

# Possible Protective Effects of Agomelatine against Paracetamol Induced Toxicity in Rats

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**Abstract-** Paracetamol has a reasonable safety profile when consumed in therapeutic doses. However, it could induce hepatotoxicity, nephrotoxicity and brain damage in rats when taken in an overdose. Agomelatine, a melatonergic antidepressant with a rapid onset of action, is one of the most recent drugs in the antidepressant category. In this study, 40 Sprague Dawley rats were divided into four groups (10 rats/group): (1) control (2) Agomelatine 40mg/kg for 2weeks orally (3) Paracetamol 1gm/kg for 1week orally (4) Agomelatine 40mg/kg for 2weeks and Paracetamol 1gm/kg for 1week. Liver and kidney functions were done in serum. Antioxidant levels (GSH, CAT, MDA and NO) were analyzed in liver, kidney and brain tissues of rats also serum IL 6. Histopathological and immuno-histochemical changes of liver, kidney and brain tissue were examined. Results showed that in Paracetamol treated group there was significant increases in the activities of liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea and creatinine. Serum interleukin 6 was increased. As regards antioxidants in liver, kidney and brain, malondialdehyde (MDA) was increased and catalase and reduced glutathione (GSH) were decreased. Co-administration of Agomelatine with Paracetamol almost reversed the abnormal levels. Liver, kidney and brain histopathological and immuno-histochemical examination demonstrate that Paracetamol exerts toxicological changes in tissues with strong expression of caspase 3 meanwhile by adding Agomelatine, histopathological abnormalities were reduced and mild expression of caspase 3 had occurred. In conclusion Agomelatine co-administration with Paracetamol in rats reduced its toxicity in different organs liver, kidney and brain through its antioxidant and immunomodulatory functions

**Index Terms-** Paracetamol, Agomelatine, Antioxidants, immuno-histochemical.

## I. INTRODUCTION

Paracetamol induced toxicity in animal is one of the most commonly experimental model to evaluate the hepatoprotective activity (Jayadevaiah et al. 2012). Paracetamol, when given at overdoses, is the leading cause of liver, kidney, and other organ damages in both humans and animals. Paracetamol is a phenacetin metabolite which is considered one of the most nephrotoxic analgesics by induction of apoptosis (LORZ et al. 2003). Concerning the effects of Paracetamol overdose in the rat brain, it causes a dramatic decrease of glutathione levels (Nencini et al., 2007)

Agomelatine is an antidepressant drug considered as melatonin agonist. Melatonin is used in therapeutic application as antioxidant, anti-inflammatory and anti-apoptotic drug. The most side-effect of Agomelatine is its association with abnormal liver function tests, jaundice and hepatitis have also been reported so, Agomelatine is contraindicated in people with hepatic impairment (Zajacka et al. 2010). Agomelatine was considered as hepatoprotective in experimentally Paracetamol induced liver toxicity. Matsura et al. (2006) suggested that orally administered melatonin significantly reduces hepatic lipid peroxidation induced by Paracetamol administration.

Agomelatine reduce the expression of inflammatory cytokines and increase muscle strength in Duchenne muscular dystrophy (mdx) mice (Carvalho et al., 2014)

It is rapidly metabolized in the liver to the extremely toxic substance N-acetyl-p benzoquinoneimine (NAPQI) which leads to the formation of reactive oxygen and nitrogen species and initiates lipid peroxidation causing damage, necrosis or apoptosis of the liver cells (Hinson et al., 2004).

Agomelatine is a melatonergic receptor agonist and a selective serotonin 5HT<sub>2C</sub> receptor antagonist that has shown antidepressant efficacy. MT<sub>1</sub> and MT<sub>2</sub> receptors are predominating in the human fetal kidney cortex area and disclosed as reno-protective agent as they are generally attributed to the debated antioxidant capability (Karaman et al., 2016). Melatonin can protect the kidneys from inflammation which can cause renal damage (Kheradpezhohu et al., 2011).

The aim of the present study is to evaluate if Agomelatine will be protective in Paracetamol induced toxicity in different organs like brain, liver and kidney or has no role.

## II. MATERIALS AND METHODS

### Animals:

Male adult Sprague-Dawley rats (150–200 g) were obtained from the breeding colony of the National Organization for Drug Control and Research and maintained in the animal house at relative humidity, with 12-h light-dark cycle, temperature 21–24°C with free access to food and water ad libitum. Experimental procedures were conducted in accordance with

the ethical guidelines for investigations in laboratory animals and were approved by the Research Ethical Committee of Faculty of Pharmacy, Cairo University (Egypt) to comply with the Guide for the Care and Use of Laboratory Animals (ILAR, 1996)

### Drugs :

**-Agomelatine** :was purchased as white powder from SEDICO Pharmaceutical Company. It was freshly dissolved in 50% DMSO in saline and daily oral administration by orogastric tube (40 mg/kg) for fourteen consecutive days.

