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HEPATO-RENAL AND NEURAL EFFECTS OF *COVICURE* HERBAL TEA, AN ACCLAIMED COVID-19 THERAPEUTIC AGENT, ON WISTAR RATS

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ABSTRACT

This study evaluated the effects of *Covicure* herbal tea, an acclaimed COVID-19 therapeutic agent, on selected hepatic, renal and brain parameters of adult Wistar rats. A total of 28 rats were divided into four groups A, B, C and D of seven rats each and treated with 0, 20, 40 and 80 mg/L of the tea, respectively for 14 days. Administration of herbal mixture tea significantly ($p<0.05$) increased levels of liver and kidney malondialdehyde, liver and brain reduced glutathione, liver catalase, serum K^+ (20 mg/L) and HCO_3^-. In contrast, levels of brain malondialdehyde, liver-body weight ratio, serum albumin, direct bilirubin, creatinine, urea, K^+ (40 mg/L) and Na^+/K^+-ATPase were significantly ($p<0.05$) reduced. However, levels of kidney and brain catalase, kidney reduced glutathione, kidney-and brain-body weight ratios, tissue and serum proteins, liver and serum alanine aminotransferase, serum total bilirubin, kidney and serum alkaline phosphatase, serum uric acid, Na^+, Cl^-, Ca^{2+}/Mg^{2+}-ATPase and acetylcholinesterase remained significantly ($p>0.05$) unchanged. Liver histology showed signs of organ damage at 40 and 80 mg/L, while kidney and brain histoarchitecture remained unaffected. In conclusion, the herbal mixture at the concentrations used had caused functional perturbations with signs of liver damage but no histological changes in kidney and brain.

Keywords: *Covicure* herbal tea, liver function, kidney function, brain function, structural toxicity, functional toxicity.

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INTRODUCTION

With the advent of the corona virus disease (COVID-19) pandemic, came several pharmaceutical and non-pharmaceutical interventions to break the chain of transmission of the infection. Corona virus disease-2019 (COVID-19), caused by the severe acute respiratory syndrome corona virus-2 (SARS COV-2), has, according to the European Centre for Disease Prevention and Control, infected 394,483,957 persons globally and 253,727 persons in Nigeria, with 5,753,799 deaths globally and 3,139 in Nigeria, as at February 2022 [1]. Post-infection signs in humans include flu-like symptoms, breathing difficulty, severe acute respiratory syndrome, fever, cough, extreme tiredness, body aches, and even severe pneumonia that can be fatal [2-6].

Though vaccines have been developed and administered, no specific treatment strategy currently exists for COVID-19 [7, 8], despite the several approved antiviral agents being used. Several researchers have reported promising *in vitro*, *in vivo*, and *in silico* results against the disease, by using metal nanoparticles, natural and synthetic flavonoids, photosynthetic aquatic algae-derived phytocompounds, herbs and mushrooms, essential oils, coconut waste, etc [7, 9-15]. These agents possess several pharmacological activities such as antioxidant, anti-inflammatory, antiviral and other activities, which are related to the pathophysiology of the disease. Several indigenous natural products formulations have also appeared in Nigeria, one of which is *Covicure* herbal tea.

Covicure herbal tea is an herbal mixture containing powdered ginger (*Zingiber officinale*), garlic (*Allium sativum*), moringa leaves (*Moringa oleifera*) and neem leaves (*Azadirachta indica*), in specific proportions and packaged as tea bags. It is acclaimed to prevent and cure COVID-19 and flu, and can detoxify and improve respiratory health, due to the anti-inflammatory, antioxidant, immunomodulatory, and free radical eliminating and antiviral activities of the component mixture.

Several reports have documented the beneficial effects of the components of *Covicure* herbal tea, with scanty reports on toxicity. *A. sativum* has been reported to possess antiviral, immunomodulatory, anti-inflammatory, antioxidant, hepatoprotective, antibacterial, anti-fungal, anticoagulant, and wound healing properties, among others [16-20]. It was also reported to be hepatotoxic and nephrotoxic when administered intravenously [18]. *Z. officinale* has also been documented for antiviral, anti-inflammatory, hepatoprotective, nephroprotective, antibacterial, immunomodulatory activities, etc [16, 17, 21-24]. *M. oleifera* leaves exhibited nutritional, antioxidant, anti-inflammatory, nephroprotective, immunomodulatory, antiviral, antibacterial and other properties [25-29]. *A. indica* leaves have been reported to be safe in mammals and possesses activities such as antioxidant, anti-inflammatory, antidiabetic, antiulcer, anticancer, antimalarial, antifertility, antipyretic, antibacterial, antiviral, antifungal, etc., but mildly toxic to aquatic organisms [30-36].

