Histopathology on the vital organs of antidepressant treated Rats.

Report of the UGC Minor Research Project

Submitted

By

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INTRODUCTION:

Depression is a medical disorder of the brain that affects feelings, thoughts, behaviors and physical health of a person. The World Health Organization (WHO) figures reveal that currently over 450 million people in the society are directly affected by mental disorders or disabilities, most of who live in developing countries. Almost 80% of people who see physicians are depressed (Escobar *et al*., 1987; Lipowski 1987; Barsky 1995). About 41% of depressed women are too embarrassed to seek help. Thus the life-time risk of developing depression is 10-20% in females and slightly less in males.

In terms of sociodemographic variables, studies in Indian population have shown that depression is more common in women than men (Bagadia *et al.,* 1973, Sethi B.B and Prakash R., 1979, Nandi *et al*., 1979, Ramachandran *et al*., 1982, Poongothai *et al*., 2009). Gender differences in depression have been due to biological factor (Parker G, Brotchie H., 2010). In view of the morbidity, depression as a disorder has always been a focus of attention of researchers in India. Up to 15% of those who are clinically depressed die by suicide. Thus depression, if untreated, may lead to suicide.

Antidepressants are drugs that relieve the symptoms of depression. The term ‘antidepressant’ is sometimes applied to any therapy (e.g. psychotherapy) or process (e.g. sleep disruption) found to improve clinically depressed mood. They are generally, if not in pharmacology, considered separately from stimulants. Despite the name, antidepressants are often useful in the management of sexual dysfunction, eating disorders, impulse control disorders, enuresis, aggression and some personality disorders. Some have also become known as lifestyle drugs, sometimes referred to as "mood brighteners. Antidepressants are safe and effective for people with depression who have additional chronic physical health problems (Price A, Rayner L., 2011, Gelder *et al*., 2005). These drugs are therefore recommended as first-line treatment for depression in patients with epilepsy. (Henning O, Nakken K.O., 2011, Okazaki *et al*., 2011). Studies suggest that antidepressants have been evaluated mainly in the acute phase treatment (Avasthi *et al.,* 2010).

Standard antidepressants, SSRIs such as Prozac, Paxil (Aropax) and Zoloft, have recently been revealed to have serious risks, and are linked to suicide, violence, psychosis, abnormal bleeding and brain tumors (Vedantam 2004; Meijer *et al*., 2004). The first SSRI, Fluoxetine (trade name Prozac), was invented at Eli Lilly Pharmaceuticals by Chemists Klaus Schmiegel and Bryan Molloy, and was originally referred to as Lilly 110140, or p-trifluoro-phenoxyphenylo N-methyl-propylamine (Wong *et al*., 1974). Antidepressants with exception of Prozac have been banned in Britain for children (Jureidini, 2004). Prozac (Fluoxetine hydrochloride) is safest of all as it can be safely used for the treatment for all the age group people.

Fluoxetine is a highly effective SSRI in vitro and in vivo (Fuller *et al*., 1991). Despite the availability of newer agents, it remains extremely popular due to its reduced side effects as compared to other TCA (tricyclic antidepressant). Fluoxetine was approved for the treatment of depression, obsessive-compulsive disorder and bulimia. In clinical trials of antidepressants, the placebo effect can be quite high in the range of 30% for subjects diagnosed with major depression (Healy D.,1997), meaning that many patients who take placebos (which contain no active ingredient) experience significant mood improvements despite not having taken any medication. The most dramatic benefits from fluoxetine tend to occur in people who have moderately severe illness (Peselow *et al.*, 1992) and the data that support clinical benefits are convincing (Burke M.J, Preskorn .S.H., 1995).Though fluoxetine is approved as most popular drug for the treatment of depression, it has some of the side effects.

Prozac (Fluoxetine hydrochloride) is extensively metabolized in the liver to norfluoxetine and a number of other unidentified metabolites. The primary route of elimination appears to be hepatic metabolism to inactive metabolites excreted by the kidney. Since these organs are the targets for metabolism and excretion, the current study will evaluate the histopathological changes in these vital organs of depressed rat model undergoing treatment with antidepressant.

RATIONALE/RESEARCH HYPOTHESIS:

Several researches had been undertaken to study the effects of various antidepressants for better treatment of depressive individuals. Though fluoxetine is approved as most popular drug for the treatment of depression, it has some of the side effects. Most of the research in the field of depression aims in understanding the brain chemistry. Studies on nervous system suggest that enhanced excitability of amygdala neurons may contribute to the increase in anxiety-like behavior observed following acute fluoxetine treatment (Ravinder *et al.*, 2011). **I**t has been reported that fluoxetine increases catecholaminergic concentration in some brain regions (Bymaster *et al*., 2002; Gobert *et al*., 1997), most notably the catecholamine outflow in prefrontal cortex (PFC), an area of brain with cognitive and affective functions (Berman and Weinberger 1990; Goldman-Rakic 1996). In vivo visualization of serotonin transporters in the human brain during fluoxetine treatment reveals that four hours after injection of the tracer more than 40% of serotonin transporters were blocked (Tauscher *et al.*, 1999).

Histological studies and biochemical assays were carried out to determine the effect of fluoxetine on the reproductive system of male rats (Unnikrishnan G, 2002). Effect of various pharmaceutical compounds on reproductive structure of albino rats had been worked out (Kondulkar S, 2002). Detailed studies on metabolic changes in rat uterus had been undertaken (Shivbalan R, 1981). Biochemical analyses were done to study the effect of fluoxetine on the reproductive system of female rats (John L, 2002). Biochemical analysis reveal that fluoxetine is a potent inhibitor of the liver cytochrome P-450 enzyme (CYP2D6) which breaks down TCA drugs; as a result, it may produce unexpected drug interactions with TCAs (Gram and Lars F, 1994). The effect seems to be a consequence of the drug and / or metabolite solubilization in the inner membrane of the mitochondria **(**Eliza et *al*, 1993).

However literature survey reveals that the histopathological effects of fluoxetine on vital organs; kidney and liver at different dose levels has not been assessed. The current study therefore shall exploit the histopathological effects of fluoxetine.

MATERIALS AND METHODS:

Model animal:

Wistar rats are an outbred strain of albino rats belonging to the species Rattus norvegicus. It is widely used in research in the field of Teratology, Nutrition, Aging, Oncology, Toxicology and General studies. Survey indicates the prevalence of female depression was 67.8 % and the female to male ratio was 2:1 **(**Benazzi 2000). Since risk of developing depression is more in females and slightly less in males, Wistar rats of female gender were used for the study. In the current study fresh stocks of female Wistar rats weighing between 100-150 kg initially (i.e., before leading them to develop depression clinically) were selected.