**-Paracetamol** :was purchased as white powder (pot. 99.1% W.C 0.1%) from cid Pharmaceutical Company 1gm/kg for 7days orally

### Groups :(10 rats/group)

Group 1 ):Control received saline and served as control.

Group 2) *Agomelatine* (Sedico) 40mg/kg for 14 days oral

Group 3) *Paracetamol* (Cid) 1gm/kg for 7 days oral

Group 4) *Agomelatine* 40mg/kg for 7days than co administrated with *Paracetamol* 1gm/kg for 7days

### Parameters to be examined

- Relative weights of liver, kidney and brain
- Liver function tests (AST, ALT, ALP, Total Protein and Albumin).
- Kidney function test ( urea, creatinine)
- Antioxidants (Catalase, MDA, GSH) in liver ,kidney and brain homogenates and
- serum NO and Serum IL-6
- Histopathology and Immuno-histochemical studies of liver, kidney and brain tissues

### Serum and Tissue Preparation

On the last day of injection , blood samples were taken under light ether anesthesia in non-heparinized tubes. Serum was separated by centrifugation for 20 min at 4000 g and stored at -20C in order to measure liver and kidney function . Stanbio Laboratory kits (Boerne, TX, USA) were used for the determination of the serum albumin and the total protein levels. Moreover,  $\gamma$ -glutamyl transferase (GGT) was estimated using a commercially available kit (Quimica Clinica Aplacada, Tarragona, Spain). Colorimetric assay kits for the measurement of blood urea nitrogen (BUN) level (Diamond Diagnostics, Cairo, Egypt) and serum creatinine level (Diamond Diagnostics) were used in this study. Determination of serum Interleukien-6(IL-6) was done using a test reagent kit (WKEA med supplies corp, China) .Serum NO performed using the reagent of kit provided by Biodiagnostic (Cairo, Egypt).All procedures were performed according to the manufacturers' instructions.

The kidney, liver and brain of rats were rapidly isolated and washed with ice-cold isotonic saline (0.9%) then stored at -80°C till they were homogenized in 50 mM phosphate buffer (pH 7.4) using electronic homogenizer (Ezister Daihan Scientific Co., Ltd., Korea) to prepare 10 % w/v homogenate. The homogenate was then made into aliquots and was used for the determination of MDA , GSH and CAT which according to the method of **Satoh, (1978)**, **Beutler et al. (1963)** and **Aebi, (1984)** respectively.

### Histopathological examination of the Kidney, Liver and brain:

Autopsy samples were taken from the organs of rats in different groups and fixed in 10% neutral buffered formalin for twenty four hour were embedded in paraffin; and tissue blocks were prepared for sectioning at 4  $\mu$ m thickness. Renal sections

were stained by hematoxylin& eosin and examined by light electric microscopy(40 $\times$ ).

### Casp-3 immunohistochemical examination

Prior to immunohistochemical staining, paraffin sections must be properly mounted onto slides of 5mm micron thickness and then deparaffinized and rehydrated through xylene and alcohol. Antigen retrieval was performed by placing the sections for 20 min in citrate buffer (Thermo Fisher Scientific, Fremont, USA; pH 6.0) at the boiling point then cooled. Active caspase-3 was detected when sections were incubated with the rabbit polyclonal anti-Casp-3 (CPP32) primary antibody (1:200; Thermo Fisher Scientific) overnight at 4°C. After washing with phosphate buffered saline (PBS), they were incubated with the biotinylated secondary antibody at 37°C for 30 min then incubated with the Vector Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) at 37°C for 30 min. After another wash with PBS, the antibody-biotin-avidin-peroxidase complex was developed using diaminobenzidine tetra hydrochloride (DAB Substrate Kit, Vector Laboratories Inc). Sections were counterstained with hematoxylin, dehydrated and, cleared in xylene, and cover slipped. The reaction appeared as a brown cytoplasmic reaction.

### Statistical analysis

Statistical significance of differences between means of groups was performed using the SPSS version 16 (Chicago, IL, USA), while the graphs were drawn using a prism computer program (Graph Pad software Inc. V5, San Diego, CA, USA). One-way analysis of variance (ANOVA) was employed to calculate the statistical significance followed by Tukey-Kramer Multiple Comparison Test. A value of P< 0.05 was considered significant.