Despite the reported beneficial properties of these components and scanty reports on their toxicity, no report has evaluated safety of this herbal formulation. Thus, the rationale for this study was to evaluate the safety of oral exposure of Wistar rats to *Covicure* herbal tea by monitoring the effects on selected biochemical parameters of the liver, kidney and brain. The parameters studied include cellular antioxidant status, oxidative stress and toxicity parameters, hepatic, renal and brain function parameters. Histoarchitectural integrity of these tissues were also examined.

MATERIALS AND METHODS

Experimental animals

Twenty-eight Wistar rats of both sexes, weighing 181.78±17.54 g, were obtained

from a private animal breeding farm in Osogbo, Osun State. The animals were kept in clean, plastic cages contained in well ventilated house conditions (22±-2°C; 12-h natural light and 12-h dark; humidity, 43±2%) with free access to rat pallets (Premier Feed Mills Co. Ltd, Ibadan, Nigeria) and tap water.

Herbal mixture, assay kits and other reagents

Covicure herbal tea bags were obtained from Microclear Impact Optimum Company Limited, Osun State, while halothane was obtained from the Department of Anaesthesia, Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Osun State. Urea, uric acid, creatinine, albumin and total protein assay kits were products of Randox Laboratories Limited, Co-Antrim, UK. Assay kits for alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were products of TECO Diagnostics Anaheim, USA. Urea, uric acid and creatinine kits were products of Randox Laboratories Limited, Co-Antrim, UK, while electrolyte (Na^+, K^+, Cl^-, HCO_3^-) kits were products of Agape Diagnostics, Switzerland GmbH, Cham, Switzerland. Other reagents used were of analytical grade and were prepared in the laboratory using all-glass distilled water.

Preparation of *Covicure* herbal tea

Covicure herbal tea bags (80 mg) were suspended in 1 L of distilled water and placed in water bath at 80°C for 15 min with vigorous, intermittent shaking, and then filtered when cool. This was labelled as 80 mg/L, which was further diluted to 40 mg/L and 20 mg/L of the herbal tea for administration to the animals.

Experimental design and administration of herbal tea

The rats were completely randomised into four groups of seven rats each and given daily oral doses of the tea, after two weeks of acclimatisation. The control group received distilled water, while the other three groups received 20, 40 and 80 mg/L, respectively, of the tea once daily for 14 days. The animals were sacrificed 24 hours after the last dose.

The Ethical Committee on the Care and Use of Experimental Animals of the College of Natural and Applied Sciences, Fountain University, Osogbo, Nigeria, granted approval for this study design, and the animals were handled humanely as recommended by the National Institutes of Health (NIH; Bethesda, Maryland, USA), Guide for the Care and Use of Laboratory Animals [37].

Collection of tissue samples and preparation of serum

Twenty-four hours after the last dose (Day 15), the animals were weighed and sacrificed as described by Salau et al. [38] under halothane anaesthesia and the blood was collected into test tubes for serum preparation. The clotted blood was centrifuged at 1431 x g for 5 min and the serum was carefully aspirated with a Pasteur pipette into sample bottles, which were kept frozen until used for biochemical assays. A midline incision was made for access to visceral organs to harvest the liver and kidneys, while the skull was incised to remove the brain of each animal. The tissues were cleaned with blotting paper, weighed, homogenised in ice-cold 0.25 M sucrose solution (1:4 w/v) and the supernatants were also kept frozen after homogenates were centrifuged at 1398 × g for 15 min. Another portion of the tissues were stored in 10% formalin (10% formaldehyde in normal saline) at room temperature for histological examination.

