
CD (0.5%)	0.08708		0.10184		0.14678		0.00280	

Table 2. Influence of season on physiological parameters in mango cultivars.

Varieties	IAA oxidase activity (µg g^{-1})		Gibberellic acid content (µg g^{-1})		Soluble protein (mg100g^{-1})		Total phenols (mg100g^{-1})	
Seasons	**Main season**	**Off season**	**Main season**	**Off season**	**Main season**	**Off season**	**Main season**	**Off season**
Alphonso	216.55	238.40	1.15	1.21	9.27	8.95	1.647	1.450
Bangalora	202.95	215.30	1.01	1.08	8.85	8.57	2.417	2.200
Kalepad	178.10	192.70	1.07	1.10	10.05	9.60	3.100	2.900
Himayuddin	222.35	235.80	1.27	1.28	7.62	7.23	1.725	1.515
Sendura	220.95	225.50	1.10	1.17	8.40	8.10	2.137	1.762
Mulgoa	233.60	240.45	1.31	1.28	8.05	7.84	1.385	1.152
Neelum	169.85	178.20	1.05	1.06	12.55	11.94	3.510	3.250
Rumani	214.50	217.95	1.40	1.43	7.07	6.76	2.667	2.425
Banganapalli	230.60	235.60	1.07	1.11	8.50	8.35	1.957	1.737
Swarnarekha	275.35	282.60	1.25	1.29	8.26	7.39	1.700	1.582
SEd	0.18354		0.00080		0.00942		0.00904	
CD (0.5%)	0.37124		0.00162		0.01904		0.01211	

high leaf N level in the month of February (flowering stage) which exhibited a clear and positive correlation with percentage of hermaphrodite flower per cent. These results confirmed the earlier studies (Anonymous, 1982) and revealed that per cent hermaphrodite flowers increased when the nitrogen level was increased from leaf and same observations were also made by Rajput and Tiwari (1975) and reported that high N level improved the hermaphrodite flower percentage and in term fruit set per cent in mango. Increased N level of leaves during flowering resulted with more production of hermaphrodite flowers, that is, 63% of total flowers per mango panicle.

Flowering and fruit set of the different cultivars and seasons were associated with the reduced vegetative growth, often induced by lower level of gibberellin (Voon et al., 1991). The lowest IAA oxidase activity level was observed within the present study. Reduction of vegetative growth required physiological changes, which resulted in higher in terms of flowering. Following the reduction in vegetative growth parameters, there was a higher chlorophyll content, carbohydrate content and carbohydrate-nitrogen ratio in leaves and shoots at three phases of growth and development viz., vegetative, flowering and harvesting (Table 2). A higher accumulation of required reserves in the current year or main season shoots before flowering was also observed by Stassen (1997). The hormonal content of flowering in mango implies that the cyclic synthesis of floral stimulus in the leaves and the difference between two such cycles would determine the flowering behaviour of mango cultivars (Kulkarni, 1988). The development of hermaphrodite flowers needed more reserves from the tree than male flowers. The number of inflorescence per

