EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 2
Issue: 6
138-144
Year: 2015

CONSEQUENCES OF BIOGENIC AMINES IN *SETARIA DIGITATA* ON CATTLE FILARIAL PARASITES

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Article Received on 24/08/2015 **Article Revised on 15/09/2015** **Article Accepted on 06/10/2015**

ABSTRACT

The present study expressed the analysis of biogenic monoamines and their metabolites in *Setaria digitata*. It is almost dominantly present in the South East Asia and Western Pacific Regions. The present study was highlighted the lymphatic filarial worm of *S. digitata* in India. From the present study clearly revealed about the effect of diethyl carbamazine (DEC) on the levels of neurotransmitter amino acids and on the activities of related enzymes of *S. digitata* also accompanied with its efficiency on different parts of the body parts have been studied. Moreover, the present result clearly revealed about the four kinds of biogenic amines such as Serotonin, Histamine, Dopamine and Ephinephrinand. Based on the Diethyl Carbamazine level, whenever increased the concentration effect of Serotonin also been increased such as 38.7±3.1, 45.4±3.9, 51.4±3.7 range on 0.25, 0.5 and 0.7 Concentration of DEC (mM) respectively. Similarly the Dopamine, Histamine and Epinephrine or adrenaline also showed the when the concentration of DEC increased effect on the experimental sample possessed biogenic amines were increased. It was statistically significant result at the level of 0.01% of correlation co-efficient. Furthermore, the present work has been explained the activity of certain aminoacid metabolizing enzymes present in *S. digitata*. Apart from the result GOT^b, GOT^c, GDH^a and GDH^b maximum activity noticed on the whole worm, though the other two enzymes didn't showed much more variations such as 0.046 ±0.013 and 0.145±0.01 of GOT^b, GOT^c respectively. Eventhough, amoung the four experimental parts reproductive tissue possessed GDH^a maximum level 68.03±10.3 than remaining body parts also GOT^b also manimum observed on fluid than the other three parts followed by GOT^c maximum noted on muscle tissues.

KEYWORDS: Effect of diethyl carbamazine (DEC), Serotonin, Histamine, Dopamine and Ephinephrin.

INTRODUCTION

The diseases caused by many infectious agents, the magnitude of the public health problems posed by these infections, and the paucity of effective control measurers from obstacles to economic progress and better life most of the developing countries of the tropical world. Long-term exposure and repeated infections can cause severs damages to the lymph system and serious debilitating complication (Lazdins and Kron, 1999). Filariasis afflicts more than 100 million people in the world, mostly living in the tropical countries. A larger number are prone to infection owing to their proximity to endemic areas (Lacey, 1988; Lee and Atkinson, 1976)). The World Health Organisation (WHO) estimated that at least 120 million people in 73 countries worldwide are estimated to be infected with filariasis parasites. The most widespread is Wucherera Bancroft which affects about 100 million people in Africa, India, Southeast Asia, the pacific islands, South America, and the Caribbean. The Brugia malayi and Brugia timori parasites affect about 12 million people in Southeast Asia.

The serious nature of this disease and the magnitude of the problems posed by it were so well recognised by the WHO that the world body included filariasis in its special programme of Tropical Diseases Research and Training (Dorozyski A, 1976). It was estimated by the National Filariasis Control Programme (NFCP) in 1980 that in India at least 14 million people had chronic symptomatic lymphodema and elephantiasis and that the figure would go up to 30 million in 1990.

Lymphatic filariasis is endemic in many tropical and subtropical countries of Africa, central and South America and South and South East Asia from India in

the west to Korea in the East (Hawking F, 1979). It continues to be of great concern in the developing towns of Asia, where it is increasing in prevalence (Sasa, 1976).

In 1958 it was estimated that 65 million people were exposed to the risk of filariasis. The figure rose to 125 million in 1969, to 236 million in 1976 (Joseph *et al* 1967) and to 300 million in 1982, according to a survey undertaken in that year (Manson *et al* 1987). In kerala *Bancroftian filariasis* is prevalent in the Southern and Northern parts of the state, and Brugian filariasis, in the central coastal region (Denham *et al*; 1977). The biogenic amines are widespread in invertebrates,. Besides their ro0le in neurotransmission they regulate many other functions in the helminths.