Determination of biochemical parameters and histological examination

Standard procedures were used for the determination of alanine aminotransferase [39], malondialdehyde [40], catalase [41], reduced glutathione [42], total proteins [43], albumin [44], direct and total bilirubin [45], alkaline phosphatase [46], creatinine

[47], urea [48], uric acid [49], electrolytes [50], sodium-potassium adenosine triphosphatase (Na^+/K^+-ATPase) [51], calcium-magnesium adenosine triphosphatase (Ca^{2+}/Mg^{2+}-ATPase) [52], and acetylcholine esterase [53]. Organ/tissue-body weight ratios were computed by dividing the organ/tissue weight by the total weight of the animal. Histopathological examination of the liver, kidney and brain was carried out as described by Owolarafe et al. [54] with haematoxylin and eosin staining technique, examined with Leica DM750 microscope (× 400 magnification) and photographed with Leica ICCSOHD camera [54].

Statistical analysis

Data were represented as mean ± SD of seven determinations. The data were analysed by one-way analysis of variance using IBM statistical package for social sciences version 19; 'P' value less than 0.05 was considered statistically significant.

RESULTS

Malondialdehyde was significantly ($p<0.05$) elevated in liver (at 40 mg/L) and kidney (at 20 and 40 mg/L), but significantly ($p<0.05$) reduced in brain at 40 and 80 mg/L, while other experimental groups were not significantly ($p>0.05$) different when compared with control (Table 1). Catalase activity was also significantly ($p<0.05$) elevated in liver at 20 and 40 mg/L only, while its activity in kidney and brain were not significantly affected, compared to control (Table 1). Reduced glutathione concentration was significantly higher than the control values at 20 mg/L in liver and brain but unaffected in the kidney when compared with control (Table 1). Liver-body weight ratio was significantly lower than the control values at all concentrations but the kidney- and brain-body weight ratios were not significantly different from their respective controls (Table 1). Total protein concentrations were unaffected in all experimental groups in comparison to the respective controls for each tissue (Table 1).

Specific activities of liver and serum ALT compared well ($p>0.05$) with their respective controls in all the experimental groups, as well as serum total bilirubin and total protein (Table 2). Significant ($p<0.05$) reductions were observed in serum albumin (at 40 mg/L) and direct bilirubin (at 40 and 80 mg/L) concentrations (Table 2).

Kidney and serum ALP specific activities, serum uric acid, Na^+ and Cl^- concentrations were not significantly ($p>0.05$) different from their respective controls (Table 3). There were significant ($p<0.05$) reductions in the concentrations of serum creatinine (at 20 and 80 mg/L), urea (at 40 mg/L) and K^+ (at 40 mg/L), while significant ($p<0.05$) elevations were observed in the concentrations of serum K^+ (at 20 mg/L) and HCO_3^- at 80 mg/L (Table 3).

No significant ($p>0.05$) changes were observed in the specific activities of Na^+/K^+-ATPase at 80 mg/L, and in all the experimental groups of Ca^{2+}/Mg^{2+}-ATPase and acetylcholinesterase (AChE) in the brains of the rats, while significant ($p<0.05$) losses were observed in the rat brain Na^+/K^+-ATPase activities at 20 and 40 mg/L doses of the herbal tea (Table 4).

Histopathological examination of the liver shows normal tissue architecture in the control and 20 mg/L groups (Plate 1). The 40 and 80 mg/L groups show liver sections with preserved architecture composed of cords of normal hepatocytes, normal portal tracts and central vein, but with few foci showing peri-portal and lobular lymphocytic infiltration, along with features of acute or chronic damage (Plate 1). Kidney and brain sections had normal unaltered histoarchitecture in the control and experimental groups (Plates 2 and 3).

DISCUSSION

Malondialdehyde is a metabolite that results from peroxidation of lipids and have been used as an index of oxidative stress [55, 56]. The observed, inconsistent, increase in liver and kidney MDA could

have resulted from oxidation of cellular lipids caused by the mixture. In contrast, decreased MDA level in the brain would indicate decreased cellular lipid oxidation. *Z. officinale* has been previously reported to reduce MDA levels [57], but the increase here might have been due to the fact that the herbal tea is a mixture of several components and might have induced cellular adaptation and stress. The adaptation hypothesis is supported by the reduced MDA levels as the dose increases to 80 mg/L in the liver and kidney.