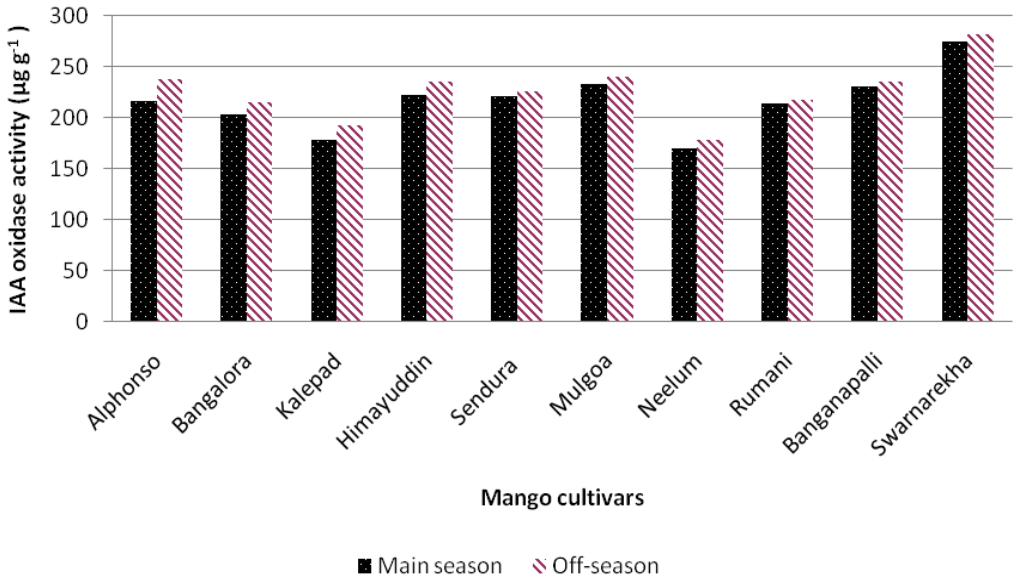

Figure 1. IAA oxidase activity (µg/g^{-1}) of mango.

metre square, the percentage of hermaphrodite flowers and fruit set percentage had the favourable environmental factors which resulted in higher reserves, that is, carbon-nitrogen ratio (Vijayalakshmi and Srinivasan, 2002). The high humidity and rain prevalence at the time of bloom or late rain appeared to influence flower bud differentiation and fruit set development. Shanmugavelu et al. (1987) opined that wide (1.25 to 70%) ratio of hermaphrodite to male flower was observed in varieties with the highest number of inflorescence per metre square particularly in Neelum. Sex expression in mango was influenced by temperature, where higher temperature seems conducive for production of more perfect flowers (Singh, 1990). Thimmappaiah and Suman (1987) stated that among 13 different cultivars, evaluated maximum percentage of hermaphrodite flowers was found in Neelum. The significant differences in sex ratio noticed among the cultivars studied may be due to their genetic makeup, time of flower, response to prevailing environmental conditions and the level of endogenous growth hormones.

In the present study, indications of physiological parameter, the highest values for total phenol content, soluble protein content and lowest IAA oxidase activity and gibberellic acid content were registered by Neelum during main season followed in Kalepad during main season (Table 2, Figures 1, 2, 3 and 4). Pal and Ram (1978) opined that the activity of gibberellin (GA) like substances was found to be greater in the 'off' year and postulated that high levels of gibberellin inhibit flowering in mango. The similar results suggested that Chandler (1950) proposed a hypothesis that flower induction in mango could occur only when the cell division had started and that a flower inducing hormone played no part in the initiation of growth; but when presented with insufficient amount at the beginning of growth, it determined the course of differentiation of tissue in the axillary buds. He also proposed that if a hormone induced flowering in plants and the source of hormone was the leaf or some precursor formed in the leaf, then the leaf surface rather than the accumulation of carbohydrates might have the dominant influence on flowering. This might be due to environmental factors that influence the accumulation of total phenol, and it might be due to the excess production of hydrogen peroxide by increased respiration (Farkas and Kiraly, 1962) or due to the activation of hexose mono phosphate (HMP) shunt pathway, acetate pathway and release of bound total phenols by hydrolitic enzymes. A reverse trend was observed in respect to IAA oxidase activity which was lower in the flowering shoots than in the vegetative shoots, thus indicating higher content of ascorbic acid, RNA and total phenolics. Lower IAA oxidase activity, may have a positive association in the flowering of mango. Besides, a lower level of gibberellin-like substances and higher levels of cytokinin-like substances, growth inhibitors and ethylene have been indicated to be the prime factors favourable for induction of flowering in mango (Tekchand, 1980).

The depletion in sugar level was found to be responsible for the accumulation of total phenols since the sugars are utilized for the synthesis of total phenols. Total phenol exhibited the highest levels during flower bud differentiation. The results are in conformity with the findings of Misra and Dhillon (1981). Total phenols and soluble protein content were reportedly increased during the period of fruit bud differentiation (Patel et al., 1992). Del Rio et al. (1978) confirmed that the above results nitrogen content in the leaves could enhance the soluble protein synthesis throughout the growth phase of the