Laboratory maintenance of animals:

All experimental animals were treated and cared for in accordance with the guidelines recommended by the Animal House, R. Jhunjhunwala College, Ghatkopar, CPCSEA registration number 525/02/.a. Upon receipt, the animals were housed in polyurethane cages with wire mesh tops and rice husk bedding and acclimatized to the laboratory conditions for one week prior to dosing. The temp maintained at 28 ± 2^0C and exposed to 10-12hrs of day light and a relative humidity of 60-70%. The animals were supplied with water *ad libitum*. Commercially available rat feed was supplied by 'Amrut' laboratory animal feed. The specification of the food was crude protein-20-21% min., crude fibre-4%max., ash-8% max.,calcium 1-2%,phosphorus -0.6%min., NFE 54% enriched with stabilized vitamins such as vit.A,vit.D3 and vit.B12, thiamin, riboflavin, niacin, folic acid and supplemented with all minerals and microelements, ME Kcal/kg-3600 and pellet size 12mm.

Drugs administered:

Reserpine:

Reserpine is a drug used to treat mild to moderate hypertension. It is a post ganglionic sympathetic nerve terminal blocker. It impairs the storage of bioamines by interfering with an uptake mechanism and results in depletion of norepinephrine, dopamine and serotonin in both central and peripheral neurons. As reserpine enters the brain, depletion of cerebral amines causes sedation and mental depression. Reserpine induced depression is used in an animal to screen antidepressants (Rein, H.J, 1978). The use of reserpine induced depression is one of the basic models for assessing antidepressants in studies of new pharmaceuticals and is still used in many studies which require depression induction in animal models (Yamashita *et al*., 1998; Minor *et al.*, 2003). The rats were clinically depressed by injecting 3mg /kg of reserpine (Bernardi *et al*., 1968; Rant W.P, 1978; Whitlock F.A, 1978; Rein H.J, 1978; Unnikrishnan G, 2002; Benton *et al*; 2007). Reserpine puriss (LOBA Chemicals) was injected intraperitoneally at morning hours as per the experimental design.

Fluoxetine Hydrochloride:

Studies comparing fluoxetine 20, 40, and 60 mg/day to placebo indicate that 20 mg/day is sufficient to obtain a satisfactory response in MDD in most cases. Consequently, a dose of 20 mg/day for adults, administered in the morning, is recommended as the initial dose. In rats a single dose of fluoxetine (20 mg/kg, i.p.) when induced, there is a significant increase in the cerebral cortical and plasma concentrations of steroids in both the brain and plasma of rats.

A preliminary study of 21 day dose of fluoxetine was undertaken to study the sub acute toxicity of the drug. There was no mortality of animals at 20 mg/kg and 80mg/kg. Fluoxetine antidepressant drug (PROZAC) was administered to the reserpine-induced depressed organisms in groups III and IV at lower dose of 20mg/kg and high dose of 80mg/kg respectively. All doses were freshly prepared in distilled water and given intraperitoneally in the morning hours.

Experimental Design :

The female Wistar rats were sorted into four groups of 10 each and were treated as follows;

Group I (Control):
Sterile distilled water was injected intraperitoneally for 28 successive days in morning hours.

Group II (Depression induced):
Sterile distilled water was injected intraperitoneally for 21 successive days. This was followed by administration of reserpine for 7 successive days.

Group III (Depression induced + Treated):
The rats were initially given reserpine for 7 successive days. Following doses of reserpine, Fluoxetine hydrochloride (20 mg/kg body weight) dissolved in distilled water was given for 21 successive days.

Group IV (Depression induced + Treated):
The rats were initially given reserpine for 7 successive days. Following doses of reserpine, Fluoxetine hydrochloride (80 mg/kg body weight) dissolved in distilled water was given for 21 successive days.

Parameters studied:

At the end of each treatment blood samples were collected from retro-orbital plexus under mild etherization. The animals were sacrificed, dissected and organs; liver and kidney were isolated.

For histological analysis, excised pieces of liver and kidney were fixed in neutral formalin for histological analysis. Processing of tissue samples for histology assessment followed established procedures. In brief, the tissue samples were rinsed with 0.9% saline solution, fixed in 10% formalin. Then the diagonal section of the liver, the transverse section of the kidneys were obtained and processed as follows: (1) 10% neutral buffered formalin for 1 h, twice; (2) 70% alcohol for 1.5 h; (3) 80% alcohol for 1.5 h; (4) 90% alcohol for 1.5 h; (5) absolute alcohol for 1.5 h, twice; (6) xylene for 1.5 h, twice; (7) in molten wax at 65°C for 2.5 h two changes. The processed tissues were embedded in paraffin and sectioned at 6 microns thickness, placed on frosted glass slides and dried on a 70°C hot plate for 30 minutes. The tissues were stained using the hematoxylin and eosin (H&E) stains. The sections were dewaxed in two changes of xylene (3 min each), hydrated in two changes of 100% ethanol, followed by 90% ethanol and 70% ethanol, for 3 min each, rinsed with water (3 min) and stained. The stained tissues were dehydrated with 70% ethanol followed by 90% ethanol, placed in two changes of 100% ethanol for 3 minutes each and cleaned with two changes of xylene (3 min each). The fixed tissues were observed under compound microscope.

For biochemical estimations, serum samples and tissue extracts were used. Tissue was extracted in ice cold phosphate buffer, centrifuged so as to make a 20% homogenate. Organ function tests were carried out using standard diagnostic kit from span diagnostics Pvt, Ltd.

RESULTS AND DISCUSSION:

Histopathology of Liver:

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It is also an organ of excretion, essential in the removal of the wastes and the toxic products from the blood (Tortora and Grabowski, 2002). It has great capacity to detoxicate toxic substances and synthesize useful principles (Shanmugasundaram and Venkataraman, 2006). Hepatocytes, which make up the majority of the liver structure, are very active in the metabolism of exogenous chemicals, and this is one of the major reasons why the liver is a target for toxic substances (Timbrell, 2001). The liver is necessary for survival; there is currently no way to compensate for the absence of liver function over long term, although liver dialysis can be used short term.

Thus liver being one of the most remarkable organs in the body, very often it is imperative to investigate biochemical changes that might occur in this organ during various toxicological and clinical studies. Alterations observed in the tissue biochemistry are very often due to the dysfunction at the cellular level and such studies would definitely help in assessing the side effects that may be caused due to the treatments under question. The liver tissue biochemistry was planned in such a way that the parameters analyzed would reflect upon the physiological condition of the tissue due to the treatment with fluoxetine. Hence observations regarding marker enzymes for the hepatic function viz. acid phosphatase and alkaline phosphatase were studied and discussed. As might be predicted from its primary site of metabolism, liver impairment can affect the elimination of fluoxetine. The elimination half-life of fluoxetine was prolonged in a study of cirrhotic patients, with a mean of 7.6 days compared with the range of 2 to 3 days seen in subjects without liver disease. This suggests that the use of fluoxetine in patients with liver disease must be approached with caution. If fluoxetine is administered to patients with liver disease, a lower or less frequent dose should be used.

