Starvation And Associated Neuropathies In The Hippocampal Formation Of Adult Wistar Rats (Rattus Norvegicus)

Fakunle P. B, Ajibade A. J

ABSTRACT:- Neurohistological integrity of hippocampus when exposed to starvation was investigated in thirty adult wistar rats of average weight 200g . They were acclimatized for 2weeks on standard laboratory mouse chow and provided water ad libitum. They were subsequently randomly distributed into 3 groups (n=10) of Control C, Treatment T1 and Treatment T2. Treatments T1 and T2 animals were exposed to starvation periods of 3 and 14days respectively and sacrificed by whole-body intracardiac perfusion fixation under gravity for routine histological techniques. Statistical analysis showed significant body weight loss P0.05 in T1(Mean±sem, 194.8±6.1)g compared to the control section with Mean±sem,225.0±6.21g.Histoarchitecture of treatment group T2 revealed distinct hippocampal subfields of CA1,CA2,CA3 and the DG (Dentate Gyrus) with pyknotic pyramidal cells and scantily distributed glial cells compared to the control, these findings were less marked in the T1 cells while the control group cells appeared normal. Also, significantly($P<0.05$) reduced neuronal population of 11%, 8% and 14 % in CA1, CA2 and CA3 subfields respectively for Treatment group T1 compared to the control group.The neuronal transverse diameter for the pyramidal cells revealed statistically significant($P<0.05$) values Mean±SEM (1.20±0.01)µm was obtained in treatment group T1 compared to the control group.Long term starvation has severe and deleterious effects on the cellular integrity of hippocampus, it may thus underline problems associated with memory and learning.

KEYWORDS:- Hippocampus, Starvation, Rats, Pyramidal Neurons, Memory

INTRODUCTION

The existence of acute and chronic malnutrition in populations across the world is of concern both because of the immediate effects on morbidity and mortality and because of the possible long-term implications[1].One of the most important advances in the understanding of eating disorders is the recognition that severe and prolonged dietary restriction can lead to serious physical and psychological complications [2].A situation whereby there is serious or total lack of nutrients needed for the maintenance of life, or a severe reduction in vitamins, nutrients and energy intake is termed starvation [3] and is the most extreme form of malnutrition [3].However, a cell's function depends not only on receiving continuous supply of nutrients and eliminating metabolic waste products but also on the existence of stable physical and chemical conditions in the extracellular fluid bathing it [4]. As far as public health is concerned, its gravest threat is hunger. Adequate nutrition has two components—necessary nutrients and energy in the form of calories. It is possible to ingest enough energy without a well-balanced selection of individual nutrients and produce diseases that are noticeably different from those resulting from an overall insufficiency of nutrients and energy [5].

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It is possible to consume a seemingly adequate amount of food without getting the required minimum of energy [6]. Tissues differ in the ratio of different fuels used: the brain and spinal cord, and also the red blood cells, can normally utilize only glucose as excess from the diet are converted to storage forms, mainly as lipid in fatty tissue and as glycogen in the liver. Children who get enough calories, but not enough protein have kwashiorkor. This is typical in cultures with a limited variety of foods that eat mostly a single staple carbohydrate like maize or rice. These conditions overlap and are associated with multiple vitamin and mineral deficits, most of which have specific names and set of problems associated with them . Adults can survive for many weeks without food, provided they have water but for just how long depends partly on the extent of their body stores of nutrients, mainly fat. But unfortunately it is not only the fat which is broken down to simpler substances to be used for metabolic energy production and for essential repair and maintenance of the body's tissues. As soon as carbohydrate stores have run out, proteins are mobilized from muscles for the manufacture of sugars by the liver, causing progressive physical weakness [3].The physiological priorities in the face of zero food intake no doubt evolved in early millennia when hunting and gathering was an unpredictable and variable source of food. The first priority is to provide the brain with glucose, which is its staple diet, and this requires a certain level of glucose in the circulating blood [7]. The carbohydrate store in the form of liver glycogen is used first to provide this glucose but is used up in the first day or two. Then glucose has to be made from lactic acid and from amino acids derived from muscle protein, released into the blood and taken up in the liver [8]. A fall in blood glucose directly affects the endocrine part of the pancreas to change the balance of its hormonal secretions, suppressing insulin and enhancing glucagon synthesis and release. A fall in blood glucose is sensed also in the hypothalamus in the brain, which is the coordinating centre for homeostatic processes which tends to maintain the body's status [9]. This orchestrates a complex hormonal response and also switches on autonomic nervous mechanisms, which stimulate the release or synthesis of glucose in the liver; adrenaline