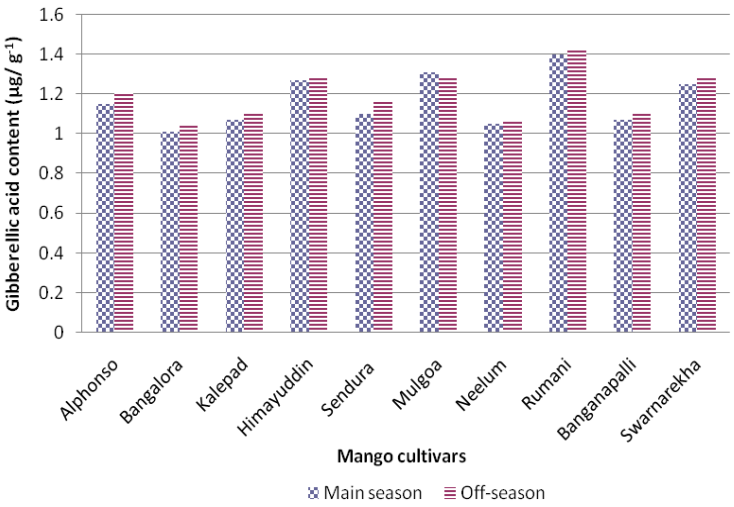

Figure 2. Gibberellic acid content (µg/g^{-1}) of mango.

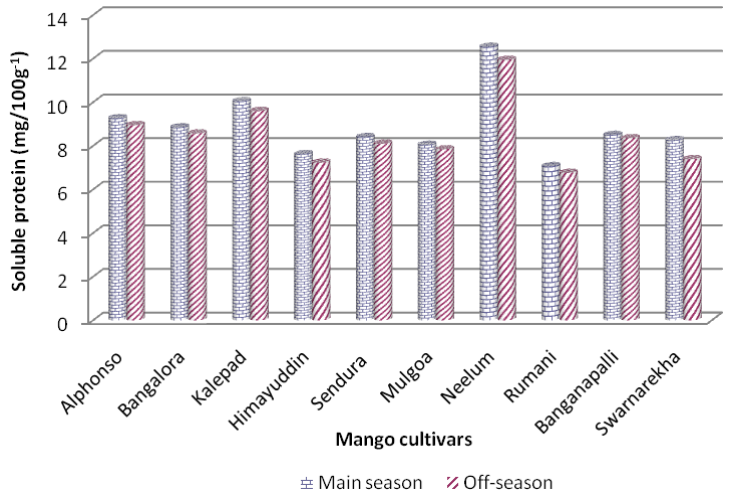

Figure 3. Soluble protein (mg/100 g^{-1}) of mango.

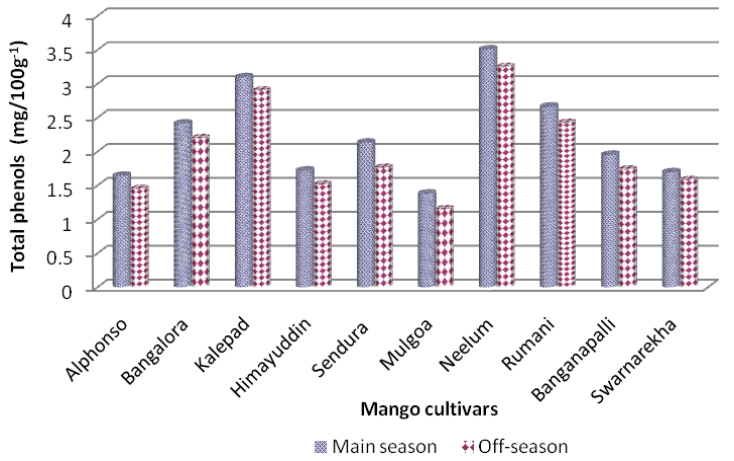

Figure 4. Total phenols (mg/100 g^{-1}) of mango.

