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Antioxidant and Histopathological Effect of Galantamine Against Paracetamol-Induced Side Effect

Gamal Shams, Shahd El-Mesiry*

Pharmacology Department, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt.

***Correspondence**
Shahd El-Mesiry
Pharmacology Department, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt.
E-mail address: shahdmesiry@gmail.com

Abstract

The goal of this research was to find out more about the possible protective role of galantamine (GAL) (0.3 mg/kg P.O) for 28 successive days against paracetamol (PCM) toxicity that was administrated on day 29 of the experiment, at day 30, blood sample were collected for evaluation of antioxidant enzymes and tissues for histopathological studies. Oxidative stress biomarkers which included serum superoxide dismutase, glutathione peroxidase, catalase, and malondialdehyde and histopathological studies indicated that GAL has protective effects by prohibiting the improvement in oxidative stress biomarkers and improved the histopathological lesions seen in livers and kidneys *in vivo* model against hepatic and renal toxicity induced by PCM in rats.

KEYWORDS
Antioxidant properties, Galantamine, Hepatotoxicity Paracetamol, Renal toxicity.

INTRODUCTION

The main role of the liver is the detoxification of any hazardous materials entering the body. Hepatotoxins, which could also originate from chemicals, dietary supplements, pharmaceutical medications, and medicinal plants, promote hepatotoxicity (Salman *et al*., 2020). The kidneys play a significant role in overall body balance. Any interruption occurred by a toxic insult to the kidneys generates acute renal failure as a side effect (Rana, 2021).

Paracetamol (PCM) also known as acetaminophen, is frequently prescribed for its safety as an analgesic and antipyretic, with no anti-inflammatory properties. It's utilized for treating high fever caused by bacterial or viral infections. PCM's hepatotoxic and nephrotoxic consequences on acute overdosage and chronic consumption have been extensively documented. coupling to develop phenolic, glucuronide, or other compounds, resulting in catechol and glutathione metabolites, are among the most significant pathways of PCM metabolism. The hepatotoxicity of PCM is increased by an increase in oxidative metabolism (Przybyła *et al*., 2021).

Galantamine (GAL) is a natural alkaloid and a long-acting specific centrally active cholinergic drug. GAL acts on the cholinergic system in two ways: it regulates the nicotinic acetylcholine receptors (nAChR) and diminishes acetylcholine receptors (ACh). GAL LadyG conjugate to nAChR to boost the nicotinic neurotransmission by a dynamic modification on nAChR. The response of nAChR is strengthened when Gal and Ach associate with specific distinct attachment sites at one time. Furthermore, the OH group of GAL gives it antioxidant characteristics because it is a hunter for reactive oxygen species (ROS) (Ibrahim *et al*., 2018).

According to the above mentioned, this study aimed to reveal the possible protecting impacts of GAL versus hepatic and renal toxicity caused by PCM in a rat model by examining the antioxidant markers and the histopathology of hepatic and kidney tissue.

MATERIALS AND METHODS

Drugs

GAL (Famalzyl, 0.3 mg/kg b.wt): It was supplied by October pharma, Giza District, Giza Governorate. PCM (Panadol, 2 g/kg b.wt): It was supplied by GlaxoSmithKline, London, UK.

Study protocols

Sprague-Dawley male albino rats (n = 40) weighing 150 to 200 g, collected from the Animal House of the Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were separated into 4 groups in each group (n=10). The 1st group: -ve control group in which animals were left without any treatment. The 2nd group (GAL): in which animals received GAL (0.3 mg/kg b.wt) orally once daily for 28 successive days (Nikiforuk *et al*., 2016). The 3rd group (PCM): Rats in this group received a single

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dose of PCM (2 g/kg b.wt) orally on day 29 of the experiment (El-Maddawy and El-Sayed, 2018). The 4th group (PCM + GAL): Rats in this group received oral doses of GAL (0.3 mg/kg) daily by gavage for 28 successive days and PCM (2 g/kg b.wt) by gavage on day 29 of the experiment. The blood samples and hepatic and renal tissues were collected and preserved until used. The study was accredited by the Institutional Animal Care and Use Committees Zagazig University (ZU-IACUC) with approval No. ZU_IACUC/2/F/186/2021.

