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# EVALUATION OF SILYMARIN ON POSTERIOR PITUITARY CELLS IN EXPERIMENTAL LIVER FIBROSIS INDUCED BY $CCl_4$ IN MALE WISTAR RATS

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## *Abstract*

*$CCl_4$-induced liver damage is a well characterized experimental model for studying liver fibrosis, and silymarin is a drug made up of mixture of flavonoids extracted from seeds of milk thistle plant characterized for its hepato-protective effect. The work was aimed at studying the effect of silymarin on pituitary cells in experimental liver fibrosis induced by $CCl_4$ in male Wistar rats. Male wistar rats were randomized into four groups viz; A, B, C and D of 5rats in each group, receiving 0.25ml/100g body weight of normal saline, olive oil, 40% $CCl_4$ and 40% $CCl_4$ with 6 mg/kg of silymarin treatment respectively. $CCl_4$ and Olive oil were given by subcutaneous injection three time a week, normal saline and silymarin were given orally daily for seven weeks. At end of the seventh week, the animals were sacrificed; Liver enzymes analysed in*

*serum, liver and general body weights were measured, and histological studies of the pituitary tissues were also assessed. The results of this study indicate significant increase **in** the plasma levels of ALT, AST and ALP. Change in body weight were significantly (P<0.05) decreased in the group receiving $CCl_4$ alone. There was increase in liver weights which were not significant. Microscopic examination of the brain sections from rats treated with $CCl_4$ showed mild abnormality in their cytoarchitecture which was reversed with silymarin treatment. Some of the abnormalities of liver fibrosis were reversible with administration of silymarin. In conclusions, this study suggests that chemically induced liver cirrhosis for eight weeks has mild effect on the pituitary cell cytoachitecture.*

**Keywords:** Liver fibrosis, Liver enzymes, Silymarin, Histology & Pituitary Cells

## 1.0 Introduction

Fibrosis is the reaction of the liver to diverse chronic insults by agents such as parasitic disease, chronic viral infection (hepatitis B and C), immunologic attack (autoimmune hepatitis), hereditary metal overload, and toxic damage e.t.c., in which excessive connective tissue builds up in the liver (Stalnikowitz and Weissbrod, 2003). Previous studies have indicated that oxidative stress can be a distinct factor in the pathogenesis of liver diseases including drug-induced hepatic damage, alcoholic hepatitis, and viral hepatitis or ischemic liver injury (Albano, 2002; Jaeschke, 2000). Increase in the production of these free radicals can be toxic to hepatocytes and initiates reactive oxygen species (ROS)-mediated cascade causing hepatocyte death, and leading to acute hepatic damage (Jaeschke *et al.,* 2002; Kaplowitz, 2002). Therefore, antioxidative and antifibrotic treatment was proposed to be a potential means of preventing or attenuating toxic liver injury in order to understand the consequences of this damage of the liver on the posterior pituitary histology.

$CCl_4$-induced liver damage has been known to be a standard experimental model for studying liver fibrosis (Wu *et al*., 2007). Silymarin is a drug made up of flavonoids extracted from seeds of Silybum marianum plant with strong hepato-protective effect (Schriewer *et al*., 1973) and antioxidant activity which scavenge and inhibit free radicals generation and lipid peroxidation in cell membrane (Schriewer *et al.,* 1973). It is known to stimulate RNA polymerase, biosynthesis of cell proteins and inhibit enzymes catalyzing the production of leukotrienes and prostaglandins such as 5-lipoxygenase and cyclooxygenase (Valenzuela and Garrido, 1994; de Groot and Rauen, 1998).

Liver diseases are implicated in some cases of brain dysfunction such as hepatic encephalolopathy. Liver-induced inflammation cause disturbances or dysfunction in the central nervous system (CNS) including some metabolic dysfunctions such as hyperthermia, somnolence, loss of body weight and some behavioural manifestations like lethargy, anhedonia and decreased social interaction, which is collectively termed as sickness behaviour (Konsman *et al.,* 2002). Without effective treatments, reversible liver fibrosis at an early stage leads to irreversible cirrhosis. Chronic liver injury leads to a progressive wound