secretion is increased from the adrenal medulla; and the anterior pituitary gland is brought into action, releasing growth hormone, and ACTH which in turn stimulates the release of cortisol from the adrenal cortex [9].It is a clear fact that the brain is a principal consumer of glucose as its energy demand is about the highest of all other body organs . The brain being among the vital body organs has several parts responsible for ensuring coordination of normal body activities among which is the hippocampus that is majorly involved in the memory and learning. Expectantly, the hippocampus will definitely be affected during starvation especially when it is on a long term note;hence this study seeks to investigate to what extent will glucose deprivation affects the cellular integrity of hippocampal formation.

MATERIALS AND METHODS

Thirty adult wistar rats of both sexes (average weight 200±12.5g) were carefully assessed, screened and confirmed to be free from any pathological conditions. The rats were maintained in the Animal Holdings of the Department of Anatomy LAUTECH, Ogbomoso, Nigeria. They were fed with standard laboratory mouse chow (LADOKUN feeds Ibadan) and were given water *ad libitum*. The animals were kept in a clean environment at room temperature before and during the experiment. Their surroundings were maintained cleaned on daily basis.At the end of acclimatization period the animals were randomly grouped into three groups (N=10) of Control C and Treatments T1 & T2 (1 animal per cage for monitoring). Group C animals were allowed a free access to feed and water the second group T1 and T2 were starved (they were allowed to feed on standard laboratory mouse chow and given water freely for 30minutes once in 2days) for 3days and 14days respectively. At the end of starvation periods, the rats were sacrificed by whole body intracardiac perfusion fixation under gravity. First they were deeply anaesthetized with an overdose of penthotal followed by 0.9% normal saline solution followed with 10% formol calcium fixative. Fixation was monitored by the decolourization of the eyeball and tongue.The skull was opened with the aid of the dissecting set and the whole brain removed en-mass, trimmed to the region of the hippocampus using the stereotaxic coordinate method [10], and then further fixed in 10% formol calcium fixative. The brain sections were processed for routine histological techniques sectioned at 6µ and stained using Cresyl violet as described by [11]for nissl'ssubstance.Qualitative observations of CA1, CA2 & CA3 areas of hippocampus were done. Every 10th section was chosen from each animal. Using brightfield compound Nikon microscope, YS100 (attached with Nikon camera), the slides were examined and photographed under 400X objective. For each slide, two areas of CA1, one area of CA2, two areas of CA3 were randomly selected. Using Image-Pro Express software, count of neurons with prominent nucleolus within a measured rectangular area was performed in the selected regions. Random measurements of neuronal cell diameter were also taken for each region. The absolute neuronal density (P) per unit area of section was estimated using the formula P = A. M / L+M postulated by [12]; M = Section thickness in micron (6 micron); L = Mean nuclear diameter of respective area; A = Crude neuronal count per sq.cm of section.

Statistical analysis:

The data were analyzed using the computerized statistical package 'SPSS Version 11'. Mean and standard error of mean (SEM) values for each experiment group was determined. The means were compared by analysis of variance at a level of significance of 95% and 99%. Independent samples t-test was performed on the counts of each area (CA1,CA2, and CA3) to determine if there is any statistically significant difference in absolute neuronal count between the control and treatment groups. The neuronal transverse diameter for the purkinje cells was also determined. The absolute neuronal density per unit area of section for each region was estimated as previously described by [13].

RESULTS

Morphometric Analysis

Body weight: The results obtained here showed that therewas a gradual significant (P<0.05) weight loss(Mean±Sem,146.6±3.3)g in group T1 and an insignificant (P>0.05)weight loss (Mean±Sem,198.8±8.2)g in group T2when compared to the control group C(**Mean±Sem,** 225.0±6.21)g(Table 1)