## III. RESULTS

### 1 ) Effect of *Agomelatine* on liver enzymes and kidney function against *Paracetamol* induced liver and renal toxicity in rats:

There were statistically significant increases in the activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase .Total protein and serum albumin were decreased. Urea and creatinine were increased .Co- administration of *agomelatine* neutralize total protein and albumin but no significant changes occurred with other parameters

### 2) Effect of *Agomelatine* on liver ,kidney and brain antioxidants MDA,GSH,CATALASE and serum NO against *Paracetamol* induced toxicity in rats

Administration of *Paracetamol* significantly reduced GSH and catalase enzyme activity in the liver,kidney and brain and increased MDA and serum NO as compared with the control group.Co- administration of *agomelatine* caused non- significant changes

### 3) Serum IL6 was Inceased with *paracetamol* and reduced by *agomelatine* but both treatment caused non significant changes

4) **Histopathological studies** showed that *Paracetamol* induced karyomegaly of hepatocytic nuclei and massive necrosis and apoptosis of hepatocytes . In the kidney , *Paracetamol* caused necrobiotic changes of renal tubular epithelium and atrophy of glomerular tuft .Protein cast were found in the lumen of renal tubules. In the brain, focal cerebral haemorrhage, necrosis of neurons and neuronophagia in brain cells had

occurred . By co-administration of *Agomelatine* the normal cellular architecture was nearly restored

5) **Immuno-histochemical studies** showed strong positive expression of caspase 3 in liver, kidney and brain .By co-administration of *Agomelatine* , immunopositivity for caspase 3 were reduced in all organs

**Table 1 : Effects of *Agomelatine* and *Paracetamol* on liver and kidney functions in rats :**

<i>Groups</i> <i>Parameter</i>	<i>AST</i> (U/L)	<i>ALT</i> (U/L)	<i>ALP</i> (U/L)	<i>Total protein</i> (g/dl)	<i>Albumin</i> (g/ dl)	<i>BUN</i> (mg/dl)	<i>Creatinine</i> (mg/dl)
<i>Control</i>	81.15±20.01	49.48±3.19	170.7±10.08	6.450±0.58	5.162±0.66	20.70±0.98	1.080±0.16
<i>Paracetamol</i>	177.1±13.21** *	126.0±22.18**	378.8±14.35*	2.853±0.65*	2.648±0.42	39.00±2.16**	4.820±1.47*
<i>Agomelatine</i>	126.6±9.83	53.87±8.25*#	116.0±9.23#	4.885±0.46	4.114±0.81	24.13±4.45#	1.880±0.66
<i>Para+Agomel</i>	165.0±9.33**	114.2±11.88*	246.6±19.57	6.335±1.03#	3.100±0.55	24.53±2.53#	2.180±0.37

Each value indicates the mean±SEM of 10-12 observations.\* P<0.05 compared to control. Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer Multiple Comparison Test.

**TABLE 2 :Effects of *Agomelatine* and *Paracetamol* on liver antioxidants:**

	<b>Catalase</b>	<b>MDA</b>	<b>GSH</b>
<b>Control</b>	7.790±0.4422	2.540±0.1424	6.616±0.4914
<b><i>Paracetamol</i></b>	3.333±0.4317**	4.166±0.5702*	3.858±0.8199*
<b><i>Agomelatine</i></b>	6.895±0.8871#	2.302±0.0954#	6.756±0.5781
<b><i>Para+Agomel.</i></b>	6.080±1.151*	3.405±0.6237	5.458±0.7313*

Each value indicates the mean±SEM of 10-12 observations.\* P<0.05 compared to control. Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer Multiple Comparison Test.

**TABLE 3 : Effects of *Agomelatine* and *Paracetamol* on kidney antioxidants:**

	<b>Catalase</b>	<b>MDA</b>	<b>GSH</b>
<b>Control</b>	4.275±0.9222	6.298±0.5976	11.94±0.1206
<b><i>Paracetamol</i></b>	1.523±0.4222*	9.506±0.8351*	6.465±0.6682***
<b><i>Agomelatine</i></b>	5.183±0.1465##	7.663±0.2712	7.158±0.3839***

