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Effects Of Ivermectin On The Brain And Kidney And Its Interaction With P-Glycoprotein Inhibitor (Verapamil) In Rats.

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ABSTRACT

Administration of permeability-glycoprotein (Pgp) inhibitors can modify the pharmacological properties or induce toxic effects of Pgp substrates. The effects of administration of ivermectin (anthelmintic drug, Pgp substrate), either alone or simultaneously with verapamil (Pgp inhibitor) on the histological structure of the kidney and brain tissues and some serum biochemical parameters were investigated. Additionally, the lipid peroxidation as malondialdehyde (MDA) and activity of the antioxidant enzymes reduced glutathione (GSH) and glutathione peroxidase (GSH peroxidase) at these tissues were determined. The results revealed that administration of ivermectin once weekly for 4 weeks induced slight elevations of the alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea and creatinine. The anti-oxidative defenses (GSH and GSH peroxidase) at these tissues were also slightly diminished and the MDA content was slightly increased. The normal histological structure of the brain and kidney tissues was mildly altered. Meanwhile, the combined treatment of ivermectin and verapamil induced stronger harmful effects on these tissues. It is concluded that ivermectin has slight toxic effects on the brain and kidney tissues, but when taken with verapamil (Pgp inhibitor) the previous effects becomes more pronounced. So, great attentions must be paid when combining drugs act either as Pgp substrates or inhibitors.

Keywords: ivermectin, verapamil, P-gp, brain, kidney, malondialdehyde, creatinine

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INTRODUCTION

Ivermectin (IVM) is an anthelmintic drug and acaricide of the avermectins family, produced by Streptomyces avermitilis cultures. It is a commonly used anthelmintic to treat various endo- and ecto-parasites [1]. The main mode of action of IVM is to bind to glutamate gated chloride channels which leads to paralysis of the nematodes [2] and may interfere with the function of gastrointestinal target parasites, resulting in parasite starvation. Additionally, new effects of IVM have been revealed, including its special effects as an anti-cancer [3-7], anti-mycobacterial, anti-plasmodial and anti-viral have been pronounced [8-10].The low IVM toxicity has been recognized to its limited access to some organs and brain tissues, especially for being a substrate of Pgp [11]. The presence of Pgp in Haemonchus contorts and other parasites plays a role in IVM resistance even at 10-fold the dose [12-15].

The drug-transporting Pgp originally revealed in multidrug resistance tumor cells [16]. Pgp was demonstrated in biologically important protective barriers, blood–brain barrier, intestinal barrier, blood- testis barrier, and maternal fetal barrier, at the apical membrane of the kidney proximal tubules and is expressed in a variety of normal tissues mostly of epithelial origin [11, 17-22]. Interactions with substances that inhibit Pgp are of great interest, as they can potentially enhance the absorption of important medicines that are generally poorly absorbed, such as chemotherapeutic medicines [23-24], increasing their levels and activities [25-26]. Theoretically, Pgp inhibition may increase the incidence of side effects or toxicity of some medicines, producing unwanted effects.

Many compounds are known to modulate the Pgp by reducing the efflux activity of the pump, e.g. verapamil, erythromycin, cyclosporine A, and their analogs [27]. Therefore, the modulation of Pgp function by Pgp inhibitors, such as verapamil, can be an important factor in modifying the pharmacological actions of certain drugs. Previously, we found that co-administration of IVM with verapamil in rats' induced adverse effects on male fertility and severely damaged fetal genetic material; their development and gene-toxic effects in somatic cells of the dams [28-29]. So, the present experiment aimed to investigate the impact of co-administration of ivermectin and verapamil on the brain and kidney tissues in rats.

MATERIALS AND METHODS

Animals

Wistar albino rats (180±20 g) of either sex, were obtained from the Animal House of the College of Pharmacy, King Saud University, Kingdom of Saudi Arabia and housed at a temperature of 22 - 28 C and relative humidity of 50–60, with artificial light from 5.00 a.m. to 4.00 p.m. Animals had free access to tap water and standard rat chow, used for the study. The local ethics committee approved the study.