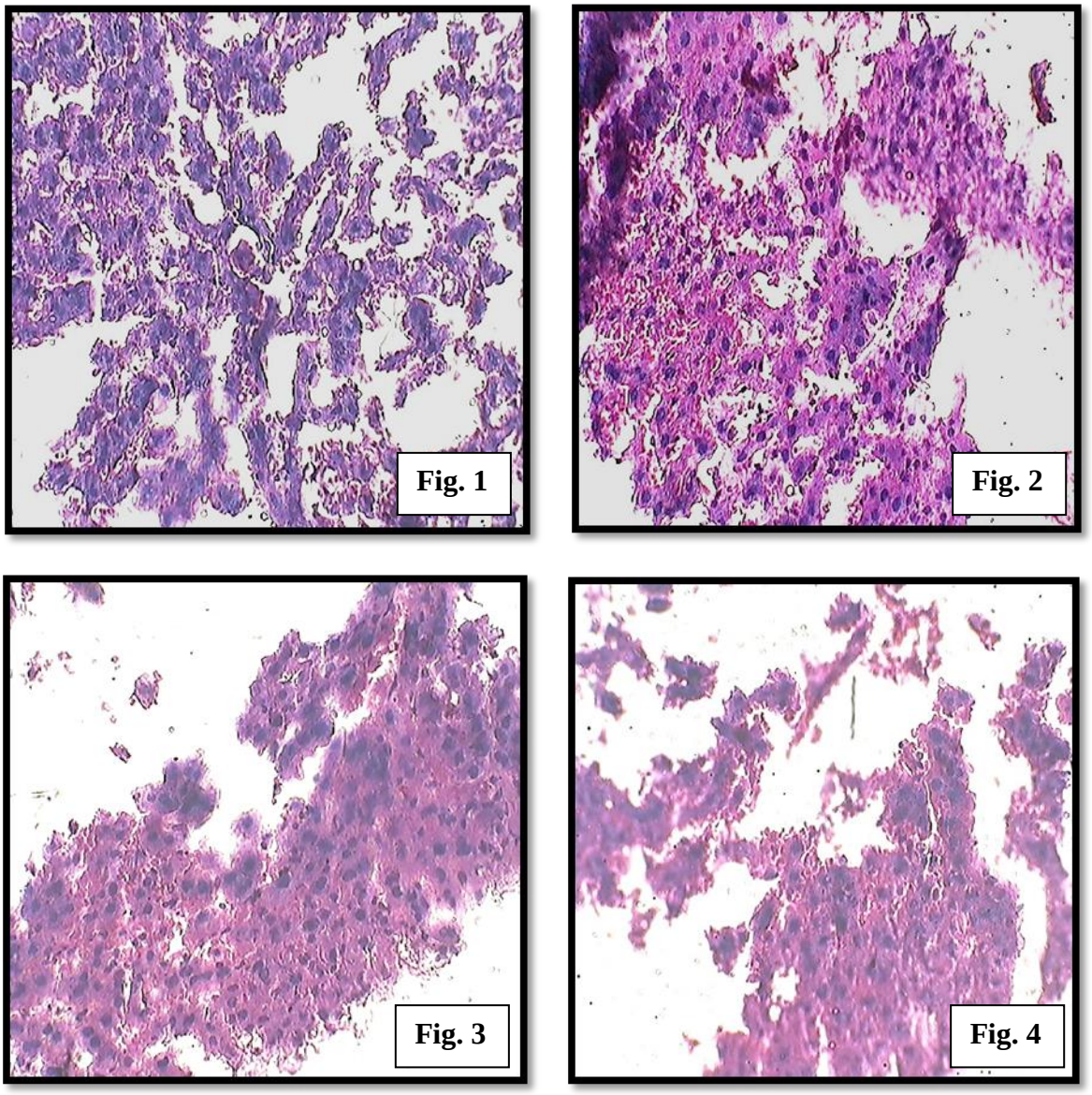


Fig 1: The liver of group I consists of a vast interanastomosing network of hepatocytes arranged in single-cell thick plates separated by vascular sinusoids.

Fig 2: The liver of group II does not show an organized interanastomosing network of hepatocytes. The hepatocytes are loosely arranged in comparison to fig.1

Fig 3: The liver of group III shows cellular distortion and the vascular sinusoids are not visible clearly.

Fig 4: The liver of group IV shows unusual morphological pattern with the degeneration of the hepatocyte. The vascular sinusoids and other structures are not visible.

Discussion:

Liver plays an important role in protein synthesis. It is also an organ of excretion, essential in the removal of the wastes and the toxic products from the blood (Tortora and Grabowski, 1993). Chronic exposure to stress contributes to the etiology of mood disorders, and the liver as a target organ of antidepressant and antipsychotic drug metabolism is vulnerable to drug-induced toxicity. The effect of administration of fluoxetine on liver injury via the measurement of liver enzymes and histopathology was studied. Various toxicological studies have shown histopathological changes associated with the biochemical changes in the liver.

Normal histology of the liver shows the parenchyma of the liver divided into lobules, which are incompletely partitioned by septa from Glisson's capsule. The hepatic lobule is roughly hexagonal in shape and has plates of liver cells called hepatocytes separated by wide vascular channels known as sinusoids. Blood flow into the sinusoids comes from the terminal branches of the portal vein and hepatic artery, bringing nutrient-rich blood from the gastrointestinal tract and oxygen rich blood, respectively. The larger branches of these two vessels course side by side in fibrous tract, known as portal tracts or portal triad, along with the bile ductules which carry bile in the opposite direction from the liver to the duodenum. The portal tracts are positioned at the angles of the hexagon. The blood from the portal vein and hepatic artery branches in the portal tract flows to the central vein (Bailey,1964).

More recently, it has been realized that the blood flow and function of the liver are more accurately represented by the unit structure known as the hepatic acinus. The hepatic acinus is a more accurate representation of the liver function although it is much more difficult to define histologically. The acinus is a roughly beery-shaped unit of the liver parenchyma centered on a portal tract. The acinus lies between two or more terminal hepatic venules and blood flows from the portal tract through the sinusoids to the venules (Young and Heath, 2002).

The liver of group I consists of a vast interanastomosing network of hepatocytes arranged in single-cell thick plates separated by vascular sinusoids which is suggestive of a normal anatomy. The liver of group II does not show an organized inter anastomosing network of hepatocytes. The hepatocytes are loosely arranged in comparison to fig.1. This indicates a change in the histology of the liver. The histopathological changes in the liver structure occur either during the hepato-cellular failure or the parenchymal damage caused due to various physiological and pathological conditions.

The liver of group III shows cellular distortion and the vascular sinusoids are not visible clearly. In case of sub acute or chronic type there is a cellular inflammation of the portal tract which may also extend into the parenchyma. The portal triads show a conspicuous infiltration with the lymphocytes and plasma cells. Lobular architecture is disturbed (Boyd, 1965). The liver of group IV shows unusual morphological pattern with the degeneration of the hepatocyte. The vascular sinusoids and other structures are not visible. Drug when administered at a dose of 80mg/kg caused disintegration, necrosis and degradation in liver. This is associated with significant alterations in liver biochemistry (Prakash *et al.*,1995). Shows intra and intercellular vacuolations, loss of hepatic architecture and fatty degeneration in the hepatocytes in centrilobular as well as periportal areas as compared to the control rats.