<i>Para+Agomel.</i>	3.043±0.7205	9.832±0.7716*	6.413±0.7121***
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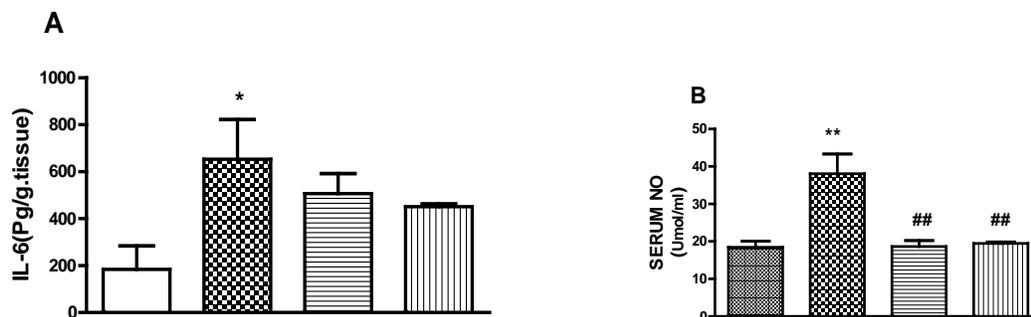
Each value indicates the mean±SEM of 10-12 observations.\* P<0.05 compared to control.

Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer Multiple Comparison Test.

**TABLE 4 : Effects of Agomelatine and Paracetamol on Brain antioxidants:**

	Catalase	MDA	GSH
<b>Control</b>	4.793±0.7395	6.224±0.6824	8.428±0.6543
<i>Paracetamol</i>	0.5135±0.2835*	10.23±0.4901**	3.963±0.9225**
<i>Agomelatine</i>	4.158±1.177#	7.050±0.5974	7.808±0.8498
<i>Para+Agomel.</i>	1.325±0.7079*	7.622±0.7026	6.703±0.6313

Each value indicates the mean±SEM of 10-12 observations.\* P<0.05 compared to control. Statistical analysis was carried out by one way ANOVA followed by Tukey-Kramer Multiple Comparison Test.