Host serotonin level was found to play a part in the curious diurnal migration which some species like *Hymnolepis diminuta* make up and down the intestine. In addition, serotonin appears to effect ovulation and circadian rhythm in insects./ however, it has now been demonstrated that *H. diminuta* has the capacity to synthesize serotonin both from tryptophan and 5-h ydroxy tryptophan (Gianutsos *et al*., 1977).

Table 1: Human Filarial Parasites

Species	Tissue	Vector
W. bancrofti	Lymphatics	Mosquito
B. malayi	Lymphatics	Mosquito
B .timori	Lymphatics	Mosquito
O volvulus	Skin	Simulium
Loa loa	Connective tissue	Chrysops
Mansonella perstansq	Serous membrane	Culicoides
M .ozzardi	Serous membrane	Simulium and Culicoides
M .streptocerca	Skin	Culicoid spp

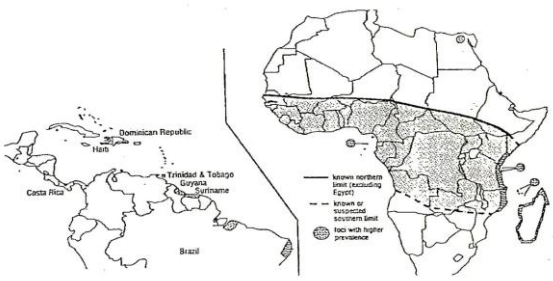

Fig- 1: Distribution of *W. Bancrofti* in the African Region, Region of the Americas and Eastern Mediterranean Region

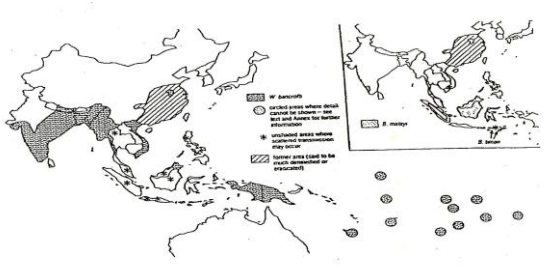

Fig- 2: Distribution of W. Bancrofti, Brugia malayi and *B. Timori* in the South East Asia and Western Pacific Regions

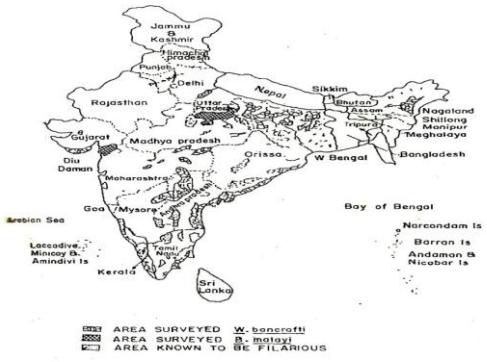

Fig.3. Filaria map of India (Distribution of Lymphatic filariasis in India)

MATERIALS AND METHODS

Composition of Tyrode Solution

Material	Gms/ 1000 ml
Sodium chloridfe	8
Potassium chloride	0.2
Sodium dihydrogen phosphate	0.5
Calcium chloride	0..2
Magnesium chloride	0.1
Sodium bicarbonate	
Glucose	5

With the help of a micro dissection needle, in ice cold condition the worm were open longitudinally the uterine tissue and the body wall (muscle along with cuticle) were separated. The pseudocoelomic fluid was collected through a minute puncture made at the anterior tip of the worm prior to dissection. Mf released by incubating adult female *S digitata* in Tyrode solution was filtered centrifuged and collected. All the chemicals used were purchased from Sigma Chemical Co USA.