Experimental Protocol

Mature male Albino rats were divided into four groups (6 rats each). Group 1(control) received once weekly saline (2 ml/kg b. wt. i. p.) and after 1 h received propylene glycol (2 ml/kg b. wt. i. p.) . Group 2 (Ivermectin) received once weekly saline and after 1 h received ivermectin, fig.1. Moramectin® 1%, obtained from Arabcomed, Egypt (0.58 mg/kg b. wt. i. p.) [30]**.** Group 3 (Verapamil) received once weekly verapamil (fig.2) Isoptin® from Knoll, Istanbul, Turkey (3 mg/kg b. wt. i. p.) [31], 1 h before and 1 h after received propylene glycol as in group 1. Group 4 (Verapamil + Ivermectin) received verapamil (3 mg/kg b. wt. i. p.) once weekly 1 h before and 1 h after received ivermectin. After 4 weeks from the beginning of treatment, the following parameters were determined:

Assay of serum AST, ALT, ALP, urea and creatinine

These parameters were analyzed using commercially available diagnostic kits of Diamond Diagnostic Co., Egypt.

Estimation of lipid peroxidation

Lipid peroxides as malondialdehyde (MDA) were measured according to the method of Iqbal et al., [32].

Measurement of glutathione

Reduced glutathione (GSH) was assayed by spectophotometric technique according to the method described by Jollow et al., [33].

Determination of glutathione peroxidase activity

GSH peroxidase was determined chemically using cummene hydroperoxide as a co-substrate according to the method of Habig et al., [34].

Histopathological observation

The brain and kidney tissues were fixed in 10% neutral formalin for photo microscopic assessment [35].

Statistical analysis

Data were analyzed using ANOVA, $p \leq 0.05$. Once a significant difference was determined, the means were compared using Duncan, multiple range tests, [36].

RESULTS

Effect of administration of ivermectin and/or verapamil on serum AST, ALT, ALP, urea and creatinine

Data presented at table 1 showed that administration of ivermectin to rats slightly increase serum AST, ALT, ALP, urea and creatinine. While, co-administration of ivermectin plus verapamil induced a significantly increased these parameters compared to other groups indicating a high degree of the kidney damage in this group.

Table 1: Effect of administration of ivermectin and/or verapamil on GSH, GSH Px and MDA in the brain homogenates of rats

Parameter	Control	Ivermectin (I)	Verapamil (V)	I+V
MDA (nmol MDA/g tissue)	9.40 ± 0.52^{b}	10.25 ± 1.40^{b}	9.11 ± 1.12^{b}	15.24 ± 1.23^{a}
GSH (umol /g tissue)	22.00 ± 3.10^{a}	19.88 ± 2.25^{a}	21.21 ± 3.27^{a}	13.00 ± 0.76^{b}
GSH Px (U/g tissue)	11.50 ± 0.82^{a}	11.15 ± 1.23^{a}	10.65 ± 0.96^{a}	6.03 ± 0.32^{b}

MDA= malondialdehyde, GSH= reduced glutathione, GSH Px= glutathione peroxidase.

Each values represent mean ± S.E. of 6 animals. Values with different litters at the same raw were significantly differed. $p \leq 0.05$

Effect of administration of ivermectin and / or verapamil on GSH, GSH Px and MDA in the brain and kidney homogenates of rats

The results presented in tables 2 and 3 revealed a slightly decrease in the level of GSH and GSH Px and slight increase in the MDA content in the group treated with ivermectin compared with verapamil treated and control groups. While, co- administration of ivermectin plus verapamil significantly increased MDA content and decreased GSH and GSH Px at the brain and kidney homogenates.

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Table 2: Effect of administration of ivermectin and/or verapamil on GSH, GSH Px and MDA in the kidney homogenates of

Parameter	Control	Ivermectin (I)	Verapamil (V)	I+V
MDA (nmol MDA /g tissue)	6.50±0.85[b]	7.10±0.80[b]	6.95±0.50[b]	11.28±0.92[a]
GSH (umol /g tissue)	19.50±2.45[a]	17.82±1.35[a]	18.60±2.51[a]	12.50±1.44[b]
GSH Px (U/g tissue)	7.30±0.45[a]	6.45±0.90[a]	7.92±0.34[a]	4.21±0.28[b]

Microscopic findings in the brain and kidney tissues

Regarding to the microscopic examination for the brain and kidneys tissue of the rat in the control group as well as the rats in the group treated with the verapamil no abnormal histologic changes could be seen. The observed lesions of rats treated with ivermectin were mild changes only (fig. 3a and fig.4a). On other hand, the microscopic examination of these tissues in the group of rats treated with ivermectin plus verapamil, revealed presence of several severe changes (fig. 3b, c & d and fig. 4b).