Hepatic maker enzymes such as acid phosphatase and alkaline phosphatase help in diagnosing the hepatotoxicity. Histological damages caused a corresponding rise in the blood biochemical parameters namely aspartate aminotransferase, alanine amino transferase, alkaline phosphatase (Gathumbi *et al.*,2002). So further the assays of these enzymes were carried out.

Activity of acid phosphatase (EC 3.1.3.2):

Assessment of acid phosphatase (ACP) levels is important in the diagnosis of liver diseases and chronic renal failure (Varley, 1988). Evaluation of liver acid phosphatase activity is important as it is one of the marker enzymes for assessing liver lysosomal functions. Lysosomes are sub-cellular particles in which several acid hydrolases of various specificities are located and have a role in cellular physiology and pathology (Suzuki S., 1979). ACP is an enzyme of the hydrolase class of enzymes. It is widely distributed and found in high concentrations in the liver, RBCs and the prostate. Acid phosphatase assays are of biochemical interest as increased ACP levels are observed in liver diseases such as Gaucher's disease, hyperparathyroidism and chronic renal failure (Bergmayer, 1984; Varley, 1988). Hence, serum ACP enzyme assay is important in diagnosis of liver and kidney dysfunction.

ACP is determined in various animal groups from serum and liver extracts samples by Mod.King's Method. ACP at an acidic pH hydrolyses diSodium Phenylphosphate to form phenol. The phenol formed reacts with 4-Amino antipyrine in the presence of Potassium Ferricyanide, as an oxidizing agent, to form a red coloured complex. The intensity of the colour formed is directly proportional to the activity of ACP present in the sample.

Results:

Experimental Groups	**Group I**	**Group II**	**Group III**	**Group IV**
ACP activity in KA units (Serum)	0.38 ± 0.12	0.29 ± 0.27	0.50 ± 0.30	1.97 ± 0.64
ACP activity in KA units (Liver homogenate)	32.49 ± 4.24	29.15± 10.43	38.05± 0.23	45.18± 1.32

Table 1: Acid phosphatase activity in the serum and liver homogenates of rats in group I to group IV.

The results obtained were statistically analyzed by using student's 't' test (unpaired). The 't' values obtained in comparative studies indicate the following;

Group I and II -Showed no significant difference in serum and liver homogenate.

Group II and III - Liver homogenate showed significant difference however serum analysis did not indicate significant difference.

Group II and IV- Both liver and serum analysis/activity exhibited significant difference.

Group III and IV- Both liver and serum analysis/activity exhibited significant difference.

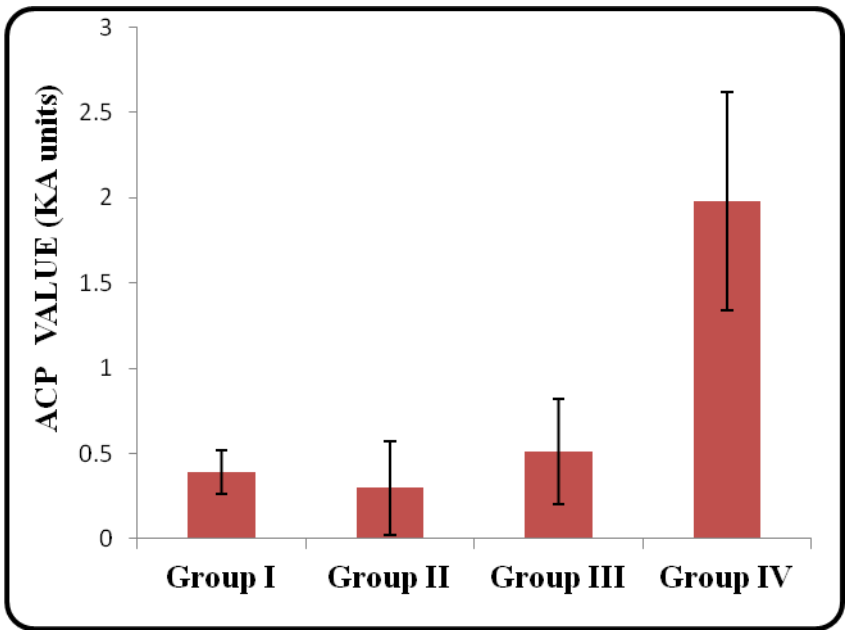


Fig. 5. Acid phosphatase activity in the serum samples of rats in group I to group IV.

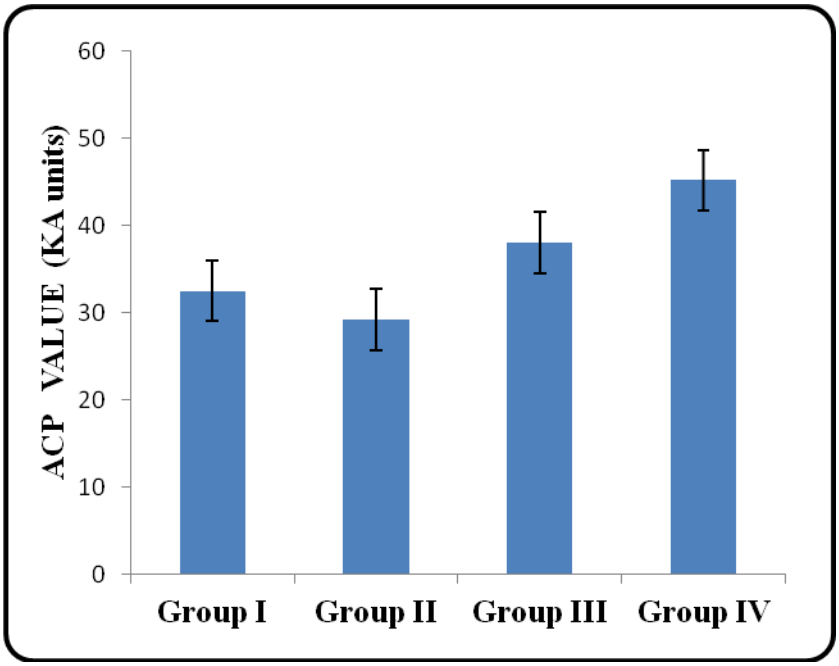


Fig. 6. Acid phosphatase activity in the liver homogenates of rats in group I to group IV.

Discussion:

From the statistical values obtained it is derived that there is significant increase in the acid phosphatase content in serum and liver tissue homogenate. Graphical analysis reveals more than 4 fold increase in acid phosphatase activity in the serum of animals in group IV treated with fluoxetine (80mg/kg) compared to marginal increase in group III treated with fluoxetine (20mg/kg). Levels of acid phosphatase in liver homogenate of group III and group IV showed around 25% to 30% increase compared to control group I and group II.