**Fig 1: A&B ):Effects of Agomelatine and Paracetamol on serum interleukin 6 and nitric oxide :**

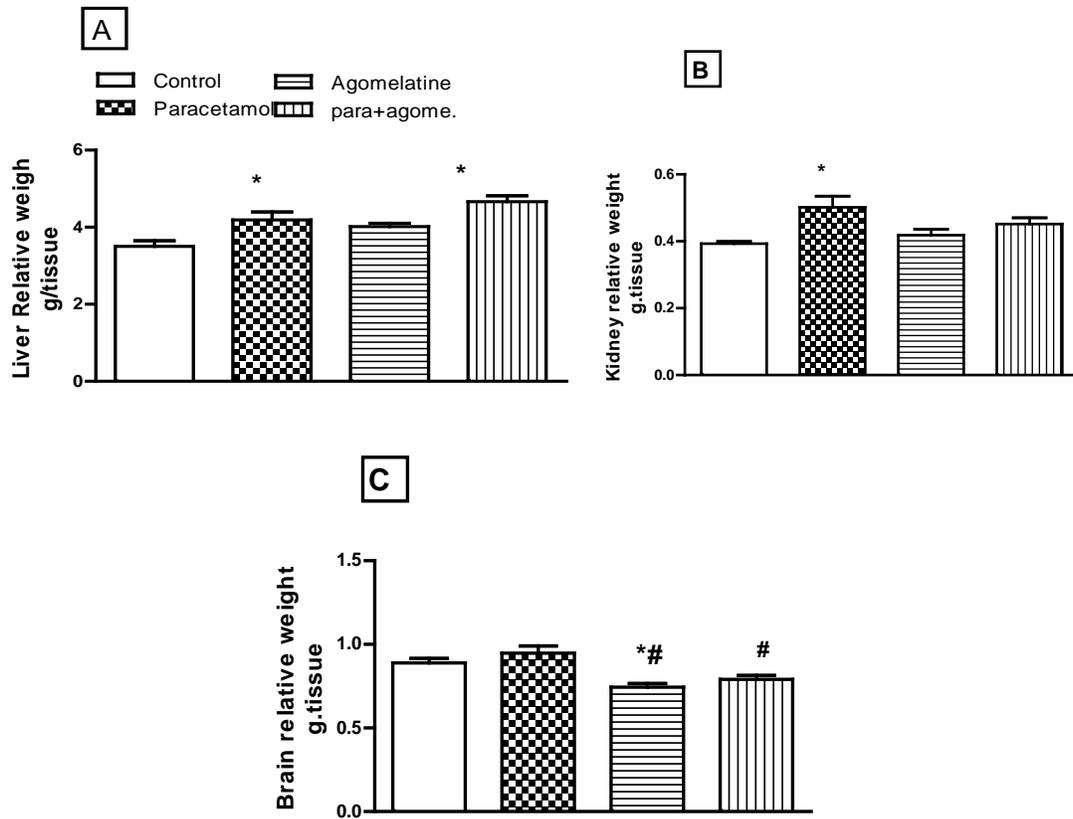
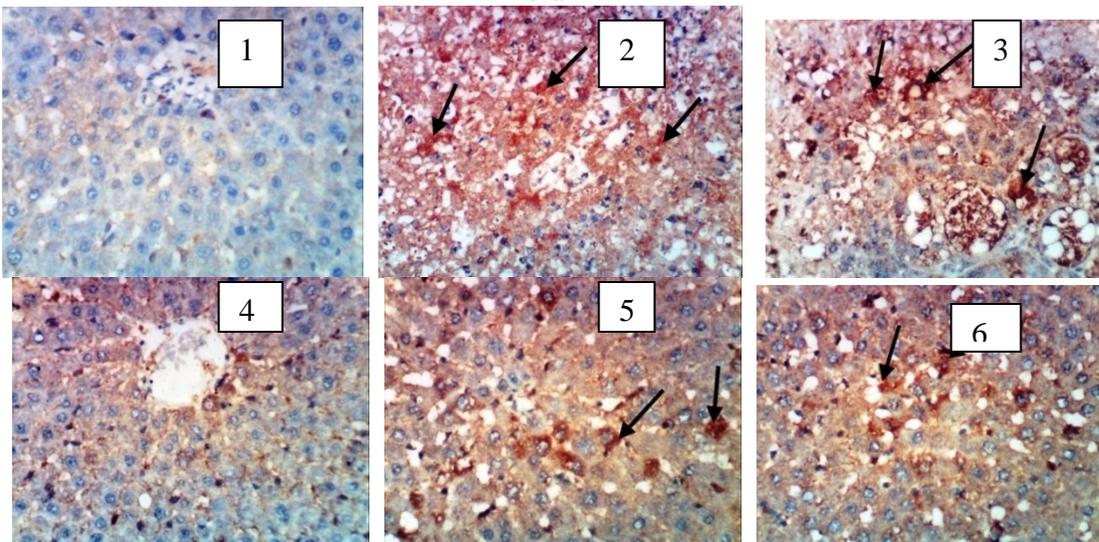
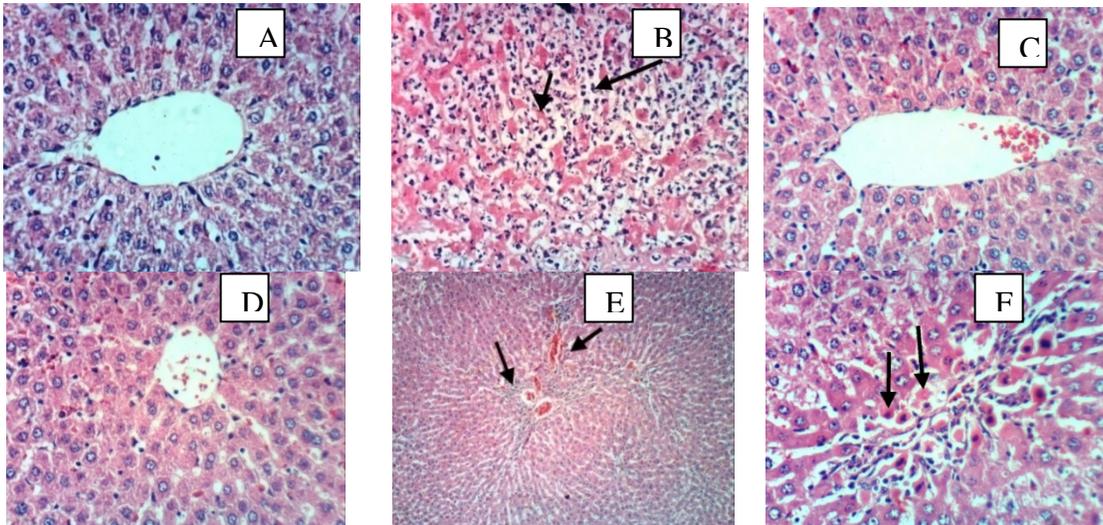


Fig 2 :(A,B&C) : Relative weights of liver ,kidney and brain of *Agomelatine* and *Paracetamol* treated groups :