Neuronal density

The three areas of hippocampus CA1, CA2 and CA3 showed higher neuronal densities per sq.cm in Treatment group T1 than in Treatment group T2compared to the control group. Treatment groups T2showed significantly reduced neuronal densities (**P < 0.05**) of Mean±sem (4467.3 ± 137.98,2483.3 ± 164.37and995.8 ± 36.97)/sq.cmcompared to the control section with Mean±sem (5654.8 ± 110.53,3065.8± 122.18 and 1778.3 ± 115.19)/sq.cm (**Table II**), while the neuronal density in Treatment group T1 was Mean±sem (5032.3± 124.07,2819.8 ±118.06 and 1529.3 ±36.97)/sq.cm but this was statistically insignificant (**P > 0.05**) compared to the control group.However the percentage reduction in the neuronal density for CA1, CA2 and CA3 subfields are 21%, 19% and 44% respectively for T2 and 11%, 8% and 14 % in CA1, CA2 and CA3 subfields respectively for Treatment groups T1 as seen in **Table III**

Neuronal diameter

The neuronal transverse diameter for the pyramidal cells revealed statistically significantly reduced values **(P<0.05**) for treatment groups T2 of Mean±SEM (0.51±0.03)µm in **Table iv** compared to Mean±SEM (1.30±0.01)µm obtained from the control section. The values obtained for neuronal transverse diameter for the pyramidal cells in treatment group T1 was Mean±SEM (1.20±0.01)µm which is insignificant **P>0.05** as compared to control group as seen in **Table iv.**

Histological Findings

The histoarchitecture in Treatment group T1 revealed distinct hippocampal layers of **CA1, CA2,CA3**and the **DG** (Dentate Gyrus) . The pyramidal cells appear distorted

while the glial cells are scantily distributed and the dentate cells appear normal compared to the control. The histoarchitecture of Treatment group T2 revealed distinct hippocampal layers of **CA1,CA2,CA3** and the **DG** (Dentate Gyrus) with the pyramidal cells appearing pyknotic while the glial cells are scantily distributed and the dentate cells shrunken when compared to the control.The control group C presents a pictorial histoarchitecture of distinct hippocampal layers of **CA1,CA2,CA3** and the **DG** (Dentate Gyrus) of evenly distributed and normally appearing glial and dentate cells including pyramidal cells .

DISCUSSION

The results of this study revealed here that long term starvation clearly affects body weight as well as neuronal cellular distribution within the hippocampal formation. The significant (**P<0.05**) mean body weight decrease seen only in the treatment group T2 as shown in **Table I** compared to the control group seemed to be duration dependent as no significant weight change was recorded in the first 7 days of exposure which is even beyond the exposure duration of treatment group T1 until the 8th day as inferred from Table I. Although starvation in animals has been reported as a set of adaptive biochemical and physiological changes that reduce metabolism in response to a lack of food [14], however insufficient amount of insulin in the bloodstream may trigger release of triglycerides from the adipose tissue and catabolism of amino acids in the muscle tissue resulting in a loss of both fat and lean muscle, which concomitantly leads to a significant reduction in in total body weight [15].This study had used computer Image-Pro Express software and the sections were stained to reveal the pyramidal neurons characteristically which were counted with the aid of a digital image software on a computer. Neuron counting from histological sections has been used to estimate the total number or cell density in brain regions. Extensive cell death in the central nervous system has resulted from neuronal degeneration and neurotoxins have been implicated as prime candidates that induce neuronal degeneration [16]. Treatment group T2 showed significantly reduced neuronal density (P<0.05) compared to the control while an insignificant reduced neuronal density was recorded in group T1 when compared to the control as seen in **Table II.** The remarkable reduced population of the Purkinje cells in the treated rats might be due to neurotoxic - effect of starvation on the Pyramidal cells which again underline the possibility of a duration dependent effect as the higher percentage neuronal loss recorded in group T2 for CA1, CA2 and CA3 subfields are 21%, 19% and 44% respectively as compared to lower values obtained in group T1 as 11%, 8% and 14 % in CA1, CA2 and CA3 subfields respectively as seen in **Table III.** A change in the dimensions of a cell has been reported to have potentials of reflecting the internal changes in the ultrastructure of the cell, hence the transverse diameter of a pyramidal cell could be used as a representative index of its size [17] and as seen in this study neuronal transverse diameter for the pyramidal cells revealed statistically significantly reduced values **(P<0.05**) for treatment groups T2 and insignificantly reduced values for treatment group T1 compared to the control section as seen in Table IV. Lack of cell generation may be a key mechanism of neurodegeneration [18]. Indeed, inmany neurodegenerative diseases, the lack of ongoing cell generation by stem cells has been hypothesized to contribute to tissue loss[19].The scantily distributed glial cells and the shrunken dentate cells with the pyramidal cells appearing pyknoticas observed in the histoarchitecture of the treatment group T2 **(Figures 5&6**) with less distortions seen in treatment group T1**(Figures 3&4**) as compared to normal histology obtained in the control sections **(Figures 1&2**) is due to the effect of long term starvation. Cell degeneration occurs by necrosis and apoptosis. Necrosis affects extensive cell population which involves cytoplasmic swelling, while apoptosis is an organized form of self destruction that is characterized by cell shrinkage [17]. Apoptotic cell death could be induced by cytotoxic drugs or physical (example, mechanical) stimulation [20]. Glia ought not to be regarded as 'glue' in the nervous system as the name implies; rather, they are more of a partner to neurons [20].The scanty distribution of the glia cells as noticed in this work will drastically affect their roles of surrounding and holding neurons in place as well as supplying nutrients and oxygen to neurons. Hence the neuronal loss will underline impairment of the functions of CA3 as an extensive excitatory recurrent connection of region in encoding and retrieval of associations, including autoassociative completion of a single pattern, or associative retrieval of the next pattern in a sequence [21]while the activities of CA1 involved in matching of CA3 output with afferent input from entorhinal cortex will also be affected [22].