There was no variation in levels of ACP in serum and liver homogenate in group I (control) and group II (depressed) suggesting that conditions of depression does not affect the activity of liver phosphatases. This indicates that animals under depression do not show symptomatic liver damage. However when the depressed rats were treated with fluoxetine doses of 20mg/kg and 80mg/kg there was significant increase in ACP not only in the liver homogenate but also in the serum. An increase in ACP is an indication of severe liver damage when treated with fluoxetine. Fluoxetine (as well as norfluoxetine) has multiple effects on the energy metabolism of rat liver mitochondria, being potentially toxic in high doses (Eliza *et al.*, 1994).

Various studies indicate the alterations in the acid phosphatase activity in liver under the drug influence. A rise in ACP activity was found in liver parenchymal cells when dextran solutions were injected into the peritoneal cavity of mice (Van *et al.*, 1959). An increased level of serum ACP during hepatocellular carcinoma induced by N-nitrosodiethylamine has been reported (Ramakrishnan *et al*., 2006). In the liver cell suspensions of female rats, with age, a very pronounced heterogeneous pattern in multiple forms of ACP was observed as a result of post-translational modifications (Sleyster E.C, Knook D.L. 1980). Studies on crystalline acid phosphatase present further evidence that the histidine residue is important for catalytic activity (Igarashi *et al.*, 1970). As per the statistical analysis mentioned earlier, the significant difference between treatments can be considered as effective.

Activity of Alkaline phosphatase (EC 3.1.3.1):

Alkaline phosphatases (ALP) occur widely in nature, and are found in many organisms from bacteria to man. Alkaline phosphatase is a marker enzyme which is membrane bound and hence evaluation of its activity could reflect upon the cellular damage which would result due to altered permeability of plasma membranes of the targeted tissue cells (Zimmerman, 1978).

The enzymes catalyze the hydrolysis of monoesters of phosphoric acid and also catalyze a transphosphorylation reaction in the presence of large concentrations of phosphate acceptors. ALPs are a group of enzymes found primarily in the liver (isoenzyme ALP-1) and bone (isoenzyme ALP-2). There are also small amounts produced by cells lining the intestines (isoenzyme ALP-3), the placenta, and the kidney (in the proximal convoluted tubules). The total amount of ALPs released from these tissues into the blood is measured in the serum. The primary importance of measuring alkaline phosphatase is to check the possibility of bone disease or liver disease.

An increased serum ALPs may be due to Liver congestion/Cholestasis or Osteoblastic/Bone Conditions. Fluctuations in liver ALP enzyme activity have been reported in various pathological conditions in liver. It is determined by Kind and King's method. Alkaline Phosphatase from sample converts Phenyl Phosphate to inorganic Phosphate and Phenol at pH 10.0. Phenol so formed reacts in alkaline medium with 4-aminoantipyrine in presence of the oxidizing agent potassium ferricyanide and forms an orange-red coloured complex, which can be measured colorimetrically. The colour intensity is proportional to the enzyme activity.

Results:

Experimental Groups	**Group I**	**Group II**	**Group III**	**Group IV**
ALP activity in KA units (Serum)	36.84 ± 9.99	1.46 ± 0.35	39.29 ± 1.62	59.66 ± 4.95
ALP activity in KA units (Liver homogenate)	11.35 ± 4.74	10.15± 9.70	8.57 ± 9.32	22.50 ± 16.71

Table 2: Alkaline phosphatase activity in the serum and liver tissue homogenates rats in group I to group IV.

The results obtained were statistically analyzed by using student's 't' test (unpaired). The 't' values obtained in comparative studies indicate the following;

Group I and II- show significant difference in serum ALP and no significant difference liver homogenate ALP

Group II and III -show significant difference in serum ALP and no significant difference liver homogenate ALP

Group II and IV- show significant difference in serum ALP and no significant difference liver homogenate ALP

Group III and IV- Both liver and serum analysis exhibited show significant difference in ALP activity.

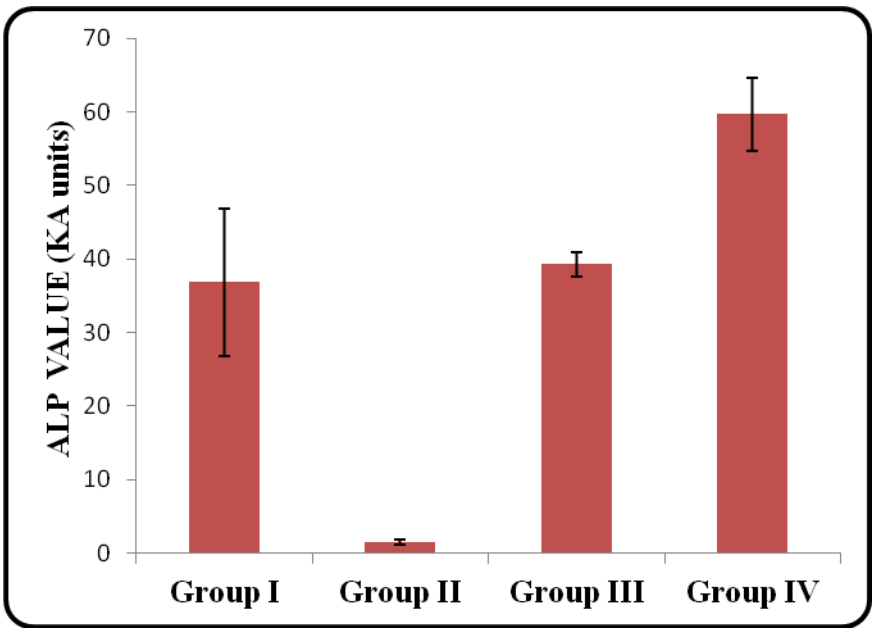


Fig. 7. Alkaline phosphatase activity in the serum samples of rats in group I to group IV.

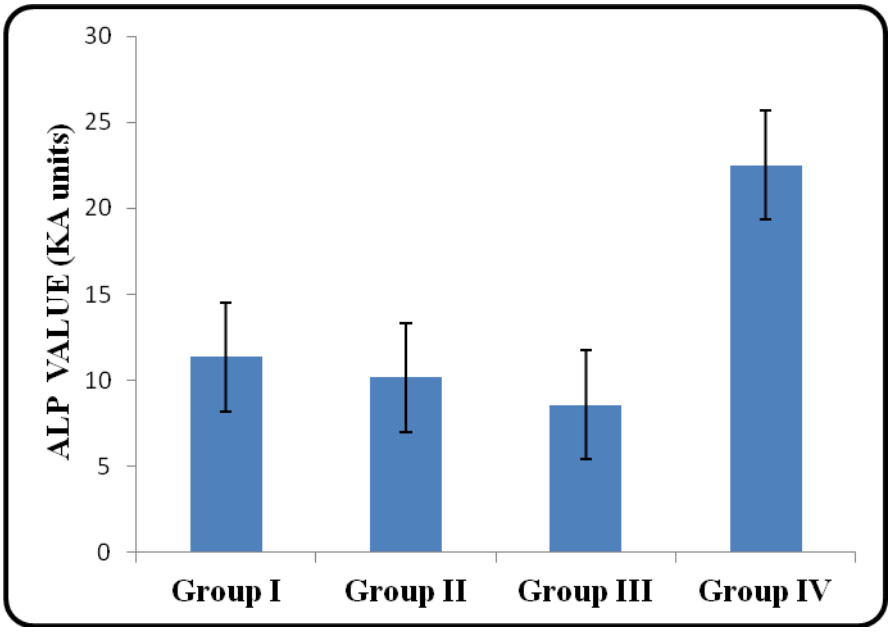


Fig. 8. Alkaline phosphatase activity in the liver homogenates of rats in group I to group IV.