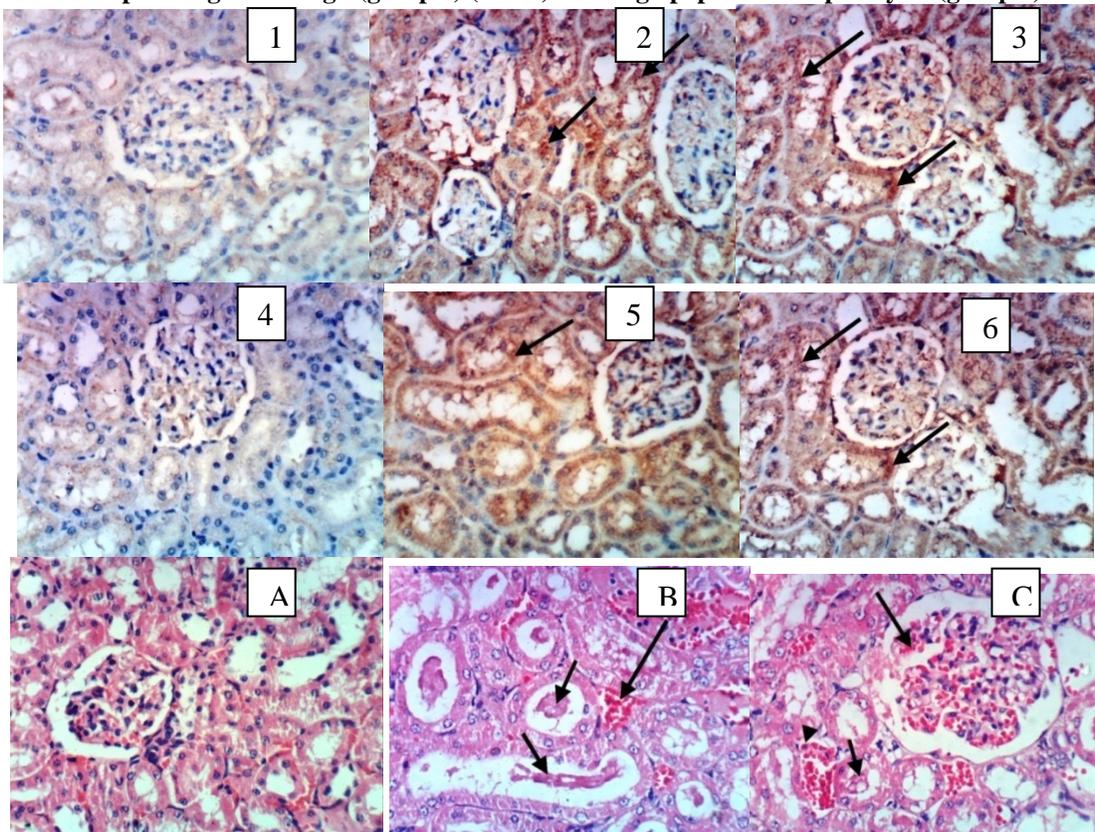
**Immunohistochemical examination (Casp-3) and histopathological examination of the Liver ,Kidney and brain**