Catalase is an antioxidant enzyme and reduced glutathione is a non-enzymic cellular antioxidant; both are involved in the reduction of cellular oxidants, which can cause oxidative damage to components of the cell. They have been used as indices of antioxidant status [58]. Their levels are reduced during oxidative assault to the cell and they are induced by antioxidant agents. The antioxidant components of the herbal mixture might have induced the catalase activity in the liver and also induced elevated glutathione levels in the liver and kidney, increase in their levels is an indication of antioxidant activity [58]. This finding corroborates the decreased MDA levels in the liver, kidney (80 mg/L) and brain.

Computed organ/tissue-body weight ratios and tissue total proteins have also been used as indices of toxicity as they indicate organ shrinkage, oedema and/or stress on the organs [59]. The reduced liver-body weight ratio might have resulted from tissue damage, due to cell shrinkage, though the tissue proteins homeostasis was still maintained. The kidney and brain showed no signs of oedema or shrinkage, and had normal cellular protein homeostasis.

The liver performs synthetic and secretory functions, being the site of xenobiotic biotransformation. ALT is an index of liver health and its activity is affected in cases of liver injury. Serum albumin and total protein are indices of the liver's synthetic activity, while serum bilirubin is an index of its secretory activity [53, 60]. The herbal tea did not alter serum and tissue ALT activities, indicating normal hepatic carbohydrate and lipid metabolism. The reduced serum albumin, without corresponding changes in serum total protein might be due to lowered *de novo* albumin synthesis suggestive of cellular stress induced by xenobiotic biotransformation, especially at higher doses. Similarly, reduced direct/conjugated bilirubin does not correspond with any change in the total bilirubin content. These biochemical alterations are indicative of cellular adaptation, especially with increasing dose, as suggested by Sulaiman et al. [61], though they reported increased albumin and bilirubin levels.

The kidneys play a role as the sites of xenobiotic excretion, which involves ultrafiltration and selective reabsorption of useful materials. ALP is a membrane-bound enzyme involved in transport of materials across the cell membrane. Its activity reflects integrity of membrane components [59]. Creatinine, urea and uric acid are markers of glomerular function, while the electrolytes are markers of renal tubular function. Alterations in levels of these molecules would indicate signs of renal impairment [62, 63]. The observed fluctuations in the levels of serum creatinine, urea, potassium and bicarbonate ions are symptoms of cellular perturbations due to assault by the herbal mixture. These fluctuations indicate glomerular and tubular functional inadequacies due to adaptation and stress as suggested by Owolarafe et al. [64]. These changes are selective because uric acid, sodium and chloride ions were not affected; the normal ALP activities in the serum and nephrons also suggests that the membrane components are not affected, and intramembrane ion transfer would not be affected in the nephrons.

Na^+/K^+-ATPase and Ca^{2+}/Mg^{2+}-ATPase are respectively involved in transport and homeostasis of sodium, potassium, calcium and magnesium ions across membranes and

maintain osmotic equilibrium and membrane potential, while acetylcholinesterase degrades acetylcholine, a neurotransmitter [65-68]. These enzymes maintain the optimum functions of the brain and central nervous system. Alterations in their activities would adversely affect brain and CNS activities. The decreased sodium pump activity could have resulted from cellular stress caused by the presence of the herbal extract. This would adversely affect the maintenance of membrane potential of the brain cells and affect normal brain function. It is probably an isolated selective effect on the sodium pump since the calcium pump and AChE were not affected, and thus, calcium and magnesium-controlled processes would not be affected. Normal neural impulse transmission by acetylcholine would not also be affected. This finding is in contrast with reports by Tyagi et al. [69] that reported increased AChE activity by an inflammatory agent, and Uner et al. [70], who reported decreased AChE activity in the presence of a pesticide.

The biochemical alterations observed did not affect the histoarchitecture of the kidney and brain at all doses, while the liver showed signs of tissue damage at the higher doses of 40 and 80 mg/L, corresponding with the cellular shrinkage (via reduced liver-body weight ratios) observed at these doses.

In conclusion, a single daily oral administration of *Covicure* herbal tea for 14 days selectively induced biochemical alterations in some cellular parameters of liver, kidney and brain of rats. These alterations caused mild perturbations in the functions of these organs and resulted in shrinkage and cellular damage to the liver at the higher doses, but the kidney and brain were not affected in size and histoarchitectural integrity. *Covicure* herbal tea should therefore, be restricted to divided lower doses, instead of single doses, if taken via the oral route.