plant by direct participation as an essential constituent of soluble protein. At flowering stage, there was a low rate of IAA oxidase activity which might have resulted in greater amount of auxins in the leaves (Vijayakumar, 2001). It was also revealed that high yielding plants had favourable auxin balance through IAA oxidative degradation. The present results corroborate with the findings of Reece et al. (1949). The hormonal studies on the mango varieties showed lower levels of IAA oxidase activity and of gibberellin favour flowering (Chacko et al., 1970). The shoot tip of Dashehari contained during flower bud differentiation several fold higher auxin in the “on” year than the “off” year. This is in conformity with the earlier findings of Lal and Ram (1977). The auxin concentration was greater in the buds of Langra during its “on” year than “off” year at flowering stage. This was in corroboration with the findings of Upreti and Murti (1993). Singh (1961) reported that newly emerged leaves in the shoot of regular bearing cultivars such as Neelum was capable of synthesizing flower inducing hormone. During floral induction period, the apical bud of an on year tree attracted photosynthates. Photosynthates moved basipetally to the main stem and root system during branches; movement was towards the developing sink in fruit (Chacko, 1984). The shoots from Dashehari “on” year and Totapuri “on” year had higher levels of growth promoting substances during the period of flower bud differentiation. This was in conformity with the findings of Chacko (1968). Studies so far have shown that during both the preceding period and floral initiation of mango shoot, leaves or xylem sap contain higher levels of auxins, abscisic acid, cytokinins and steroids when compared to non-flowering trees.

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Scientific Research and Essays

Full Length Research Paper

Effect of *Phyllanthus amarus* leaf extract on alterations of haematological parameters in *Salmonellae typhi* infested wistar albino rats

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Haematological indices provide crucial information to assessing the well-being of an organism. In this present study, the antihaematotoxic effect of *Phyllanthus amarus* leaf extract on *Salmonellae typhi*–induced haematotoxicity in rats were investigated. The experimental animals were randomly divided into three study groups. Group 1 received feed and water and was not induced with typhoid (negative control). Groups 2 and 3 received, in addition to feed and water, single dose of stock *S. typhi* at a concentration of 10^6 cfu/ml. After 7 days, Widal test confirmed typhoid infection and rats in Group 2 were not treated with the leaf extract but rats in Group 3 were treated with 750 mg/kg body weight ethanol extract of *P. amarus* for 10 days at the end of which animals were sacrificed and blood obtained for haematological indices using standard laboratory methods. In Group 2 (positive control), there were significant ($P < 0.05$) decrease in red blood cell (RBC) count, packed cell volume (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), neutrophils and increase in platelet, total white blood cell (WBC) and lymphocytes relative to the non-induced negative control. In Group 3, the rats recorded a significantly ($P < 0.05$) higher mean values in RBC count, PCV, Hb, MCH, MCV, MCHC and lower values in platelets, WBC and lymphocytes when compared to the *S. typhi* induced positive control (Group 2). The results suggest that treatment of *S. typhi* infection with ethanol extract of *P. amarus* reverses and ameliorates the haematotoxic effects induced by *S. typhi* infection in rats.

Key words: *Salmonellae typhi, Phyllanthus amarus,* Blood cells, antihaematotoxic, rats.

INTRODUCTION

Typhoid fever (also called enteric fever) is an acute life threatening febrile illness caused by the bacterium *Salmonellae enterica typhi* (Kotton, 2007). It is the second most common cause of fever, second only to malaria, particularly in the tropics (Wilcocks and Manson-Bahr, 1972). An estimated two million cases of typhoid and two hundred thousand related deaths each year have been reported (Crump et al., 2004). It is contracted through contaminated food and vegetables (Crum, 2003). In developing countries like Nigeria, *S. typhi* infection is endemic/prevalence and account for high rate of morbidity and mortality, particularly due to inefficient

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water carriage method of sewage disposal (Crump et al., 2004). One challenge of a developing country is provision/or availability of portable water for her citizens which have a negative impart on their sanitation. Poor sanitary and hygiene have been reported to increase the prevalence of *S. typhi* infection while reduced incidence in developed countries has been attributed to high level of hygiene (Kotton, 2007). Gastroenteritis, the most common disease caused by *S. typhi* infection is characterized by nausea, vomiting and diarrhoea (Parry et al., 2002). This is possible as *S. typhi* escape the macrophage cells and enter the spleen, liver and other organs where it thrives and re-enter the blood (Jones and Falkow, 1996). These tissues/organs are prone to damage by bacterial toxins which are released by bacterial cells to the host organism during the process of metabolism. This tends to disrupt the blood components/cells or blood forming tissues.