MATERIALS AND METHODS

Estimation of Biogenic Amines

(i) Histamine

This method involves the extraction of serotonin from a salt saturated alkalinized homogenate with n-butanol. This serotonin is then returned to an aqueous phase by adding heptanes to n-butanol to lower the polarity and shaking the mixed solvents with dilute (Shore, 1971). The fluorometric method is based on the coupling of histamine with O-phthalaldehyde at a highly alkaline

PH to form a fluorescent product which is rearranged upon acidification to form an even more highly fluorescent and stable flurophore by the method of (Persky, 1955).

(ii) Dopamine was oxidised by iodine at H 6. The oxidation product was protected by sodium sulphite, isomerised in alkaline solution to a dihydroxyindole and stabilised by re-acidification (Melherbe, 1971). The eluate was neutralized to pH 6 and added 0.2 ml phosphate buffer, 0.1 ml of 0.01 N HCI and 0.05 Ml of 0.02 N iodine solution. After 3 min, 0.25 ml alkaline sulphite solution was added. After 5 min it was followed by 0.1 ml glacial acetic acid. To the blank, the reagents were added in the reverse order. Heated in a boiling water bath for 40 min. Read in a spectrophotofluorometer at an activation wavelength of 335nm and emission wavelength of 380 nm.

(iii) **Epinephrine** is oxidised to adrenochrome by ferricyanide and it is rearranged to fluorescent N- methyi 3, 5, 6 trihydroxyindole (adrenolutin) which is unstable unless protected by a suitable reducing agent, mercaptothanol, (Lavrty and Taylor, 1968). Since the tripydroxyindole thus obtained is unstable and can be easily oxidised, ascorbic acid is added together with NaOH eliminating excess oxidising agent (Vendasalu, 1960) and acetylcholine present (Augustinson, 1957).

Biogenic amines from various tissue analyses

The earliest colorimetric methods such as the AOAC Official method 957.07 which required careful attention to procedural detail and were tedious are not used today. The new colorimetric assay proposed by Patange *et al*. (2004) appears to be simple; its limit of quantitation is 10 mg/kg. Most of the others colorimetric methods require prior purification by cation exchange chromatography, i.e. the reaction between purified histamine and copper which form a visible red complex (Bateman *et al*., 1994). Tissues were weighed and homogenised with 75% ethanol, centrifuged at 900 g for 10 min, supernatant was taken and evaporated to dryness. The reside was resupended in DW to the volume directly proportional to the wet weight of the sample (1 ml/4 mg). Aliquot of supernatant was taken in small test tubes and were dried. The incubation of GABA assay consists of 1 ml pyrophosphate buffer, 0.2 ml NADP, 0.2 ml ketoglutarate, 0.2 ml mercaptothanol and 0.1 ml enzyme solution. A 15 l of this mixture was added to dried sample and standards while keeping in ice. The mixture was transferred to 38^0 C water bath for 15 min, and then returned to ice and added 50ul of phosphate solution. Then the tubes were transferred to a 600 C water bath for 15 min. From these mixtures 15l was removed and added to a tube containing 100 µl or 0.03% H_2O_2 in 10 N NaOH. The tubes were than heated at 60^0C for 10 min in the water bath, 1 mt of DW was added and the fluorescence was read at 485nm and activated at 375 nm.

In order to confirm the veracity and reliability of the observed date and to list the data observed are not caused by chance variation, the data were analyzed statistically.

Correlation coefficient, $r = \frac{\Sigma(xy) - \frac{(\Sigma x)(\Sigma y)}{n}}{\sqrt{x^2 - \frac{(\Sigma x)^2}{n}\, x\, y^2 - \frac{(\Sigma y)^2}{n}}}$

Where x = variable of values of drug concentration
y = variable of values of specific activity, n = number of observation.

or $r = \frac{Convariance}{S(x)(sy)}$ Where covariance (x, y) = $\frac{1}{n-1}$ $\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}$

S (x) = standard deviation of variables of x

$$\sqrt{S^2(x)} = \sqrt{\frac{1}{n-1}\left[\Sigma x^2 \frac{(\Sigma x)^2}{n}\right]}$$

Where S^2 (x) = variance of x.