Discussion:

The results obtained indicates no significant difference in ALP levels in liver homogenates in all groups in comparison to the control groups However serum analysis indicates significant difference in ALP levels. Study indicate that the liver homogenate ALP shows no significant variation but animals treated with 80mg/kg of fluoxetine shows significant rise in the ALP levels suggestive of hepatic toxicity at organ level at higher doses. Hepatic side effects have been reported during fluoxetine therapy though widely prescribed for depression disorders. Study indicates that fluoxetine induces liver damage and mediates free radical reactions (Inkielewicz-Stępniak I., 2011).

It can be concluded that there is liver injury to the organisms during the course of fluoxetine treatment. Alkaline phosphatase from the biliary canalicular membrane often appears in serum in such cases of hepatic toxicity (Bergmeyer, 1984). Higher levels of serum alkaline phosphatase are a predictor of mortality independent of the baseline prevalence of metabolic syndrome which is also associated with higher serum alkaline phosphatase levels (Chikkappa *et al.,* 1988). The mechanical stimulation of the digestive tract during feeding, starvation and fibre ingestion appears to influence the passage of intestinal ALP to serum (Martins *et al*., 1998).

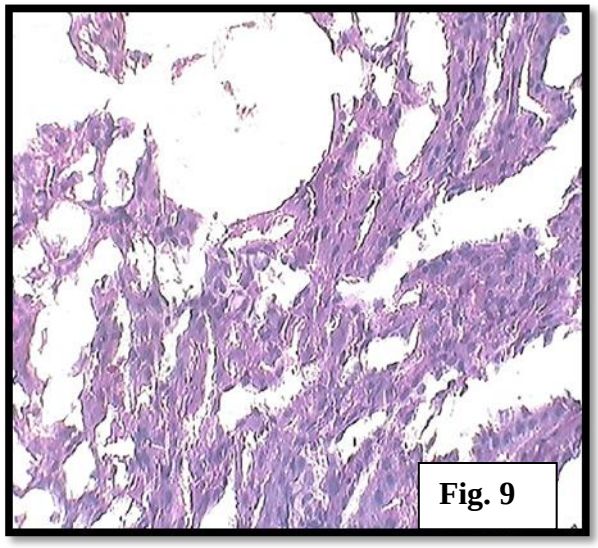
Serum alkaline phosphatase levels are very sensitive to extrahepatic as well as intrahepatic injury and show increased levels under such conditions (Varley, 1988). In one of the studies it was found that liver alkaline phosphatase activity gradually increased with age in both sexes, males showing higher activity than females at all ages (Kuwana *et al*., 1988). Seminomas, known to express eutopically placental-like alkaline phosphatase were demonstrated to contain increased levels of both intestinal and liver-bone-kidney alkaline phosphatases as compared to the normal testis (Hirano *et al.,* 1987).

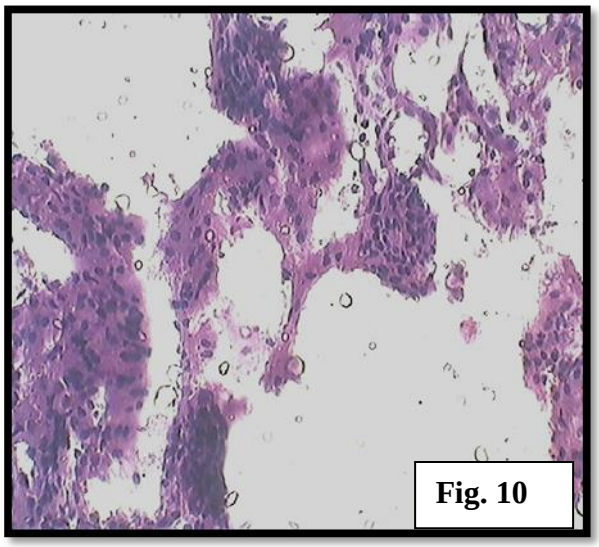
Histopathology of Kidney:

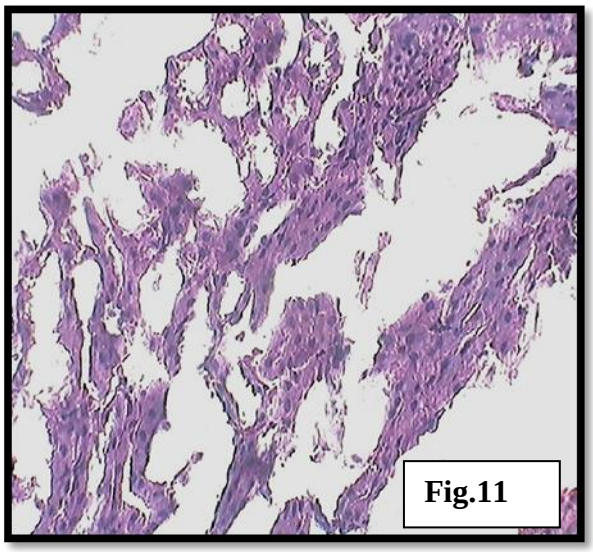
The kidney is a compound tubular gland concerned with the important function of excretion. It excretes urea and other nitrogenous waste products, eliminates substances foreign to the body and it maintains homeostasis by controlling the composition, volume and pressure of blood. Approximately one and a half quarters of blood per minute are circulated through the kidneys, where waste chemicals are filtered out and eliminated from the body (along with excess water) in the form of urine. Common causes of kidney failure in the UK are hypertension and diabetes.

Kidney also contributes to metabolism by performing gluconeogenesis, synthesis of new glucose molecules during periods of fasting or starvation (Tortora and Grabowski, 2002). It is important to note that water and other substances needed by the body are eliminated only to the extent that they exceed the needs; in other words, the kidney conserves the proper amounts of water, electrolytes and other chemicals of value to the body. However changes in the biochemical parameters and/or the histological structure of kidney would interfere with its vital functions.

Kidney function tests help to determine if the kidneys are performing their tasks adequately. One of the many methods of toxicological evaluation is the biochemical investigation of the targeted tissue. Since kidneys are reported to be adversely affected by chemical agent, biochemical assays of certain substance could throw light on the physiological status of kidney after treatment with drugs.