Blood is one of the specialized body fluid responsible for the transportation of nutrients, oxygen, hormones and other metabolites to the body's cell and metabolic waste products away from those cells to sites of elimination. It is known to be the most important body fluid that regulates various vital functions of the body. Mammalian circulation of blood transports such specific chemical substances as nutrients, gases, minerals, metabolic products and hormones between different tissues and organs (Baynes and Dominiczak, 2005). Available reports showed that haematological profiles of different species of animals may be influenced adversely by diabetic condition (Edet et al., 2011), phenylhydrazine (Sanni et al., 2005), and aqueous leaf extract of *Ocimum gratissimum* (Obianime et al., 2011).

Phyllanthus amarus is a tropical shrub indigenous to the rainforest of Amazon and other tropical areas of the world (Samraj, 2001). It belongs to the family Euphobiacea and classified as a type of *Phyllanthus nururi* (Kassuya et al., 2005). The plant has been valued in many countries for its medicinal properties and curative potentials for a variety of ailments such as asthma/bronchial infection (Lizuka et al., 2006), jaundice and hepatitis B and other viral infections (Huang et al., 2003). It exhibits inhibitory effect on human immune virus (HIV) and reverse transcriptase activity (Notka et al., 2004). Nwanjo et al. (2007) has reported the hypotensive, hypoglycaemic and hypocholestrolemic effect of *P. amarus* extract on hepatocytes of diabetic rats while Nwankpa et al. (2012) has reported the antioxidative effect of the plant extract on *S. typhi* induced oxidative stress in rats. The in *vitro* and antimicrobial activity of the plant extract against *Staphylococcus, Micrococcus* and *Pasteurella* spp has been reported (Agrawal et al., 2004).

In rural communities in Nigeria, *S. typhi* infection is endemic and people resort to the use of *P. amarus* for the management of typhoid fever and related cases without recourse to the haematological effects. This study was therefore designed to investigate the effects of *P. amarus* on haematological profiles in *S. typhi* infested albino rats.

MATERIALS AND METHODS

Plant materials

The fresh leaves of *P. amarus* were harvested from the natural habitat in Owerri, Imo State, Nigeria. They were identified and authenticated by Professor S.C. Okeke of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. A voucher samples are kept in the University herbarium for reference.

Preparation of extract

The fresh leaves of *P. amarus* were washed free of sand and debris. Large quantities were dried under shade at room temperature of 27°c for three weeks. The dried leaves were homogenized with an electric blender to get a coarse powder used for the extraction. 700 g of the powder were macerated in 1.1 L of 80% (v/v) ethanol. The mixture was allowed to stand for 24 h after which it was filtered with a chess cloth. The filtrate was concentrated in vacuo at low temperature (37 to 40°c) to 10% of its original volume using a rotary evaporator. The concentrate was placed in a water bath (40°C) to evaporate and the solid residue referred to as extract. Approximate concentration of the extract was made in 100 ml of 10% ethanol for the experiment.

Salmonellae typhi

Stock *S. typhi* was obtained from Federal College of Veterinary and Medical Laboratory Technology of the National Veterinary Research Institute, Vom, Jos, Plateau State, Nigeria. The stock *S. typhi* was sub-cultured into nutrient agar plates, cesteine lactose electrolyte deficient plate (DCA). Plates were incubated at 37°c for 24 h and examined for growth. Stock culture slants were then prepared using McCartney bottles and nutrient agar. The organism from the sub-cultured plate was then aseptically incubated for 18 h.

Animals

Albino wistar rats weighing between 150 to 200 g of both sexes maintained at room temperature in the Animal House of the Faculty of Medicine, Imo State University, Owerri, Nigeria were acclimatized for 12 days to daily handling and were fed ad-libitum with normal commercial rat chow (Product of Pfizer Nigeria Ltd) and water.

Induction of typhoid

One (1) ml of *Salmonellae typhi* at a dose of 10^6cfu/ml was orogastrically administered to the rats to induce typhoid (Kirby-Bauer, 1960).

Experimental design

Twenty-four rats used for this study were randomly assigned into three groups of eight animals each.