S(y) = standard deviation of variables of y

$$\sqrt{S^2(y)} = \frac{1}{n-1}\left[\Sigma y^2 - \frac{(\Sigma y)^2}{n}\right]$$

Where S^2 (y) = variance of y

As a positive correlation existed between the two variables the regression line of y on x was calculated by calculating the slope B

Regression y = Covariance (x, y) Then a = y-bx S^2 (y)

Where $\overline{y}$ = mean of values of y and $\overline{x}$ = mean of values of x

In order to best the significance of differences between two experimental data as an estimate of their confidence limits of the observed differences or similarities, student t-test was applied

$$t = \frac{x_1 - x_2}{SE}$$

Where x_1 = mean of observation in one set of data and
X_2 = mean of observation in one set of data
SE = standard Error

$$SE = \sqrt{\frac{n_1 s_1 + n_2 s_2}{n_1 + n_2 - 2}\left[\frac{1}{n_1} + \frac{1}{n_2}\right]}$$

at $n_1 + n_2 - 2$ degrees of freedom.

Where n_1 = number of samples in one set of data
n_2 = number of samples in the other set of data and $S^2{}_1$ and $S^2{}_2$ the variance of samples of data 1 and 2 ‘t’ values obtained were compared to standard percentage points of ‘t’ distribution according to (Merrington, 1941) and the null hypothesis was accepted or rejected.

RESULTS AND DISCUSSION

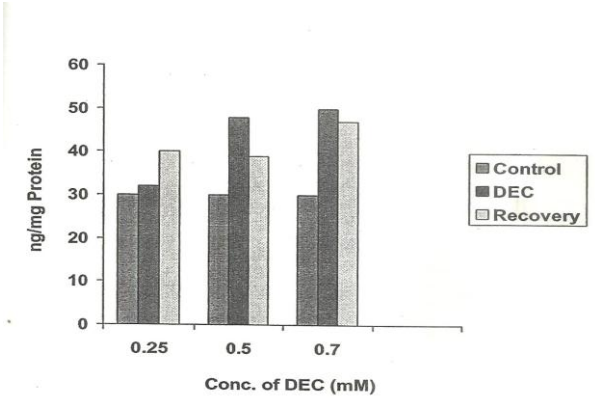

Fig:-4 Effect of DEC on the level of Serotonin

Table 2: Distribution of biogenic amines in different anatomical parts of *S. digitata*

Body Parts	Serotonin	Dopa	Dopamine	Epinephrine	Histamine
Whole	31.32±2.3	7.2± 0.34	9.4±0.63	2.9±0.3	**10.6±1.8**
Muscle	31.60 ±1.2	6.9± 0.52	7.1± 0.18	2.3±0.36	**5.3±0.4**
Reproductive Tissue	53.00± 2.5	5.3±0.43	6.7 ±0.54	2.4±0.3	**12.0±0.2**
Fluids	**18.90± 1.4**	**3.9± 0.56**	**4.2±0.73**	**4.8±0.3**	**51.3 ±0.2**

Values expressed as ng/mg/ protein n=6±SD

Table 3: Effect of DEC on the level of serotonin production in Filarial worm.

Criteria	Concentration of DEC (mM)		
	0.25	**0.5**	**0.7**
Control	9.4±0.316	9.4±0.32	**9.4±0.32**
DEC	9.88±0.26	10.08±0.27	**11.17±0.25***
Recovery	**9.36±0.24**	**9.58±0.186**	**10.4±0.8**

Values expressed as µg/mg protein are mean ± SD of 5 Separate observations. **P<0.001.*

Table 4: Effect of DEC on the level of production of histamine

Criteria	**Concentration of DEC (mM)**		
	0.25	**0.5**	**0.7**
Control	9.0±0.3	9.0±0.3	**9.0±0.3**
DEC	8.7±0.37	9.3±0.42	**8.8±0.18**
Recovery	**9.1±0.32**	**9.13±0.17**	**9.0±0.29**

Values expressed as µg/mg protein are mean. SD of 5 Separate observations. * P<0.001.