healing response that eventually results in liver fibrosis characterized by both quantity and quality alteration of hepatic extracellular matrix (ECM) (liu *et al.,* 2003). lactotrophs alteration in the presence of exogenous and endogenous hyperestrogenemia is been described in isolated cases of lactotroph hyperplasia in cirrhotic human livers, while in cirrhotic rats, a study failed to show altered serum or pituitary prolactin levels (Gonzales *et al*., 2007). However, most of these studies focus more on anterior pituitary cell and to the best of our knowledge, there is a dearth of information regarding the experimental studies evaluating the histological and biochemical analysis of the posterior pituitary cells in liver fibrosis. Therefore, the aim of the present study is to evaluate the effect of silymarin on posterior pituitary cells activity in experimental liver fibrosis induced by $CCl_4$ in male Wistar rats. It is expected that this research will provide a better and clearer information and understanding regarding the associated hazards of liver diseases that may impair neurological and biochemical function of the brain (hypothalo – posterior pituitary axis). This form one of the bases for, understanding the consequences liver diseases may causes to some part of the brain using silymarin treatment.

## 2.0 Materials and Methods

### 2.1 Animals

Twenty male Wistar rats (140–180g) used for this work were acclimatized for two weeks. They were housed in plastic boxes in a controlled environment (temperature 25±2 °C and 12 h dark/light cycle) with standard laboratory diet and water *ad libitum*.

### 2.2 Animals Grouping and Treatment

Group (A) animals served as the control group (normal saline), Group (B) animals received 0.25ml/100g olive oil orally daily, group (C) animals received 0.25ml/100g 40%$CCl_4$ subcutaneous injection three times in a week as described by Li *et al.,* (2011), while group (D) animals received 0.25ml/100g 40% $CCl_4$ subcutaneous injection three times in a week and 6mg/kg silymarin orally daily (Jamil et al., 2015). At end of the seventh week, the animals were sacrificed by cervical dislocation for investigation of liver fibrosis and posterior pituitary cells cytoachietecture using samples from the blood, liver and brain tissues. This study was done by biochemical assays of the blood and histological studies of the tissues.

### 2.3 Liver Biochemical Analyses

Blood samples were collected in Lithium heparinised sample tubes through orbital venous plexus of the animals using the approved method by Institutional Animal Care & Use Committee in year 2011 (IACUC, 2011), centrifuged at 3000 revolution per minute. Serum analysis for the presence of liver cell enzymes i.e., aspartate amino transferase (AST) alanine amino transferase (ALT), and alkaline phosphatase (ALP) were measured according to the reported methods by Atef M. Al-Attar, (2012).

### 2.4 Histopathological studies

Liver and brain tissues were fixed in 10% formalin and processed by the routine method for paraffin embedding at University College Hospital (UCH), Ibadan, Nigeria. 4-5 ìm thickness of each tissue was taken using microtome and stain with haematoxylin and eosin for histopathological examination through light microscope (Bancroft and Stevens, 1996).

### 2.4 **Statistical Analysis**

Results were presented as mean ± SEM analyzed using Student t-test and one way ANOVA. The difference of the means was considered significant at *p< 0.05.

## 3.0 Results

**Table 1 showing liver weight and change in body weight**

| Group | ALP(IU/L) | AST(IU/L) | ALT(IU/L) |
|---|---|---|---|
| **A(N/saline)** | 250.60± 16.23 | 278.20±18.08 | 138.60±8.49 |
| **B(Olive Oil)** | 194.20± 5.12 | 299.00±21.02 | 110.60±3.53 |
| **C(CCl4)** | 426.60± 7.36 | 787.60±54.31 | 586.20±89.34 |
| **D(CCl4+SM)** | 286.60± 16.73 | 222.20±62.29 | 246.40±36.70 |

**Table 2: Showing plasma levels of the liver enzymes.**

| Group | Body weight(g) | Liver weight (g) |
|---|---|---|
| **A(N/saline)** | 84.40± 3.80 | 10.70± 0.49 |
| **B(Olive Oil)** | 91.60± 3.67 | 8.05± 0.22 |
| **C(CCl4)** | 32.40± 2.38* | 12.18± 0.54 |
| **D(CCl4+SM)** | 5.20± 0.73* | 12.70± 0.26 |

Result presented as Mean ± SEM; *P<0.05; n=5

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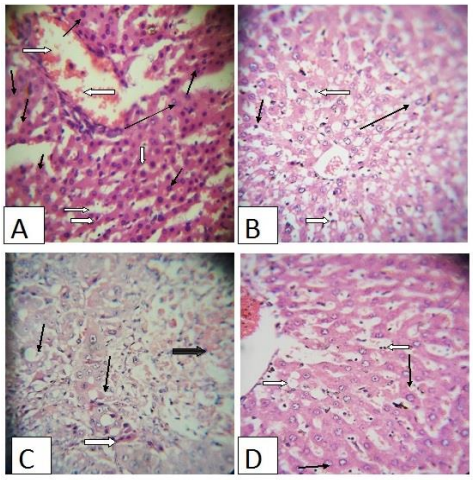