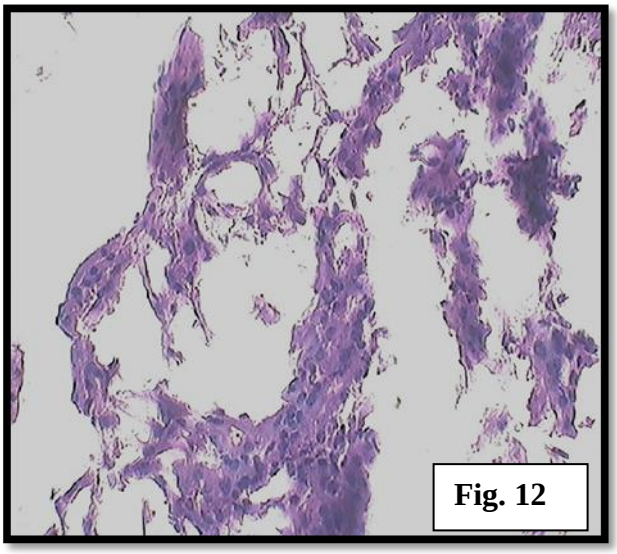


Fig 9: The kidney of group I show presence of renal corpuscle and kidney tubules. The cells are compactly arranged.

Fig 10: The kidney of group II show disorientation of the corpuscles.

Fig 11: The kidney of group III shows reduction in the renal corpuscles.

Fig 12: The kidney of group IV shows unusual morphological pattern with the degeneration of the renal corpuscles

Discussion:

Histopathology has important application in pharmacology as well as toxicology. Examination of abnormal tissue may serve to identify the cause and causative agents of pathological lesions (Sharma, 1993). Histological observations have important application in pharmacology as well as toxicology. Not only the microscopic structure but also the external morphology can be affected due to the treatment with a particular toxicant such as chemicals, drugs etc. Morphologically, kidneys of treated group and control group animals showed their typical bean shaped structure with smooth and shiny outer surface. There were no changes in size of kidneys as well. In the present study no lesions or changes were observed in the kidneys of the treated animals of all the durations with respect to their controls.

Normal histology shows the presence of functional unit of the kidney, the nephron, consisting of a renal capsule and a long folded renal tubule. The renal corpuscle has two components-a tuft of capillary loops called the glomerulus surrounded by a double walled epithelial cup, called the bowman's capsule (Tortora and Grabowski, 1993). The kidney of group I show presence of renal corpuscle and kidney tubules. The cells are compactly arranged indicative of a normal structural integrity. The kidney of group II show disorientation of the corpuscles.

The kidney of group III shows reduction in the renal corpuscles. The kidney of group IV shows unusual morphological pattern with the degeneration of the renal corpuscles Significant alterations in the histology of kidney with the renal tubular cells showing degeneration and exfoliation were observed when the effect of ethanolic extract of crotalria seeds was assessed on kidney of adult albino rats by its administration at a dose of 200mg/kg (Prakash *et al*.,1995). De angeleis Pereira *et al*.,2003 have reported the toxic effect of hydroalcoholic crude exract from the fruits of solanum following chronic treatment with a daily oral dosage of 2mg/kg in wistar rats for 65 days.

Fenoglio *et al*., (2002) have reported nephrotoxicity as one of the major side effects during antitumour treatment with cisplatin in rats. Heavy morphological damage was observed in the proximal tubules. Lead induced renal toxicity was reported by Banu *et al*.,(2006) in mice treated with lead acetate at a dose of 160mg/kg/day orally administered for 90 days a number of histopathological changes that included glomerulonephritis, atrophy and shrinkage of the renal tubules and wider intertubular spaces were noted in the kidneys of lead treated animals.

One of the many methods of toxicological evaluation is the biochemical investigation of the targeted tissue. Since kidneys are reported to be adversely affected by this agent, biochemical assays of certain enzymes as well as other parameters could throw light on the physiological status of kidney. Enzyme evaluation of changes in the activity of lysosomal enzymes in rat kidneys could be useful indicator of kidney damage as well as kidney failure (Lakowska *et al*.,2001). Hence a biochemical assay of creatinine was carried out to ascertain the effects of fluoxetine on kidney.

Activity of Creatinine:

Creatine is primarily synthesized in the liver from the methylation of glycocyamine (guanidino acetate, synthesized in the kidney from the amino acids arginine, glycine, and methionine) by S-Adenosyl-L-Methionine. It is then transported through blood to the other organs, muscle, and brain where, through phosphorylation, it becomes the high energy compound phosphocreatine. During the reaction phosphocreatine, catalyzed by Creatine Kinase, spontaneous conversion to creatinine may occur.

Creatinine is chiefly filtered out of the blood by the kidneys (glomerular filtration and proximal tubular secretion). There is little or no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which reflects the glomerular filtration rate (GFR).

The measurement of creatinine is used to aid in the determination of renal function and is estimated by Alkaline Picrate method. The principle of the measurement of creatinine is based on the Jaffe reaction. That is, under alkaline conditions creatinine reacts directly with picric ions forming a reddish complex, the absorbance of which can be measured at 520 nm. However, several interfering substances including proteins, ketones, glucose, and ascorbic acid also react with picric acid, producing similar colored complexes. Serum proteins are precipitated with tungstic acid solution before measuring for creatinine.

Results:

Experimental Groups	**Group I**	**Group II**	**Group III**	**Group IV**
Creatinine activity in mg/100ml (Serum)	9.36 ± 1.13	9.43 ± 1.58	10.38 ± 0.47	11.76 ± 1.29
Creatinine activity in mg/100ml (Kidney homogenate)	4.37 ± 0.55	9.74 ± 0.71	11.67 ± 1.43	11.98 ± 0.93

Table 3 : Creatinine Phosphatase activity in the serum and kidney homogenates of rats in group I to group IV.

The results were statistically analyzed by using student's't' test (unpaired).
The 't' value obtained for various groups are as follows;

Group I and II -No significant difference in serum and significant difference in kidney homogenate in creatinine levels

Group II and III-No significant difference in serum but significant difference in kidney homogenate in creatinine levels

Group II and IV- Significant difference in serum and kidney homogenate in creatinine levels.

Group III and IV- Significant difference in serum and no significant difference in kidney homogenate in creatinine levels.