Table 5: Effect of DEC on the level production of Adrenalin

Criteria	Concentration of DEC (mM)		
	0.25	**0.5**	0.7
Control	2.6±0.80	2.6±0.80	**2.6±0.80**
DEC	3.0±0.42	3.4±0.21	**4.0±0.56***
Recovery	**2.8±0.51**	**3.0±0.12**	**3.3±0.49**

Values expressed as µg/mg protein are mean. ± SD of 5 Separate observations. * P<0.001.

Table 6: Activities of certain amino acid metabolizing enzymes in different regions of *S. digitata*

Body Parts	**GOT[b]**	**GOT[c]**	**GDH[a]**	**GDH[b]**
Whole worm	0.046±0.013	0.145±0.01	51.50±5.3	ND
Muscle	0.046±0.02	0.18±0.04	48.58±8.04	ND
Reproductive Tissue	0.057±0.1	0.11±0.01	68.03±10.3	ND
Fluid	0.0064±0.003	0.04±0.01	21.89±6.12	ND

The occurrence of amines in the parasite indicates that these biologically active molecules may be playing an important role in the life processes of this worm. The presence of serotonin, histamine, dopamine and epinephrine observed in *S. digitata* from the present study is similar to that observed in *Litmosoides carini* and *D. viteae*. But *S. digitata* adults had higher levels of different amines than the other two filarids. The earlier observations revealed the similar kind of findings by Vijayanathan and Raj. (1991); Lewis *et al*., (1980) the the presents of three biogenic amines viz serotonin, norepinephrine and dopamine in the trematode *Schistosoma japonicum* dopamine in *F. hepatica* and *Paragonimics westermani* and serotonin in *H diminuta*.

Though all the above mentioned amines and amino acids are found, a great variation occur in the distribution pattern in different body parts certainly points out towards the specific role of some of the amino acids and amines in a particular organ (Lacey and Pritchard, 1986). Transminases is often involved in amino acid synthesis and relevant systems are widely distributed in parasites especially a- ketoglutaric- glutamic acid and pyruvic alanine system (Von Brand, 1973). In *S. digitata* ketoglutaric acid- glutamic acid and the oxaloacetic- acid aspartic acid system were found to be present in the different tissues studied (Table-5). Whether aminotransferase systems are mediated by a common enzyme or a specific enzyme is not known. Transminase activity in the parasite cast some light on the possible function of the amino acids in the free amino acid pool and their relationship to those bound in the parasdite protein (Liebau *et al*., 1994a,b).
GDH showed very high activity in the reproductive tissue of the parasite (Table 5) although GDH has been demonstrated in a range of parasitic helminths as regulatory properties have only been studied in nematode *H. contortus* (Rhodes and Ferguson, 1973; Sasa, 1976) and in the cestode *H. diminuta*. Enzymes from both these helminths were found to differ considerably from typical animal sources. Presence of GDH indicates towards the possibility of a direct link between carbohydrate and amino acid metabolism. The high rate of decarboxylation of 5- hydroxytrytophan by the aromatic amino acid decarboxylase may account for the high concentration of serotonin in *S digitata*. The high level of serotonin in the reproductive tissue could perhaps be due to the high, activity of decarboxylase in this region. When the DEC inhibited both GOT and GPT in the parasite at a particular concentration inhibition of GPT was higher than GOT. At 0.7 mM DEC GOT had an inhibition of 32.8% while GPT had 55.7%. Activity of glutamate dehydrogenase was enhanced was enhanced and there was an increase of 42.89% in the worms which was incubated in 0.7 mM DEC for 4 hr.