Conclusion

Long term starvation has a great tendency of inducing major learning, memory and emotional deficits as a consequence of inducing heavy loss of pyramidal neurons in the CA1 and CA3 subfields of the hippocampus .

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Table I: Mean±SEM weight distribution at the end of experiment

		GROUPS		
DAYS	NO OF RATS	C	T1	T2
0	10	202.8 ± 3.5	209.3 ± 7.0	210.8 ± 7.9
1	10	204.8± 2.1	206.8 ± 8.0	207.6 ± 1.3
2	10	207.3 ± 5.1	200.8 ± 6.9†	201.0 ± 2.2
3	10	210.3± 2.0	198.9 ± 8.2†	196.6 ± 6.2
4	10	211.2 ±1.7	-	194.5 ± 3.3
5	10	211.3 ±4.1	-	190.6 ± 2.6
6	10	213.6 ±1.5	-	186.1 ±.1.4
7	10	215.5 ±2.3	-	182.5 ±2.6
8	10	215.2 ±0.3	-	179.7 ±1.4*
9	10	218.2 ±3.3	-	172.2 ±2.4*
10	10	220.7± 4.3	-	167.1 ±2.0*
11	10	222.5 ±2.3	-	160.8 ±4.2*
12	10	222.7 ±1.6	-	157.5 ±2.6*
13	10	224.2 ±1.3	-	152.2 ±0.1*
14	10	225.0 ±0.6	-	146.6 ±3.3*

* ($P < 0.05$)Significanct difference when compared with control using t-test

† ($P > 0.05$)insignificant difference when compared with control using t-test

Table II: Mean+SEM of Neuronal density per sq. cm of section

GROUPS

CORTICAL AREA	C	T1	T2
CA1	5654.8 ± 110.53	5032.3± 124.07	4467.3 ± 137.98
CA2	3065.8± 122.18	2819.8 ±118.06	2483.3 ± 164.37
CA3	1778.3 ± 115.19	1529.3 ±36.97†	995.8 ± 36.97ϕ