Group 1: Rats in this group were not induced with typhoid fever and were fed with normal commercial rat chow and has free access

Table 1. Physical examination/observation of the rats in both experimental and control groups.

Group	Treatment	Soft and mucous feacal matter	Loose and erect hairs	Vomiting
1	Negative control (water)	-	-	-
2	*Salmonellae typhi* (positive control)	+	+	-
3	*Salmonellae typhi* + *Phyllanthus amarus*	-	-	-

+= Present; - = Absent.

Table 2. Serology test of rats in Groups 2 and 3 infected with *Salmonellae typhi* before treatment with *Phyllanthus amarus* leaf extract.

Group	*Salmonella* antigen	Antibodies	
		O	H
2	*Salmonella Paratyphi* A	-	-
	Salmonella Paratyphi B	-	-
	Salmonella Paratyphi C	-	-
	Salmonella typhi	+	+
3	*Salmonella Paratyphi* A	-	-
	Salmonella Paratyphi B	-	-
	Salmonella Paratyphi C	-	-
	Salmonella typhi	+	+

+ = agglutination (*Salmonella typhi* present), - = No agglutination (*Salmonella typhi* absent); Titre values ≥ 1/160 were considered positive.

to water throughout the period of the experiment. They were used to monitor successful induction of typhoid.

Group 2: The rats in this group served as control. They were fed with normal rat chows and orogastrically given single dose of *S. typhi* at 10^6cfu/ml. After 7 days of infection, the rats were observed to have loose and erect hairs as well as soft mucous feacal matter signifying signs of infection and diarrhoea. The rats in this group were not treated with the plant extract.

Group 3: The rats in this group were fed with normal rat chow and orogastrially given single dose of *S. typhi* at 10^6cfu/ml. After 7 days of infection, the rats showed signs of infection as the rats in Group 2. Serology test, tube agglutination method (Cheesborough, 2005) were used to test for O & H antibodies using a commercial prepared antigen suspension (BSL Global Plasmatic, UK. Code FAT 1010 and 1002 for O & H respectively) to confirm *S. typhi* infection after which they were orogastrically given 750 mg/kg ethanol leaf extract of *P. amarus* daily for 10 days.

Collection and preparation of blood samples for analyses

Twenty-four hours after the last treatment was given, all the rats were weighed and quickly sacrificed under chloroform vapour anesthesia. With a sterile syringe and needle, 5 mls of blood was collected from each animal by cardiac puncture into EDTA treated screw-cap sample bottles. The anti-coagulated blood samples were used for haematological analyses which were carried out within 24 h of sample collection.

Haematological analysis

Full blood counts such as packed cell volume (PCV), red blood cell (RBC), haemoglobin (Hb), total white blood cells (TWBC), platelet count, differential white blood cell (lymphocytes, monocytes, neutrophils, eosinophils) and red cell indices including mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean cell haemoglobin concentration (MCHC) were estimated using the Sysmex® Automated Haematology Analyzer KX-2IN, Sysmex Corporation, Kobe, Japan.

Statistical analysis

Statistical evaluation of the data generated was carried out using one-way analysis of variance of the SPSS window Statistical software Programme. This was followed by the student's t-test of significance. Values were declared significantly different at $P<0.05$.

RESULTS

The results of physical examination of the rats in uninfected and untreated, infected and untreated as well as infected and treated groups are shown in Table 1. The infected and untreated rats (Group 2) were observed to have loose and erect hairs (a sign of fever) and soft and mucous feacal matter (a sign of diarrhoea). These were not observed in uninfected and untreated (Group 1) and infected and treated rats (Group 3). However, the rats in all the groups showed no sign of vomiting. Serology result of the rats in Group 3 is shown in Table 2. The result confirms the presence of O and H antibodies in the serum of the rats indicative of typhoid fever.

Tables 3 and 4 shows the effect of *S. typhi* infection and subsequent treatment with 750 mg/kg body weight daily ethanol leaf extract of *P. amarus* on haematological parameters in albino rats. The results showed a significant ($P < 0.05$) decrease in red blood cells (RBC) count, packed cell volume (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and percentage nuetrophil levels in *Salmonellae typhi* infested rats compared to the non-infested group (Tables 3 and 4). On the contrary, the total white blood cell (WBC), platelets and lymphocyte levels in rats infested with *S. typhi* showed a significant ($P < 0.05$) increase compared to the non-infested group (Table 3). Treatment of the rats in Group III with ethanol leaf extract of *P. amarus* showed a

Table 3. Effect of *Phyllanthus amarus* on mean values of red blood cells, packed cell volume, hemoglobin and red cell indices in both experimental and control groups.