Assay of serum Antioxidant enzymes activities and MDA level

Determination of Malondialdehyde (MDA) concentration in serum as a marker of lipid peroxidation was determined by using Rat Malondialdehyde ELISA Kit (MyBioSource, Catalog No: MBS738685), according to manufacturing instructions (Esterbauer, 1982). Catalase (CAT) activity was determined in serum by using Rat catalase ELISA Kit (MyBioSource, Catalog No: MBS701908) according to manufacturing instructions (Aebi, 1984). Glutathione peroxidase (GPx) activity was determined in serum by using GPx ELISA Kit (Biodiagnostic, Catalog No: GP 2524) according to manufacturing instructions (Paglia and Valentine, 1967). Superoxide dismutase (SOD) activity was determined in serum by using the SOD Activity Assay Kit (Abcam, Cat. No: ab65354) according to manufacturing instructions (Misra and Fridovich, 1972).

Histopathology

The desired organ tissues of the rats were obtained and preserved with formaldehyde solution (10 % w/v) after they were dissected. Following that, follow-up techniques were used to do a histological study of the tissue's toxicity. The tissues were then fixed in paraffin blocks at the end of the operation. They were then cut into 5–6 µm thick paraffin sections and blemished with hematoxylin-eosin.

Statistical analysis

The analysis was done by using the computerized SPSS version 16 application. The data are reported as mean standard deviation. One-way ANOVA was used to examine these data, and Duncan's test was used to reveal the significance. $P < 0.05$ was considered significant (Billard and Diday, 2003).

RESULTS

Effect on oxidative stress markers (SOD, GPX, CAT, and MDA)

The results showed in Fig. 1 revealed a substantial downregulation in the value of SOD in the PCM treated group in comparison with the -ve control group. Meanwhile, the group of rats administrated both PCM and GAL showed a substantial upregulation ($p \leq 0.05$) in the level of SOD compared to PCM treated group. Moreover, the group of rats administrated a GAL showed no significant difference in the SOD level compared to the -ve control group.

Also, the obtained data demonstrated a remarkable downregulation in the value of CAT in the PCM treated group compared to the -ve control group. Meanwhile, the group of rats administrated both PCM and GAL demonstrated a remarkable downregulation in the value of CAT compared to PCM treated group. Moreover, the group of rats administrated a GAL showed no significant difference in the CAT level compared to the -ve control group Fig. 2.

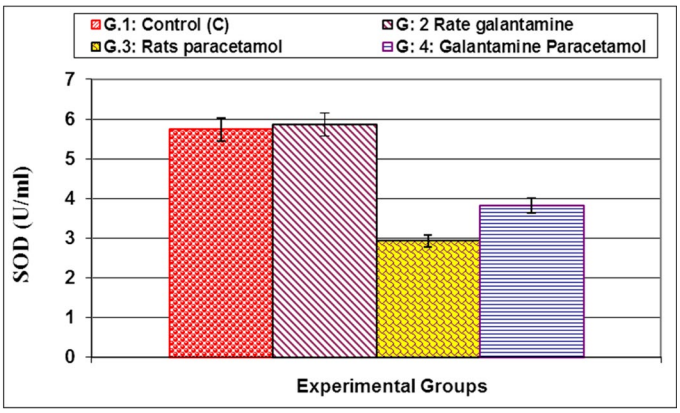

Fig. 1. The effect of oral supplementation of PCM, GAL and their coupling on SOD (U/ml) in male albino models.