ϕ

(P < 0.05) Significance difference when compared with control using t-test

†(P > 0.05) Insignificance difference when compared with control using t-test

Table III:- Percentage Neuronal loss per each hippocampal subfield

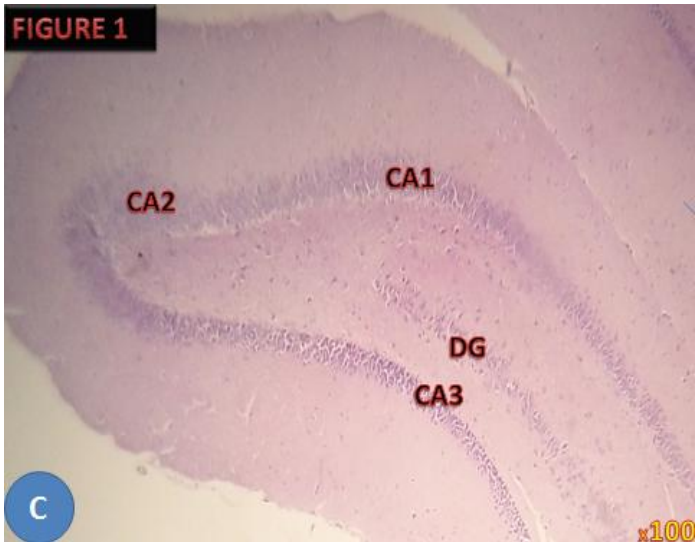
GROUPS

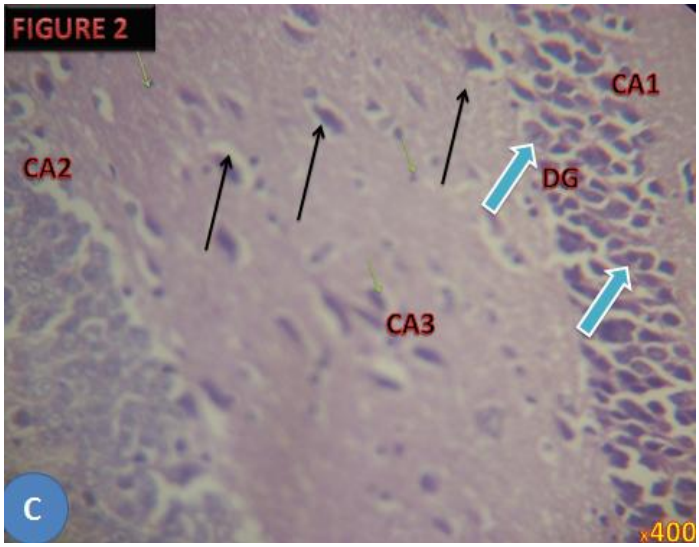
CORTICAL AREA	C	T1	T2
CA1	-	11	21
CA2	-	8	19
CA3	-	14	44

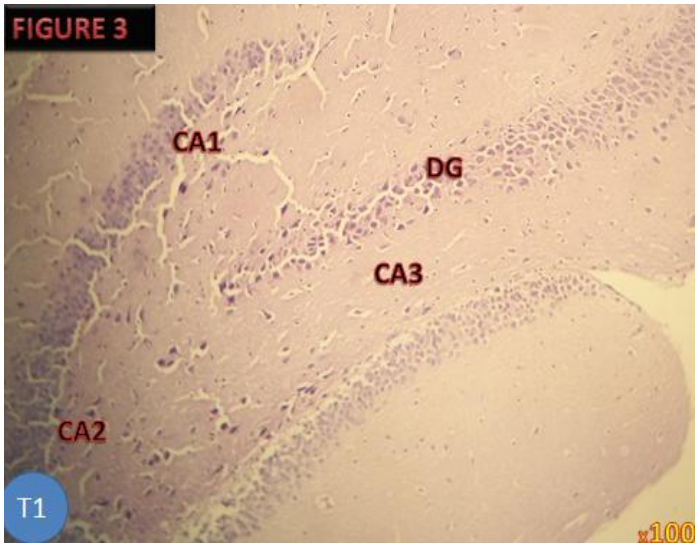
Table IV: Mean ± SEM (Neuronal diameter) of Pyramidal cells

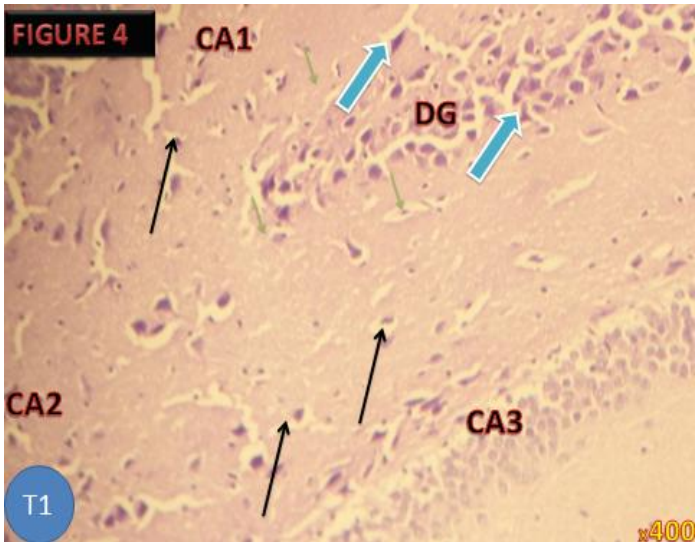
Group	Pyramidalcells diameter (µm)
C	1.30±0.01
T1	1.20±0.03
T2	0.51±0.03ϕ