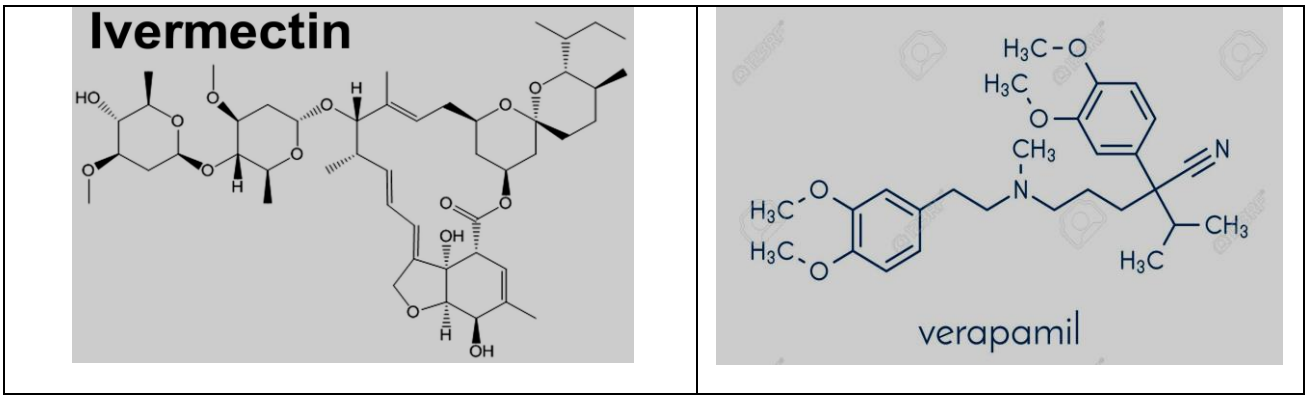

Fig1: Structural formula of Ivermectin ($C_{48}H_{74}O_{14}$) and Verapamil ($C_{27}H_{38}N_2O_4$).

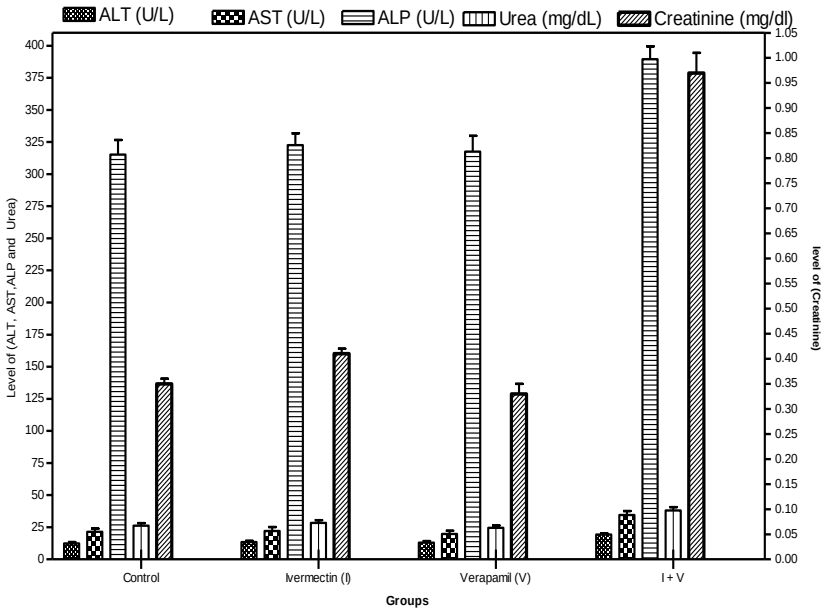

Fig 2: Effect of administration of ivermectin and/or verapamil on serum AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), urea and creatinine.