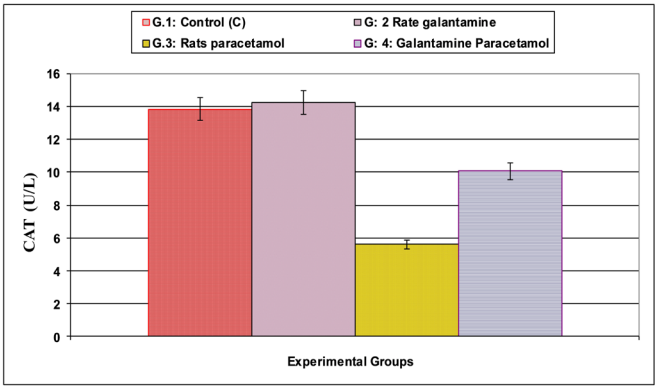

Fig. 2. The effect of oral supplementation of PCM, GAL and their coupling on CAT (U/L) in male albino models.

Data shown in Fig. 3 demonstrated that there was a highly statistically different among groups in GPx value. The PCM-treated group revealed the lowest GPx reading. Meanwhile, the group of rats administrated both PCM and GAL revealed a notable upregulation in the value of GPx compared with PCM treated group. Moreover, the group of rats administrated a GAL showed no difference in the GPx level in comparison with the -ve control group.

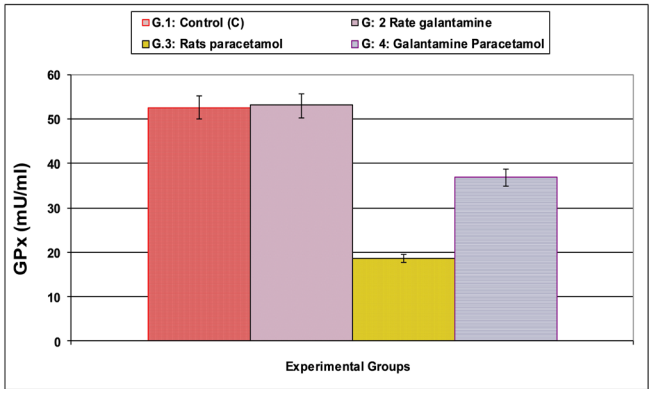

Fig. 3. The effect of oral supplementation of PCM, GAL and their coupling on GPx (U/ml) in male albino models.

The obtained result of Fig. 4 revealed a meaningful upregulation in the MDA value in the PCM treated group compared to the -ve control group. Meanwhile, the group of rats administrated both PCM and GAL revealed a meaningful downregulation in the MDA value compared to PCM treated group. Moreover, the group of rats administrated a GAL showed no significant difference in the MDA value compared to the -ve control group.

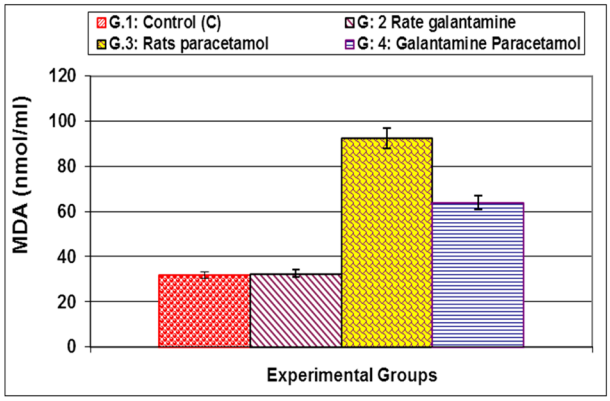

Fig. 4. The effect of oral supplementation of PCM, GAL and their coupling on MDA (nmol/ml) in male albino models.