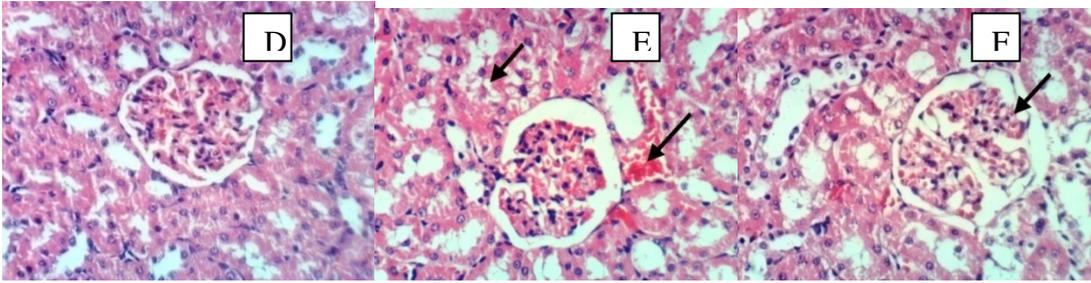




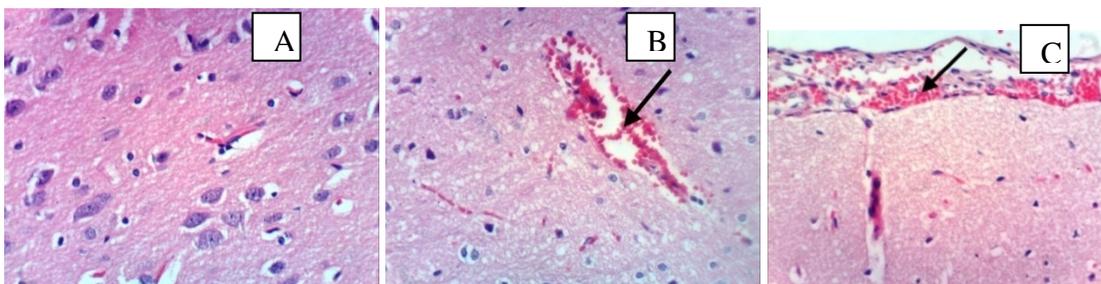
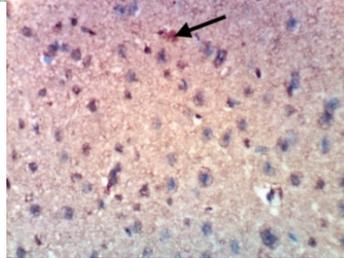
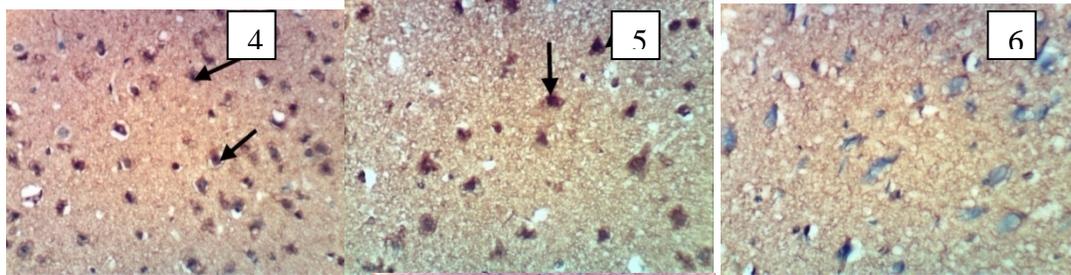
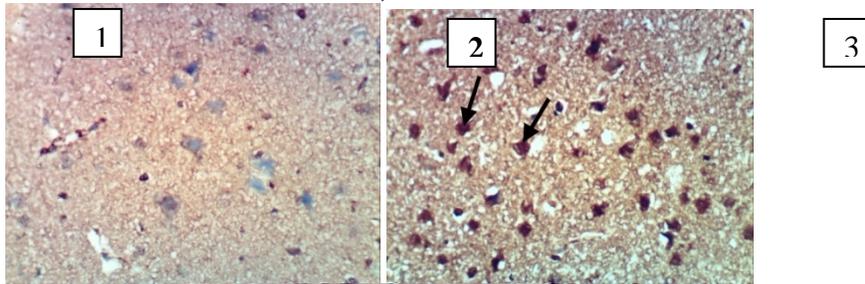
**Fig 1)** control liver showing no expression of caspase 3 (group1). ( 2 & 3) showing strong positive expression of caspase 3 (group2). (4) showing no expression of caspase 3 (group 3). (5&6) showing moderate positive expression of caspase 3(group 4)

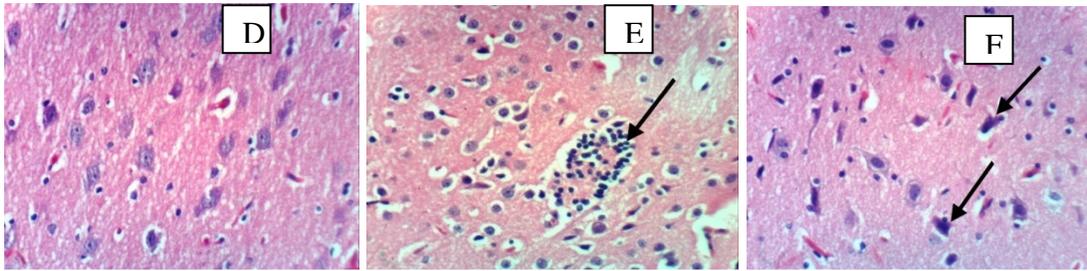
**Fig A)** showing the normal histological structure of hepatic lobule (control group). (B&C ) showing karyomegally of hepatocytic nuclei and marked apoptosis of hepatocytes (group2) .D) showing no histopathological changes(group 3).( E&F) showing apoptosis of hepatocytes (group 4)





**Fig 1) control kidney .(2&3) showing strong positive expression of caspase 3 (strong immunopositivity for caspase 3 indicated by brown colour)( group 2). 4) showing no expression of caspase 3 (negative immunohistochemical reaction) (group3). (5&6) showing moderate positive expression of caspase 3(group4). Fig A) control group showing the normal histological structure of renal parenchyma (B&C) showing protein cast in the lumen of renal tubules, congestion and vacuolation of glomerular tuft as well as congestion of intertubular blood vessels ( group 2). D) showing no histopathological changes (group 3). (E&F) showing slight vacuolation of renal tubular epithelium and congestion of intertubular blood vessels and glomerular tuft (group 4)**