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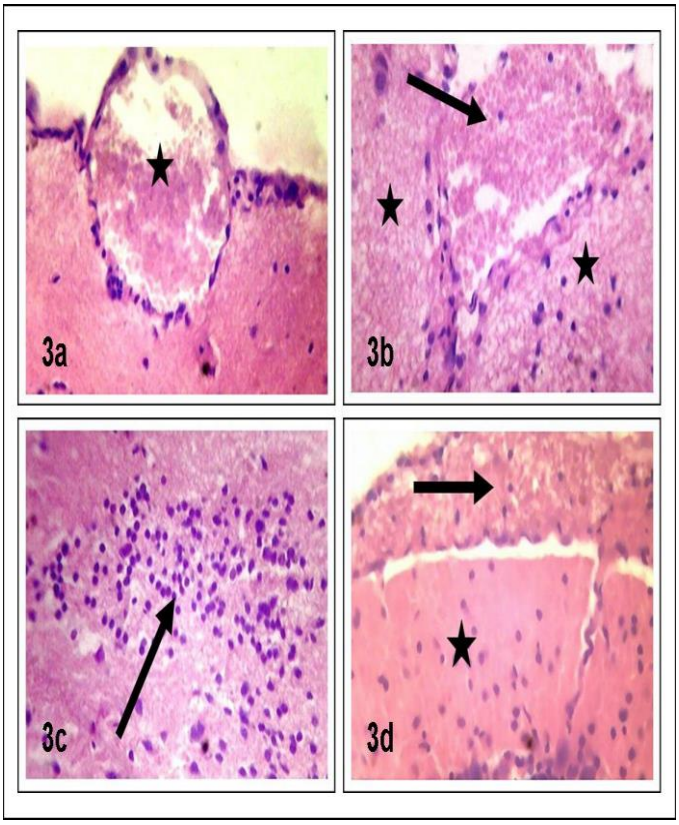

Fig 3: Brain of the rat, 3a.Treated with ivermectin showing congestion of the meningeal blood capillaries (asterisk).3b.Treated with ivermectin + verapamil showing congestion and hemorrhages from the meningeal blood vessels (arrow) at the cerebral hemisphere (asterisk), 3c.showing diffuse area of gliosis and diffuse lymphocytic aggregation (arrows) and 3d. showing thick meninges with an excess of the edematous contents (arrow) at the cerebellum (asterisk). H and E. X 400

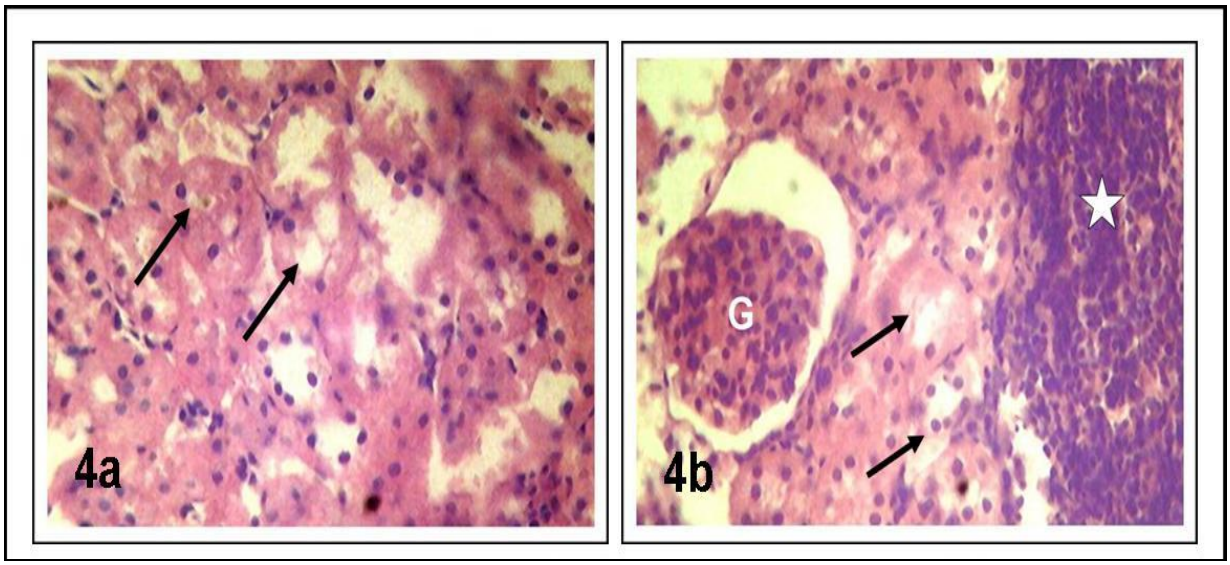

Fig 4: Kidney of the rat ,4a.Treated with ivermectin showing vacuolar and hydropic degeneration of the lining epithelium of some renal tubules (arrows) and 4b.Treated with ivermectin + verapamil showing damaged glomerulus (G), degenerated and necrotic renal tubules (arrows) and wide area of replacements with extensive mononuclear cell aggregations (white asterisk). H and E X 400