Histopathological examination

The liver section of the -ve control group showed a typical hepatic form (Fig. 5A). Rat's H&E-stained liver section of the GAL treated group (2) showed a normal histological picture of hepatocytes as control (Fig. 5B). Meanwhile, the liver section of PCM treated group (3) revealed aggregative coagulative necrosis reintegrated by mononuclear infiltration (Fig. 5C). Moreover, liver sections of drug PCM + GAL treated group (4) showing mild mononuclear (lymphocytes, monocytes, and plasma cells) aggregation with hepatocytes regeneration (Fig. 5D).

Renal slides of the negative control group show typical architecture (Fig. 6A). Also, the kidney section of the GAL treated group showed normal renal glomeruli and tubules as control (Fig. 6B). Meanwhile, the kidney section of the PCM treated group showed necrotic glomeruli, extensive hemorrhage and destructed renal tubules with little vascular congestion (Fig. 6D). Moreover, the stained section of PCM + GAL treated group showing tubular debris with regenerated tubules (Fig. 6E).

DISCUSSION

Excessive formation of ROS and a depleted antioxidant system generate free radicals, which leads to pathological illnesses like cancer, CVD, and diabetes. The antioxidant system's second line of defense is made up of CAT and GPx. All live cells that require oxygen for optimal metabolism produce catalase. Catalase is a metalloenzyme with iron as a cofactor that catalyzes the breakdown of H_2O_2 molecules into H2O and O2 molecules (Ighodaro and Akinloye, 2018).

PCM is an analgesic and antipyretic that is widely considered

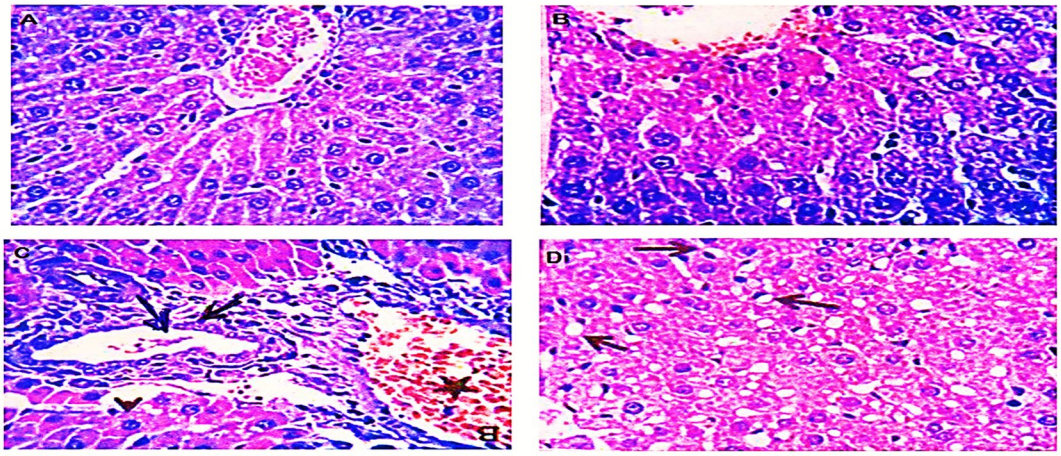

Fig. 5. Histopathological evaluation in rat liver. (A) Photomicrograph of liver, negative control group showing normal liver (H&E, 400x). (B) Photomicrograph of liver, of GAL treated group (2) showing normal histological picture of hepatocytes as control (H&E, 400x). (C) Photomicrograph of liver, of PCM treated group (3) showing aggregative coagulative necrosis reintegrated by mononuclear infiltration (H&E, 400x). (D) Photomicrograph of liver, of PCM and GAL treated group (4) showing mild mononuclear (lymphocytes, monocytes, and plasma cells) aggregation with hepatocytes regeneration (H&E, 400x).