Group	Treatment	RBC (×10^{12}/L)	Hb (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
1	Negative control (water)	4.02 ± 0.13	15.6 ± 1.35	48.33 ± 1.14	60.13 ± 1.52	18.54 ± 1.21	33.27 ± 1.11
2	*Salmonellae typhi* (positive control)	1.70 ± 0.65^{a}	10.53 ±1.20^{a}	32.16 ± 1.12^{a}	49.18 ± 1.13^{a}	12.78 ± 1.38^{a}	25.16 ± 0.89^{a}
3	*Salmonellae typhi* + *Phyllanthus amarus*	3.89 ± 0.21^{bc}	14.95 ± 0.51^{bc}	47.13 ± 0.8^{bc}	58.85 ± 1.28^{bc}	17.18 ± 0.82^{bc}	32.16 ± 1.22^{bc}

Mean ± SD (n = 8); ^{a} Significantly different compared with negative control ($P < 0.05$); ^{b} Significantly different compared with positive control ($P < 0.05$).; ^{c} No significant difference compared with negative control ($P > 0.05$).

Table 4. Effect of *Phyllanthus amarus* on mean values of platelets, total white blood cells and differential cell counts in both experimental and control groups.

Group	Treatment	Platelets (×10^{3}µL^{-1})	TWBC (×10^{3}µL^{-1})	Lymphocytes (%)	Neutrophils (%)	Eosinopils (%)	Monocytes (%)
1	Water (Negative control)	850.18 ± 1.51	14.15 ± 0.81	73.65 ± 1.56	22.56 ± 1.30	2.85 ± 0.67	2.68 ± 0.72
2	*Salmonellae typhi* (positive control)	872.56 ± 1.61^{a}	23.82 ± 1.40^{a}	85.72 ± 1.23^{a}	12.52 ± 1.12^{a}	3.21 ± 0.13^{c}	2.90 ± 0.33^{c}
3	*Salmonellae typhi* + *Phyllanthus amarus*	848.17 ± 1.35^{bc}	16.47 ± 0.6^{bc}	72.56 ± 1.56^{bc}	21.68 ± 0.81^{bc}	2.75 ±0.15^{c}	2.79 ± 0.75^{c}

Mean ± SD (n = 8); ^{a} Significantly different compared with negative control ($P < 0.05$); ^{b} Significantly different compared with positive control ($P < 0.05$); ^{c} No significant difference compared with negative control ($P > 0.05$).

significant ($P < 0.05$) increase in RBC count, Hb, PCV, MCH, MCV, MCHC and percentage neutrophil levels compared to the *S. typhi* infested non-treated (positive control) group (Tables 3 and 4). However, the treatment of rats in Group III with ethanol leaf extract of *P. amarus* showed a significant ($P < 0.05$) decrease in platelets, WBC and lymphocyte levels compared to the non-treated *S. typhi* infested group (positive control) Table 3. The results obtained in this study showed no significant ($P > 0.05$) difference in RBC, Hb, PCV, MCV, MCH, MCHC, platelets, WBC, and lymphocytes in *S. typhi* infested rats treated with the plant extract, compared to the non-infested rats (Tables 3 and 4).