**Figure 1 H&E staining of liver(x400) in**

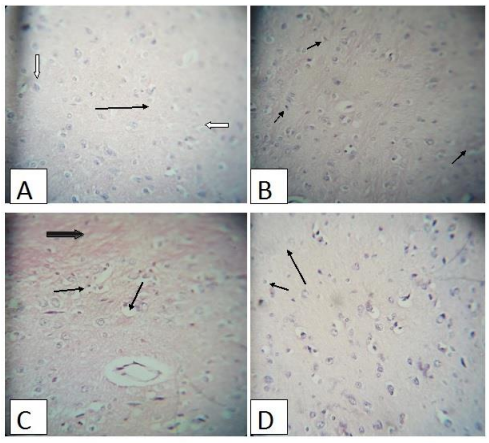


**Figure 2: H&E staining of Pituitary(x400) in**

**group A (normal saline),B(olive oil), C($CCl_4$) (fibrotic) and D($CCl_4$ + SM)**

**group A (normal saline),B(olive oil), C($CCl_4$) (fibrotic) and D($CCl_4$ + SM)**

In figure 1, the control group (A) shows normal histology of the liver, the hepatocytes appear normal (slender arrow),the sinusoid (white arrow) appear normal and there is no congestion of the venules. There is no evidence of inflammation. Group (B) liver section shows moderate mirovesicular steatosis. There is mild infiltration of inflammatory cells(slender arrow), the hepatocytes are seen with deposits of fat in their cytoplasm (white arrow) i.e mild faty changes was observed. Group (C) which is the fibrotic group, the liver section shows macro and mirovesicular steatosis. There are numerous granuloma with multinucleated giant cells (white arrows), that are surronded by lymphocytes. The hepatocytes are seen with heavy deposits of fat in there cytoplasm pushing the nuclei to the edges of the cells(slender arrow). There is evidence of acute infiltration of inflammatory cells and bridging fibrosis. There were focal area of necrosis (big black arrow) with features that are tending towards liver cirrhosis. Silymarin treatment as indicated in group (D) liver section shows normal liver histology with scanty inflammatory cells. Majority of the hepatocytes appear normal even though there is evidence of mild vascular congestion.

In Figure 2, the photomicrograph of a brain section in the control group (A)shows normal histology, the stroma as indicated by "Slender arrow" appear normal and the glial cells shows no degeneration. Group (B) receiving only olive oil which serves as the vehicle which carbon tetrchoride is been diluted, pituitary section shows neurohypophysis with moderate glial cells, however, all the neuronal cells appear normal. However, in the fibrotic group (C) focal gliosis as indicated by the "big arrow" was observed, while some cells also appear degenerated (slender arrow). Treating the fibrotic group with silymarin (SM) treatment as seen in Group D, the level of glosis in the neurohypophysis A & B, becomes very mild gliosis, with fewer degenerated cells seen.

## 4.0 Discussion

Different pharmacological agents or chemical substances are known to cause hepatic injuries such as acetaminophen, CCl4, D- galactosamine and dimethylnitrosamine. This is as a result of liver been one of the major organ responsible for the metabolism of drugs and toxic chemicals, and therefore makes it the primary target organ and most vulnerable for nearly all toxic chemicals (Lee, 2003). Excessive dose of hepatotoxins exposure to the liver may induce acute liver injury characterized by abnormality of hepatic function, and degeneration, necrosis or apoptosis of hepatocytes, etc.