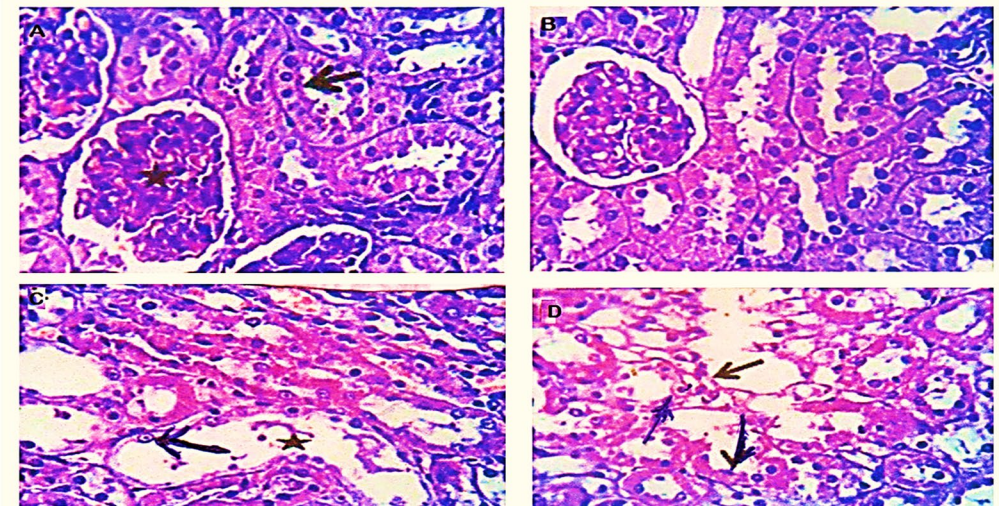

Fig. 6. Histopathological evaluation in rat kidney. (A) Photomicrograph of kidney, negative group revealing typical architecture (H&E, 400x). (B) Photomicrograph of kidney, of GAL treated group showing normal histological picture of kidney as control (H&E, 400x). (C) Photomicrograph of kidney, of PCM treated group showing necrotic glomeruli, extensive hemorrhage and destructed renal tubules with little vascular congestion (H&E, 400x). (D) Photomicrograph of kidney, of PCM and GAL treated group showing tubular debris with regenerated tubules (H&E, 400x).

to be safe. It is known for causing dose-dependent hepatotoxicity in overdose conditions. Unless adequate antidotes are supplied early after the overdose, medical conditions can develop into acute liver failure, which is usually coupled with renal failure and multiple failures in different organs (Moshaie-Nezhad *et al*., 2019).

In this study, we demonstrated that the supplying of PCM to albino male rats caused a remarkable downregulation in CAT, SOD, and GPx with a remarkable upregulation in MDA activity levels when in comparison with the -ve control group, while a low dose of GAL significantly alleviates oxidative stress with restoring the normal histological structures in liver and kidney sections. This is the first study to evaluate the impacts of GAL against hepatic and renal toxicity induced by PCM in animal models.

Results from the present study coincide with Ajith *et al*. (2007), who recorded a remarkable reduction in hepatic CAT, SOD, and GSH with a remarkable increase in hepatic MDA content in female rats which received a single oral dose of PCM (3 g/kg body wt) in comparison with the -ve control group.

Soliman *et al*. (2014), studied the overdose of PCM for liver and renal toxicity in male Wistar rats. Overdosing on PCM raised MDA levels considerably in the PCM-treated rats in comparison with the -ve control group. CAT value was substantially lower in the PCM group than in the -ve control group, and these data are in agreement with the current study. Motawi *et al*. (2020), studied the impact of PCM toxicity in rat models. When compared to the normal group, PCM caused kidney impairment by increased MDA and a decrease in CAT, SOD, and GSH antioxidants demonstrating oxidative stress induction in tissues.

The recorded data in this study are in line with the report of Gupta *et al*. (2014), who reported that PCM induced a decrease in CAT, SOD, and GPX in a male rat that injected 350 mg/kg of PCM. The same result was recorded by Yen *et al*. (2007), in male Wistar-albino rats intoxicated with 835 mg/kg APAP on day seven of the experiment.