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DISCUSSION

The use of Pgp inhibitors as a means of enhancing systemic and tissue bioavailability of drugs has been demonstrated in vitro [37] and in vivo [25, 26, 38, 39], and reversal of multidrug resistance with this plan may be beneficial or harmful. At the present experiment administration of ivermectin to rats induced a slight increase in serum AST, ALT, ALP, urea and creatinine. While, co-administration of ivermectin plus verapamil significantly increased these parameters compared to other groups indicating severs damage of the kidney (urea and creatinine), brain and other organs (AST, ALP and ALP) in this group. At the same time, the microscopic examination for the brain and kidney tissues in rats treated with the verapamil no abnormal histologic changes could be seen. The observed lesions of rats treated with ivermectin were mild changes only. Many studies recorded the safety of several folds of the therapeutic doses of ivermectin in different animals [26,40] and its adverse effects at different organs appear only after folds of therapeutic doses in several animals [12,41-43]. On the contrarily, Wang et al. [44] recorded that Ivermectin induced a damage effects on structures of cerebellum, cerebrum and optical lobe in the pigeon brain. Meanwhile, rats treated with ivermectin plus verapamil, revealed presence of several severe changes in the normal histological structure of the brain and kidney. Furthermore, the obtained results revealed a slight decrease in the level of GSH and GSH Px and slight increase in the MDA content in rats treated with ivermectin compared with verapamil treated and control groups. Ivermectin administration to pigeons at higher doses induced brain damage and elevates oxidative stress in brain and serum [44-48]. Additionally, co-administration of ivermectin plus verapamil significantly increased MDA content and decreased GSH and GSH Px at the brain and kidney homogenates.

Inhibition of Pgp allowing drug penetration into the pharmacological reserves behind the blood–tissue barrier such as the brain and the abnormal drug accumulation in renal tubular cells may result from the inhibition of renal drug transporters, leading to drug-induced nephrotoxicity [24]. P-gp is localized at the apical membrane of the kidney proximal tubules, which plays a role in the efflux of drugs into urine [49, 50]. Similarly, verapamil reduced renal clearance of digoxin [51, 52]. In spite of the approval use of ivermectin in all dogs, it cause neurotoxicity at very low doses to genetically sensitive canine breeds collies. Increased ivermectin levels in the brains of sensitive collies appear to be due to ineffective brain-to-blood efflux caused by Pgp transporter [53].

All of these findings and the present study explain the potential harmful toxicity of IVM in presence of verapamil by inhibiting Pgp transporter. Additionally, ivermectin has slight effects on male fertility, but when taken with verapamil induced adverse effects on meiosis and fertility [29]; the mixed treatment of pregnant rats with ivermectin and verapamil severely affect fetal genetic material and development and induced genotoxic effect in somatic cells of the dams [28]. Many authors recorded the undesirable effects of avermectins and ivermectin on fetuses from genetically-modified animals. Animals with wild-type (+/+) produce Pgp or deficient genotypes differ markedly in their sensitivity to the neurotoxicity induced by abamectin and ivermectin, attributed to differences in accumulation of these compounds in the brain [54, 55].

It is also known that verapamil increase the cytotoxic effects of some antitumor antibiotics [56] and augmenting parasitic sensitivity to ivermectin.in equines [14]. This was explained as verapamil act as inhibitors of P-glycoprotein substrate of the cells which is either interferes with the passive diffusion of drugs into the cell [57] or is involved in their active transport out of the cell thus reducing their intracellular concentration [58]. In broilers, the E. coli infection induces intestine P-gp expression, altering the absorption of orally administered enrofloxacin in both healthy and infected broilers; the treatment with verapamil significantly improved the absorption of enrofloxacin [59]. Hence, concomitant administration of substrates and Pgp inhibitors would modify drug pharmacokinetics by increased bioavailability and organ uptake leading to increased efficacy or more adverse reactions and toxicities and these findings agree with this study.

CONCLOSION

This is the first study that shows the interaction between ivermectin and verapamil on the brain and kidney tissues. It has been found that, ivermectin has slight toxic effects on the brain and kidney tissues, but co-administration with verapamil (Pgp inhibitor) the adverse effects becomes more pronounced. So, great attention must be paid when combining drugs, either as Pgp substrates or inhibitors.

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