The survival of most parasites in their natural habitual is largely department on their ability to remain in situ when exposed to peristaltic movement in the case of intestinal parasites or the movement of blood or lymph in the case of systemic parasites when kept in vitro these parasites show well co-ordinated rhythmical movements and these movements help the organism to locate and maintain themselves in the host this kind of similar study already described by (Bradley, 1961). Again, Baldwin and Moyles, (1949). Several chemotherapeutic agents that are effective against6 helminths affect specific mechanisms that regulate motility (Hiliman and Giblee, 1975). A difference between species with respect to resoponses to neuromuscular drugs is well demonstrated among parasitic helminthes (Del Castillo, 1969).

Motility is an important factor in maintaining a successful parasite life and it is generally agreed that the nerves system in the most vulnerable target to aim such agents (Liu *et al*., 1992). Nematodes are unusual in that their muscular cells are innervated by processes which pass from muscle to nerve and not as in other animals by nerves sending fibers to the muscle (Mansour, 1970; Molina, 2001). Certain neurotransmitters have shown to affect motility Acelycholine acts as a neurotransmitter throughout the animal kingdom. Acetycholine though an excitatory transmitter in certain nematodes acts as a inhibitory transmitter in certain cestodes and nematodes (Lazdins and Kron, 1999). Addition of minimum concentration of Acetylcholine into the bathing medium results in a marked increase in the movement of *S digitata*. Similar results have been reported in Ascaris (Bryant, 1975; Chandler, 1978). The stimulate effect of Acetylcholine was partially antagonized by high concentration of tubocurarine but not by atropine suggesting that the receptors are different from the mammalian system discussed by Pratt, (1977). GABA produced a flaccid paralysis in *S. digitata*. Biochemical evidence for GABA being a ganaglionic transmitter in any other invertebrate phyla is still limited though there are certain evidences implicating GABA to be a neurotransmitter agent in nematodes (Smyth and Manus, 1989). Acetylcholine probably results in depolarization and increased frequency of firing and GABA causes hyperpolarization of the membrane and brings about complete suppression of the rhythmic action potential and relaxes the musculature (Sasa, 1976; Piessens *et al*., 1987). These two neurohumors probably acts as excitatory and inhibitory neuromuscular transmitter respectively (Lacey, 1988). All the four important biogenic amines viz serotonin, histamine, dopamine, adrenaline were detected in *S. digitata* level of serotonin was found to be ten times higher than that of epinephnine. Under in vitro condition biogenic amines had no effect on the motility of the parasite (Liebau *et al*., 1994). Acetyl choline and GABA are probably the excitatory and inhibitory neurotransmitters in *S digitata*. DEC enhanced the level of amino acids and amines of *S digitata* (Liu *et al*., 1992).

CONCLUSION

From the present study revealed the conclusion of when the amines content of the parasite treated with DEC was characterized by significant increase in the level of all

the amines studied except histamine. The latter showed no change in level. The level returned to normal during the phase of recovery it was also encouraging to note that there was no change in the level of these constituents when the worms were incubated in a control medium in the absence of DEC for entire duration of the experiments. Meanwhile, Transminases of aspartic acid and glutamic acid were studied in the parasite. Oxyglutarate- glutamate amino transferase activity (GOT) was low when compared with the activity of pyruvate alanine transferase (GPT). The activity of GOT activity compared to other tissues. The accumulation of Acetylcholine in the parasite which might have desensitized the cholinergic synapses thereby causing paralysis of the parasite. Regulatory process involving the motility and metabolism of the parasite are different from those of the host. So it can be possible to exploit these strategies for the chemotherapy of these parasites. Pharmacological research on the properties and the mode of action of biogenic amines and receptor sites may also help to develop new drugs which will interface with these natural hormones. Hence, the present study was concluded that the effect of DEC of the activities on enzymes appeared to the account for the maximized aminoacid and particular Aminoacids.

ACKNOWLEDGEMENT

The authors are gratified to the management of Correspondent (**Rev, Fr. Jose Bright**) and The Principal (**Dr. Desayar**) in Malankara Catholic College, Mariagiri, Kaliyakkavila, for granting the permission to publish this paper. Then first author expressed the sincere gratitude to her Guide for all direction for successfully completed this research manuscript.

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