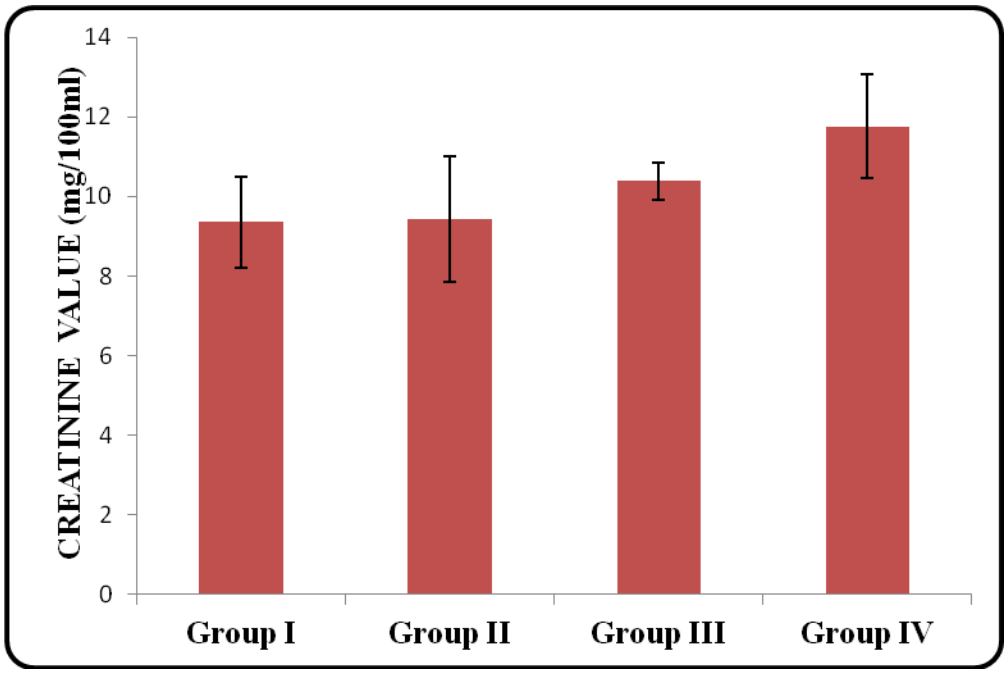


Fig. 13: Creatinine Phosphatase activity in the serum samples of rats in group I to group IV.

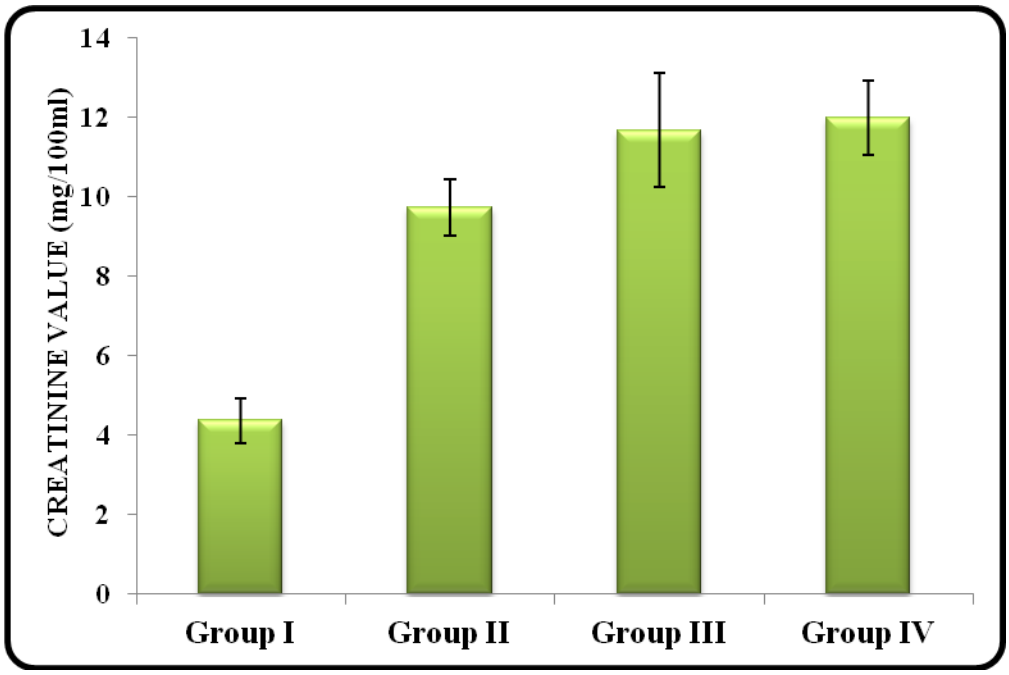


Fig.14: Creatinine Phosphatase activity in the serum samples of in the liver homogenates of rats in group I to group IV.

Discussion:

Though there was no significant difference in the creatinine levels in Group I (control) and Group II (depressed) animals the level seemed to increase under influence of fluoxetine treatment. In all the treated animals the creatinine level is significantly high in comparison to the control animals. Minimal elevations of serum creatinine at 12 hours are highly predictive of contrast-induced nephropathy and 30-day renal damage after exposure to contrast media (Ribichini *et al*., 2010).

In a predominantly hypertensive population, kidney disease identified by elevated albumin-to-creatinine ratio (ACR) was more concordant with elevated serum creatinine than with reduced glomerular filtration rate (eGFR) (Rule *et al*., 2006). In one of the study a neural network model that seemed to predict a delayed decrease in serum creatinine among pediatric kidney recipients (Santori *et al.,* 2007) was done.

In untreated hypertensive subjects with preserved renal function, arterial stiffness and plasma creatinine were found to be positively related, independent of age and ambulatory BP, suggesting an independent link between the two parameters at an early phase of hypertensive vascular disease (Gosse P. & Safar M.E, 2005).

GENERAL DISCUSSION AND CONCLUSION :

Antidepressants have been in use for a long period of time. Although it has been used effectively to treat depression, its side effects are also known. The current study is aimed to determine the effects of antidepressants on vital organs such as liver and kidney. The histological observation of liver of group I is indicative of a normal structure. Liver histology of Group II shows slight distortion in the structure of hepatocytes. The group III and IV shows unusual morphological pattern with the degeneration of the hepatocyte. The vascular sinusoids and other structures are not visible. Drug when administered at a dose of 80mg/kg caused disintegration, necrosis and degradation in liver.

This histological observation is associated with significant alterations in liver biochemistry. Graphical analysis reveals more than 4 fold increase in acid phosphatase activity in the serum of animals in group IV treated with fluoxetine (80mg/kg) compared to marginal increase in group III treated with fluoxetine (20mg/kg). Levels of ACP in liver homogenate of group III and group IV showed around 25% to 30% increase compared to control group I and group II. The serum analysis indicates significant difference in ALP levels in treated groups in comparison to the control group. The ALP level in liver homogenates in all groups in comparison to the control groups indicates no significant difference. However group IV shows significant rise in the ALP levels suggestive of hepatic toxicity at organ level at higher doses. The enzyme assays are indicative of tissue damage which is in accordance with the histological studies. It can be concluded that there is liver injury to the organisms during the course of fluoxetine treatment.

The evaluation of effects of antidepressants on kidney reveals that group I organisms do not show any significant observations. The cells are compactly arranged indicative of a normal structural integrity. The kidney of group II show disorientation of the corpuscles. The kidney of group III shows reduction in the renal corpuscles. The kidney of group IV shows unusual morphological pattern with the degeneration of the renal corpuscles. Significant alterations in the histology of kidney with the renal tubular cells showing degeneration were observed in this group.

In all the treated animals the creatinine level is significantly high in comparison to the control animals. This suggests a renal dysfunction and plasma creatinine were found to be high in correlation with the histological observation. The study concludes that any treatment with antidepressants may have negative effect on the vital organs. Thus these effects have to be considered while administering dose of the antidepressants the depression patients.

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