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Table 1: Antioxidant status and toxicity parameters in liver, kidney and brain of rats administered various doses of *Covicure* herbal tea

Parameter	Organ/tissue	Control	Concentration of *Covicure* herbal tea (mg/L)		
			20	40	80
Malondialdehyde concentration (nmol MDA/mg protein)	Liver	4.29 ± 0.60^{b}	3.43 ± 0.39^{b}	5.39 ± 0.69^{a}	4.17 ± 0.47^{b}
	Kidney	1.29 ± 0.34^{c}	2.69 ± 0.67^{b}	3.44 ± 0.07^{a}	1.64 ± 0.55^{c}
	Brain	7.32 ± 1.09^{a}	9.94 ± 1.81^{a}	2.72 ± 0.44^{b}	3.06 ± 0.11^{b}
Catalase (U/L)	Liver	7.11 ± 1.01^{b}	10.56 ± 1.48^{a}	11.27 ± 1.11^{a}	7.23 ± 1.11^{b}
	Kidney	3.03 ± 0.05^{a}	3.01 ± 0.69^{a}	3.44 ± 0.65^{a}	3.42 ± 0.59^{a}
	Brain	35.65 ± 3.82^{a}	33.78 ± 2.23^{a}	32.16 ± 1.75^{a}	32.82 ± 2.93^{a}
Reduced glutathione (µg/mg protein)	Liver	3.35 ± 0.27^{b}	4.17 ± 0.50^{a}	3.84 ± 0.44^{ab}	3.61 ± 0.86^{ab}
	Kidney	3.03 ± 0.75^{a}	3.01 ± 0.69^{a}	3.44 ± 0.65^{a}	3.42 ± 0.59^{a}
	Brain	3.71 ± 0.53^{b}	5.02 ± 0.20^{a}	3.55 ± 0.39^{b}	3.48 ± 0.35^{b}
Tissue-body weight ratio	Liver	0.07 ± 0.01^{a}	0.02 ± 0.01^{b}	0.03 ± 0.02^{b}	0.03 ± 0.01^{b}
	Kidney	0.007 ± 0.001^{a}	0.008 ± 0.002^{a}	0.006 ± 0.001^{a}	0.006 ± 0.001^{a}
	Brain	0.008 ± 0.002^{a}	0.005 ± 0.001^{a}	0.008 ± 0.001^{a}	0.007 ± 0.001^{a}
Tissue protein (g/L)	Liver	116.76 ± 21.53^{a}	116.76 ± 21.53^{a}	89.81 ± 22.97^{a}	117.33 ± 11.89^{a}
	Kidney	70.38 ± 10.67^{a}	71.27 ± 10.84^{a}	63.34 ± 8.11^{a}	75.05 ± 13.31^{a}
	Brain	35.56 ± 4.00^{a}	38.34 ± 9.60^{a}	36.45 ± 2.32^{a}	37.19 ± 3.37^{a}

Values are mean ± SD; n=7. Values with different superscripts across the row for each parameter are significantly different ($P<0.05$).

Table 2: Hepatic function parameters of rats administered various doses of *Covicure* herbal tea

Parameter	Control	Concentration of *Covicure* herbal tea (mg/L)		
		20	40	80
Liver alanine aminotransferase (U/L)	21.72±2.62[a]	25.78±4.74[a]	23.55±3.25[a]	22.23±1.57[a]
Serum alanine aminotransferase (U/L)	2.34±0.35[a]	2.31±0.36[a]	2.97±0.68[a]	2.56±0.35[a]
Serum albumin (mmol/L)	10.38±1.80[a]	9.17±1.02[ab]	7.25±2.41[b]	8.22±1.89[ab]
Serum total bilirubin (µmol/L)	33.01±3.37[a]	30.69±2.85[a]	29.91±3.34[a]	28.77±3.54[a]
Serum direct bilirubin (µmol/L)	22.07±2.25[a]	18.47±2.92[ab]	16.67±2.44[b]	16.33±1.11[b]
Serum total protein (g/L)	20.10±3.01[a]	20.06±2.88[a]	16.09±2.38[a]	17.77±2.12[a]

Values are mean ± SD; n=7. Values with different superscripts across the row for each parameter are significantly different (P<0.05).