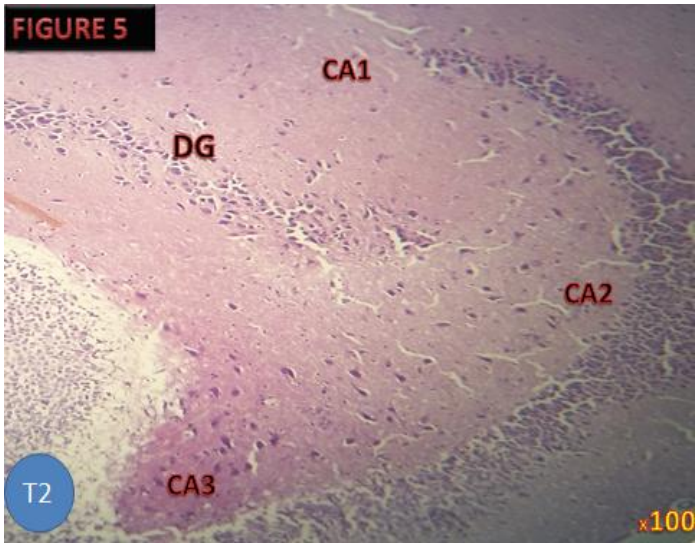
ϕ Significant difference **P<0.05** when compared to the control

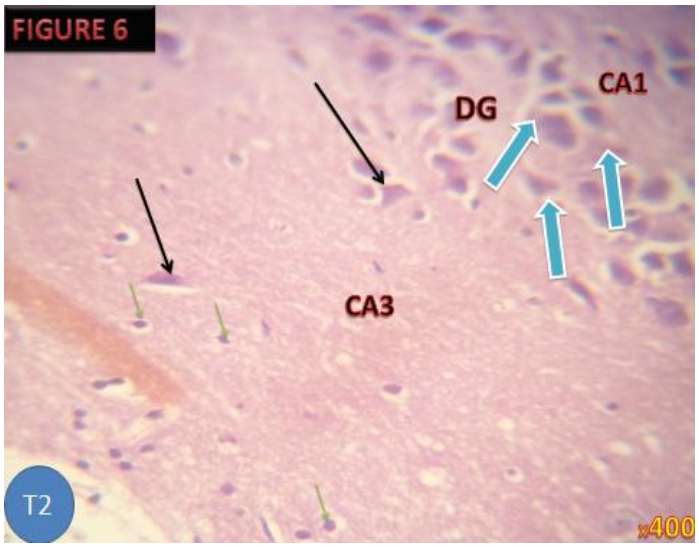












LEGENDS

Figure 1: Photomicrograph of Hippocampus (control section C) showing the distinct different layers of **CA1,CA2,CA3** and the **DG** (Dentate Gyrus) *H&Estain x100*.

Figure 2: Photomicrograph of Hippocampus (control section C) showing the distinct different layers. Note the normal large pyramidal cells(black arrows),evenly distributed glial cells(green arrows)and the dentate cells(blue arrows) *H&Estain x400*.

Figure 3 : Photomicrograph of Hippocampus (Treatment section T1) showing the distinct different layers of **CA1,CA2,CA3** and the **DG**(Dentate Gyrus) *H&Estain x100*.

Figure 4: Photomicrograph of Hippocampus (Treatment section T1) showing the distinct different layers. Note the few pyknotic pyramidal cells(black arrows),scantily distributed glial cells(green arrows)and the slightly shrunken dentate cells(blue arrows) *H&Estain x400*.

Figure 5 : Photomicrograph of Hippocampus (Treatment section T1) showing the distinct different layers of **CA1,CA2,CA3** and the **DG** (Dentate Gyrus) *H&Estain x100*.

Figure 6: Photomicrograph of Hippocampus (Treatment section T2) showing the distinct different layers. Note the pyknotic pyramidal cells(black arrows),scantily distributed glial cells(green arrows)and the distorted dentate cells(blue arrows) *Nisslstain x400*.