Previous research has shown that cholinesterase inhibitors protect patients with sepsis and acute liver failure by lowering proinflammatory cytokines and oxidative stress while raising anti-inflammatory cytokines and antioxidant levels (Bondok *et al*., 2013; Steinebrunner *et al*., 2014).

GAL's impact on oxidative stress in people with metabolic syndrome had previously been studied. Following GAL treatment, the SOD and CAT activity were greatly boosted, whereas MDA, another biomarker of oxidative damage, was significantly lowered. GAL also reduced plasma nitrite levels considerably. GAL dramatically reduced inflammation and insulin resistance, as well as the low ratio of heart rate variability, and this also supported the obtained results (Sangaleti *et al*., 2021).

GALs as a potential anti-diabetic activity were evaluated in the diabetic n5-STZ rat model for 4 weeks and the data revealed that there was a slight increase in MDA in the diabetic model and an exceptional reduction in TAC in the liver homogenate (Ali *et al*., 2015).

GAL possesses anti-inflammatory properties that limited the synthesis of cytokines (Consolim Colombo *et al*., 2017). The interaction of Ach with the α-7 nAChR inhibited inflammation (Satapathy *et al*., 2011). α-7 nAChR is found in hepatic stellate cells. Furthermore, the OH group of GAL is a hunter for ROS, indicating that it has antioxidant potential (Koola *et al*., 2019).

Results from this study demonstrated that the alternations in the biochemical results were confirmed by the histopathological studies in the liver and kidney. Supplementation of PCM showing aggregative coagulative necrosis reintegrated by mononuclear infiltration in the liver and the kidney section showed necrotic glomeruli, extensive hemorrhage, and destructed renal tubules these findings meaningful decreased in the group treated with GAL and PCM. These results are supported by the following studies.

There was considerable coagulative necrosis of hepatocytes in the PCM-treated group's liver, as well as large neutrophilic and lymphocytic infiltration. Immunohistochemistry for MMP-8 in the liver indicated an upregulation in MMP-8 activity in the hepatic lobules in PCM-treated rats (Soliman *et al*., 2014).

In the PCM-treated group on Albino rats, the hepatotoxic and nephrotoxic impact of PCM on histopathological examination of the liver revealed evidence of deterioration, sinusoidal congestion, and occasional mononuclear cell infiltration. Treatment with PCM caused mild histological alterations in the kidney, in the form of congestion, and aggregative coagulative necrosis reintegrated by mononuclear infiltration (Dahiya *et al*., 2014).

These findings are consistent with the study done by Pathan *et al*. (2013), who found that PCM-treated rats' showed a loss of renal tubular architecture as well as a reorganization of renal tubules and glomeruli. Renal sections revealed alterations, with cellular enlargement in the renal tubules typically accompanied by a significant narrowing of the lumen.

The effects of GAL were investigated by detecting inflammatory mediator levels and analyzing histological characteristics associated with LPS-induced acute lung injury. At 12 hours after receiving LPS, the rats demonstrated development in the alveolar wall, edema, hemorrhage, and inflammatory cell infiltration, indicating the onset of acute lung injury. When rats were given GAL before receiving LPS, they had considerably less inflammation and lung architectural distortion than rats who were not given GAL (Li *et al*., 2015).

Furthermore, histopathological evaluation of the kidney demonstrated that the GAL-treated group exhibited mild vacuolization of epithelium covering renal tubules, as well as minimum glomerular tuft congestion in acute kidney damage caused by zymosan in mice ((Ibrahim *et al*., 2018). GAL treatment of obese mice was studied and the microscopic investigations revealed that HFD mice had more hepatocyte fat accumulation, but GAL reduced lipid accumulation in HFD mice (Satapathy *et al*., 2011).

CONCLUSION

Co-supplementation of PCM and GAL has a better result than PCM alone. As GAL has protective effects against antioxidant markers and histopathological studies. Future studies should be carried out using different concentrations of GAL and other standard drugs.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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