Table 3: Renal function parameters of rats administered various doses of *Covicure* herbal tea

Parameter	Control	Concentration of *Covicure* herbal tea (mg/L)		
		20	40	80
Kidney alkaline phosphatase (U/L)	23.23±4.49[a]	24.13±4.03[a]	25.74±3.62[a]	21.33±3.44[a]
Serum alkaline phosphatase (U/L)	7.35±1.25[a]	8.39±1.30[a]	9.93±1.42[a]	7.45±1.79[a]
Serum creatinine (mmol/dL)	7.24±0.87[a]	5.56±0.58[b]	6.29±0.84[ab]	4.93±0.78[b]
Serum urea (mmol/L)	153.15±17.99[a]	146.45±17.57[a]	117.68±12.38[b]	171.02±19.94[a]
Serum uric acid (mg/dL)	1.17±0.21[a]	1.16±0.13[a]	1.07±0.12[a]	1.08±0.07[a]
Serum Na^+ (mmol/L)	59.22±3.67[a]	60.42±1.76[a]	56.83±7.02[a]	59.47±4.47[a]
Serum K^+ (mmol/L)	4.70±0.27[b]	5.39±0.37[a]	3.26±0.88[c]	4.39±0.55[bc]
Serum Cl^- (mmol/L)	27.05±3.28[a]	27.67±2.49[a]	22.25±3.76[a]	23.69±3.39[a]
Serum HCO_3^- (mmol/L)	35.23±1.34[b]	35.48±0.45[ab]	35.89±0.51[ab]	36.09±0.32[a]

Values are mean ± SD; n=7. Values with different superscripts across the row for each parameter are significantly different (P<0.05).

Table 4: Brain function parameters of rats administered various doses of *Covicure* herbal tea

Parameter	Control	Concentration of *Covicure* herbal tea (mg/L)		
		20	40	80
Brain Na^+/K^+-ATPase (U/L)	1.62±0.05[a]	1.41±0.08[b]	1.25±0.08[b]	1.58±0.01[a b]
Brain Ca^{2+}/Mg^{2+}-ATPase (U/L)	1.47±0.17[a]	1.22±0.15[a]	1.55±0.30[a]	1.51±0.26[a]
Brain acetylcholinesterase (U/L)	6.96±1.61[a]	7.77±1.59[a]	9.06±1.84[a]	9.19±1.55[a]

Values are mean ± SD; n=7. Values with different superscripts across the row for each parameter are significantly different (P<0.05).