DISCUSSION

Blood is known to be the most important body fluid that regulates various vital functions of the body including transport of metabolic substances and defence against foreign substance, among others. Nutritional, environmental and bacterial infection are among several other factors which have been shown to have adverse effects on the haematological indices of most organism (Jee et al., 2005; Uboh et al., 2009; Savithri et al., 2010). Bacterial infection in living cells cause cellular damage to the host organism by the release of toxins which alter the process of host metabolism and in most cases lead to an increase in free radical species (Stipanuk, 2000). In this study *S. typhi* infection significantly decreases the mean levels in RBC, PCV, Hb, MCV, MCH, MCHC, neutrophils and increase in WBC and lymphocytes which agrees with the symptoms of fever and diarrhoea observed in physical examination of the rats. The observation made in this study agrees with the report of Wlicocks and Manson-Bahr (1972) on *S. typhi* infection and Kumar and Kuttan (2005) on cyclophosphamide – induced toxicity. The haematotoxic effect of *S. typhi* infection was due to the interaction of the bacterium or its toxins with the blood forming tissues/organs which inhibit the rate at which some specific or generalized haematopoeitic committed stem cells are synthesized by the tissues. This was connected to the damage of the tissues, particularly haematopoeitic tissues by the bacterium. Benzene and cyclophosphamide-induced haematotoxic effects have been reported to be associated with the interaction of their metabolites with the haematopoeitic tissues which cause suppression and depression of their haematopoeitic activities (Synder and Hedli, 1996; Kumar and Kuttan, 2005). The reports showed that the metabolites of these chemicals can interact with the red blood cell membrane proteins to increase the rate of red blood cell destruction. Therefore, the decrease in RBC counts, Hb and PCV observed in this study were due to retarded haematopoeisis, destruction and shrinkage of RBC while the decrease in MCV, MCH and MCHC may likely be due to destruction of RBC and decrease in Hb synthesis and haemoglobin content. The observed result is an indication of anaemic condition. Significant increase in total white blood cell and lymphocytes as well as decrease in neutrophils observed in this study is consistent with the reports on the effect of insecticides and pesticides such as fenvalerate, aldrin and lidane on total while blood cells and the differential counts in experimental animals (Synder and Hedli, 1996; Kumar et al., 1996; Savithri et al., 2010). This was explained by increased lymphopoeisis and or enhanced release of lymphocytes from lymph myeloid tissue (Das and

Mukherjee, 2003). This response may be a direct stimulatory effect of toxic substance on lymphoid tissue or chemical (toxin) induced tissue damage and disturbance of the non-specific immune system leading to increase in production of leukocytes. Neutrophils are known to be involved in the phagocytosis of foreign chemical substances in the body during which some of them are ruptured. This explains the observed decrease in neutrophil count on infection with *S. typhi*.

Ethanol leaf extract of *P. amarus* significantly increased the level of RBC, Hb, PCV, MCV, MCH and MCHC thereby reversing/ameliorating the anaemic condition induced by *S. typhi* infection. The rats were observed to recover from fever and diarrhoea. The observed increase in RBC, Hb, and PCV recorded in this study on administration of ethanol leaf extract of *P. amarus* were due to reversal of bone marrow depression thus improving haematopoeitic activity of the cells and the improvement in erythrocyte membrane integrity through the antioxidant potential of the extract, thereby reducing haemolysis (Naaz et al., 2007; Nwankpa et al., 2012). Also bacterial infection causes deoxyribonucleic acid disintegration and has been shown to be ameliorated by the bacteriocidal effects of the extract (Okigbo and Ajalie, 2005), leading to an increase in protein synthesis and cell proliferation (Rajinder et al., 2008). Increase in protein synthesis may as well explain the increase in the level of Hb observed in this study. Expectedly, increase in RBC count on administration of *P. amarus* extract results to increase in MCV while increase in Hb results to increase in MCH and MCHC. In this investigation, it was observed that there were significant decrease in total white blood cell, lymphocytes and an increase in neutrophils on administration of *P. amarus* extract on *S. typhi* infected rats. The decrease in WBC and lymphocytes may be due to the inhibition of growth of *S. typhi* (bactericidal effect) by the plant extract leading to the destruction of WBC and lymphyocytes. Similar results have been reported on the inhibition of growth of some human pathogens by the plant extract (Notka et al., 2004; Agrawal et al., 2004). However, the increase in neutrophil may be explained by reduced phagocytosis of the microbial cell by neutrophil due to the drastic reduction in microbial growth.

Conclusion

Adverse effects on haemotological profiles of an individual may predispose the individual to anaemia. This study has established that ethanol leaf extract of *P. amarus* reverses anaemic condition induced by *S. typhi* infection in albino rats. This lends credence to recovery from fever and diarrhoea.

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