1) Brain of control group showing no expression of caspase 3 (negative immunohistochemical reaction). (2 &3) showing strong positive expression of caspase 3 indicated by brown colour ( group 2) . 4 ) showing no expression of caspase 3 (group3). (5&6) showing mild immunopositivity for caspase 3 (group 4).  
A) Control brain . (B&C) showing focal cerebral and meningeal haemorrhage. ( group 2). D) showing no histopathological changes ( group 3). (E&F) showing focal gliosis and necrosis of some neurons and neuronophagia

#### IV. DISCUSSION

This study evaluated the effects of administration of *agomelatine* on liver, kidney and brain in a model of toxicity induced by *paracetamol* in rats. In our study, *paracetamol* increased serum levels of AST, ALT, ALP, BUN and Creatinine and decreasing levels of total protein and serum albumin. Furthermore, *paracetamol* induced a state of oxidative stress in liver, kidney and brain tissues of rats with increase of MDA and NO and decrease in GSH and Catalase.

An acute *paracetamol* overdose can lead to potentially lethal liver and kidney failure in humans and experimental animals (Kheradpezhohu et al. 2010). In the liver, *paracetamol* toxic metabolite, n-acetyl-p-benzoquinone-amine, binds to the sulfhydryl group of protein resulting in cell necrosis and lipid peroxidation causing leakage of the plasma membrane, and an increase in serum levels of liver enzymes (Kapoor et al. 1994). Also calcium desregulation was induced by *Paracetamol* in the endoplasmic reticulum leading to induction of apoptosis (Nakagawa et al. 2000) and this explain the apoptosis present in the liver cells in our study. *Paracetamol* overdose imposed severe proximal tubular damage leading to apoptotic cell death through decrease in Nuclear factor erythroid 2-related factor (2Nrf2) (Mathur et al. 2016) and this explain the rise in serum urea and creatinine and the apoptosis detected in the renal cells of rats in our study.

The brain is considered more vulnerable to oxidative stress than other tissues because it metabolizes 20% of total body oxygen and has limited antioxidant capacity (Halliwell., 2006). *Paracetamol* can cross blood brain barrier and its increase caused a state of oxidative stress (Courad et al., 2001 & Nencini et al 2007) and this coincide with the values of antioxidants in our results.

In our study, co-administration of *Agomelatine* with *paracetamol* decreased oxidative stress and improved apoptosis in liver, kidney and brain tissues. It has been reported that melatonin protects mice against *paracetamol*-induced hepatotoxicity probably via the inhibition of oxidative stress, including lipid peroxidation and protein oxidation (Sener et al., 2003). Also Melatonin caused immunomodulation in the liver Guerrero and Reiter (2002). *Agomelatine* decreases the levels

of cytokines such as TNF- $\alpha$  and IL-6, and by displaying antioxidant activity reverses *paracetamol*-induced hepatotoxicity, thus showing protective effects for the liver (Karakus et al., 2013) and this goes with our study in which *agomelatine* decreased serum IL6.

*Agomelatine* has nephro-protective effects against contrast-induced nephrotoxicity in rats. This effect can be attributed to its properties of reducing oxidative stress and inhibiting the secretion of proinflammatory cytokines (Karaman et al., 2016). In our study, *agomelatine* has antioxidant effects in brain tissue. Stein et al. (2012) evaluated the efficacy and tolerability of *agomelatine* in the prevention of relapse in patients with generalized anxiety disorder due to its antioxidant activity. The antioxidant role of *agomelatine* in neurons was studied by Akpinar et al. (2014) in which glutathione and glutathione peroxidase values were significantly higher in neuronal cells exposed in cell culture to *agomelatine*.

Histological evaluation confirm the biochemical parameters in circulation and tissues. In the liver, kidney and brain sections of the rats intoxicated with *Paracetamol*, signs of toxicity in the cells and loss of the normal cellular architecture had occurred and was restored by *Agomelatine* confirming its protective effect. Roomi et al. (2008) found mild and significant lobular focal hepatitis in *paracetamol* studied group. Madhukiran et al. (2011) reported that kidneys of *paracetamol* treated rats, showed interstitial inflammatory cell infiltration and significant tubular damage..

**In conclusion**, *paracetamol* toxicity in rats affects liver, kidney and brain, co-administration of *agomelatine* reduce this effects by its antioxidant and immunomodulatory properties.

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