The results of this study show the presences of liver fibrosis in the group treated with carbon tetrachloride, this was evidenced by the increasing activities of liver enzymes, ALT, AST and ALP in the groups receiving only carbon tetrachloride. This finding is in agreement with the

work of Mir Asif *et al.* (2010), who studied the curative role of Solanum nigrum in carbon tetrachloride - induced liver cell toxicity in rats models and Jamil *et al*., curative role of silymarin on altered reproductive variables in $CCl_4$ induced liver fibrosis (Mir Asif *et al*., 2010 and Jamil *et al*., 2015). However, the animals in this group appeared ill looking with decreased appetite for feeding, indicating the possibility of inhibition of feeding centre or stimulation of satiety centre in the brain, which might have been infiltrated by toxin produced as a result of liver damage, that is crossing over through the circumventicular organs (**CVOs)** or the inflamed blood brain barrier. This would have been a predisposing factor that might have leaks toxin into the pituitary which disrupts its function (Baluchnejadmojarad *et al*., 2010). More so, these observations were also in support of the report of van Thiel *et al*., (1981) on the common complication of advanced liver disease and can also be associated with sickness behavior (Konsman *et al*., 2002; Rita Garcia-Martinez and Juan Cordoba, 2012). When the liver is exposed to an acute or chronic insult it generates an inflammatory response that may affect other organs. This Liver-induced inflammation can cause disturbances within the central nervous system (CNS) which includes metabolic manifestations such as hyperthermia, somnolence, loss of body weight etc and behavioural manifestations such lethargy, anhedonia, decreased social interaction, these are attributed to dysfunction of the CNS that lead to sickness behavior (Konsman *et al*., 2002; Rita Garcia-Martinez and Juan Cordoba, 2012) .

There was increased liver weight due fat accumulation and extracellular fibrotic tissue which was in agreement with Das and Vasudevan, (2008) obsrvations. However, histopathological studies of the rats receiving only carbon tetrachloride show the presence of vesicular steatosis, and infiltration of inflammatory cells. The hepatocytes are seen with heavy deposits of fat in their cytoplasm, presences necrosis and there is bridging fibrosis (figure C). The histopathological finding in liver correlate with the biochemical values in the groups. The histology of the posterior pituitary gland in the group receiving normal saline appears normal; while those rats that were administered with $CCl_4$ shows presence of focal gliosis and few inflamed and degenerated cells, this is in accordance to the discussion of the data published Rita Garcia-Martinez and Juan Cordoba, that neuroinflammation may also participate in more subtle neurological manifestations of liver disease (Rita and Juan, 2012).
In the group receiving silymarin at the same time with carbon tetrachloride, the activities of ALT, AST and ALP significantly decreases toward the normal value of control group receiving normal saline, confirming the hepatoprotective property of silymarin when compared with the fibrotic group receiving only carbon tetrachloride. This result is supported by histopathological studies done in the liver of animal in this group showing moderate microvesicular steatosis, mild infiltration of inflammatory cells (figure D). The hepatocytes are seen with mild deposits of fat in their cytoplasm without fibrosis, this reveals hepatoprotection by the silymarin when compared to the group receiving only carbon tetrachloride. Posterior pituitary gland in the group that received $CCl_4$ with

silymarin treatment shows some few focal gliosis and few degenerated cells. Thou, the pituitary gland is refractory to the toxicity of most substances other than hormone analogues or antagonists (Saeger, 1992), this might have come to be as result of prevention of protein oxidation by silymarin which is in line with some previous studies were by, silymarin when administered at a dose of 200mg/kg/day, strongly reduced the proteins oxidation in hippocampus and the CNS via the blood–brain barrier (BBB) (Galhardi *et al.,* 2009; Nencini *et al.,* 2007; Davies, 2000; Metodiewa and Koska, 2000). Silymarin is also known to elevate some neurotransmitters concentration in brain. Silymarin has antidepressant effect in animal models when its aqueous extract was administered to mice forced in swimming test. This resulted into significantly reduced duration of mice immobility (Karimi and Saradeghi, 3007), which may explain the recovery from sluggishness; depression and loss of weigh observed in the group receiving $CCl_4$ alone. Hypothalamus-pituitary-testis axis is affected by positive and negative control factors. Norepinephrine is one of the factors influencing the axis (Selvage and Johnston, 2004; Ca³ka, 2006). It has been shown that silymarin have increased the concentration of norepinephrine, serotonin and dopamine in certain areas of the brain of laboratory white mice (Osuchowski *et al.,* 2004). It appears in the current study that increase of gonadotropin hormones from pituitary gland is related to increased release of norepinephrine by silymarin. Norepinephrine by increasing the synthesis of nitric oxide will increase releasing of GnRH from hypothalamus and LH and FSH hormones from pituitary gland (Selvage and Johnston, 2004; Ca³ka, 2006).

**Conclusion**

This study suggests that chemically induced liver cirrhosis for a short period of time has no significant effect on the posterior pituitary cytoachitecture.

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