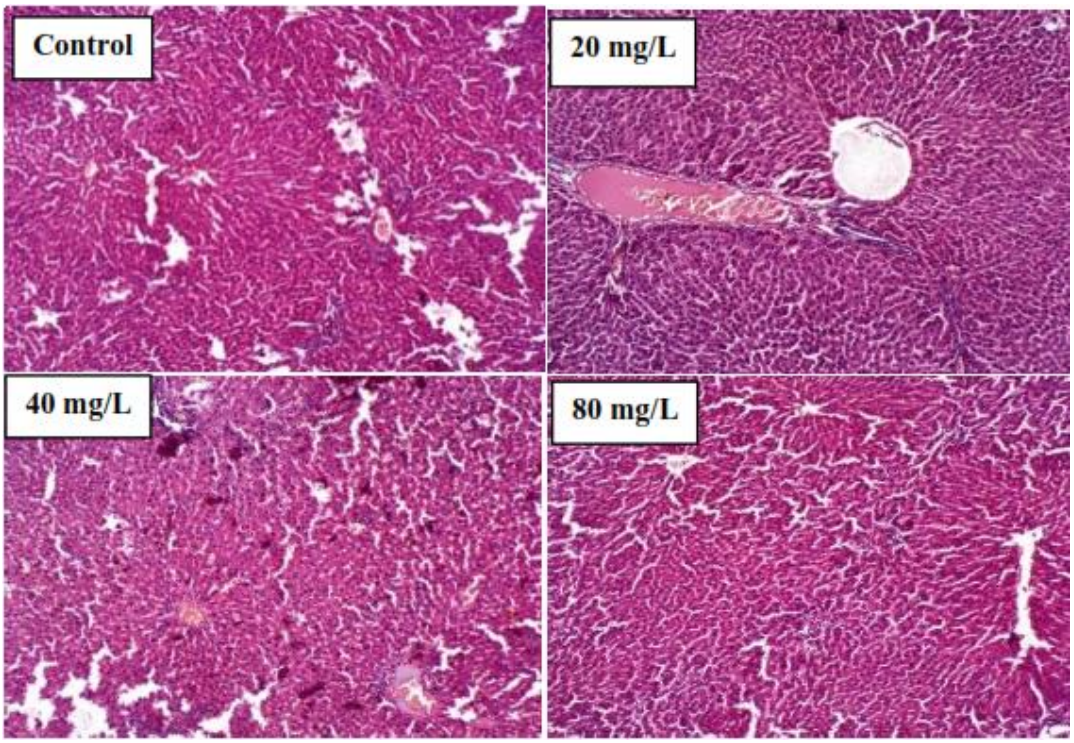

Plate 1: Photomicrograph of liver in rats administered *Covicure* herbal tea. **Control and 20 mg/L:** Preserved architecture composed of cords of normal hepatocytes, normal portal tracts and central vein. There is no significant inflammation or features of acute or chronic damage. **40 mg/L and 80 mg/L:** Preserved architecture composed of cords of normal hepatocytes, normal portal tracts and central vein. There are few foci showing peri-portal and lobular lymphocytic infiltration. There are features of acute or chronic damage. H&E (x400).

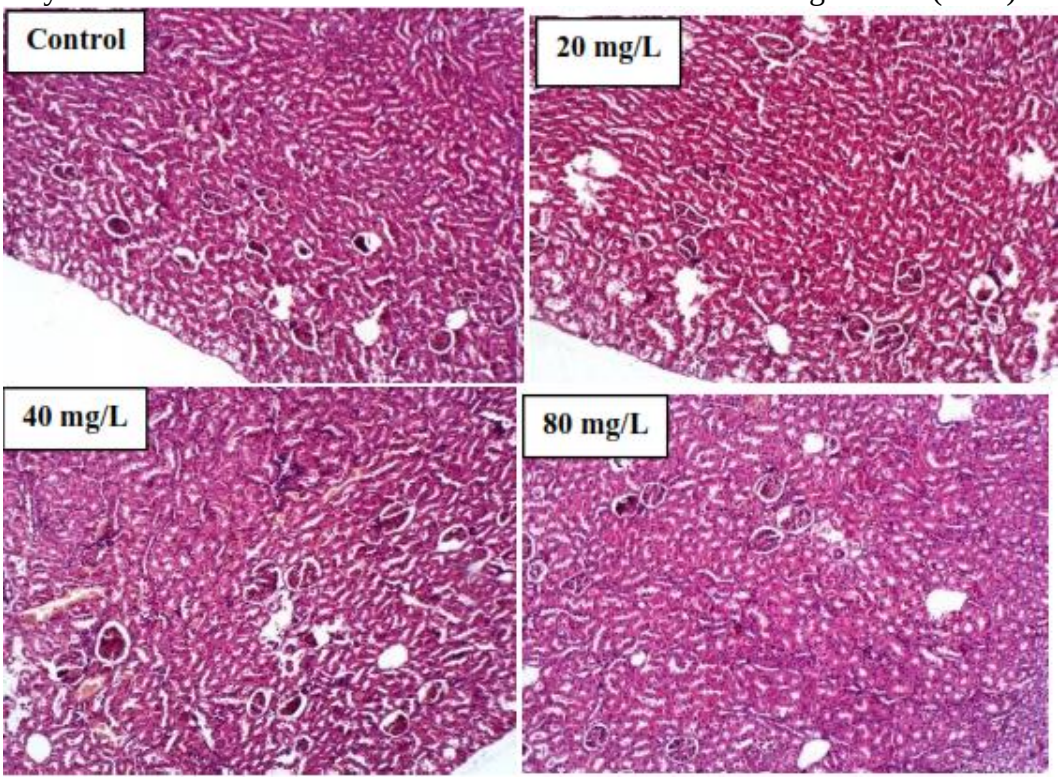

Plate 2: Photomicrograph of kidney in rats administered *Covicure* herbal tea. **Control, 20 mg/L, 40 mg/L and 80 mg/L:** Preserved architecture composed of normal glomeruli and tubules. There are no features of acute or chronic damage. H&E (x400).

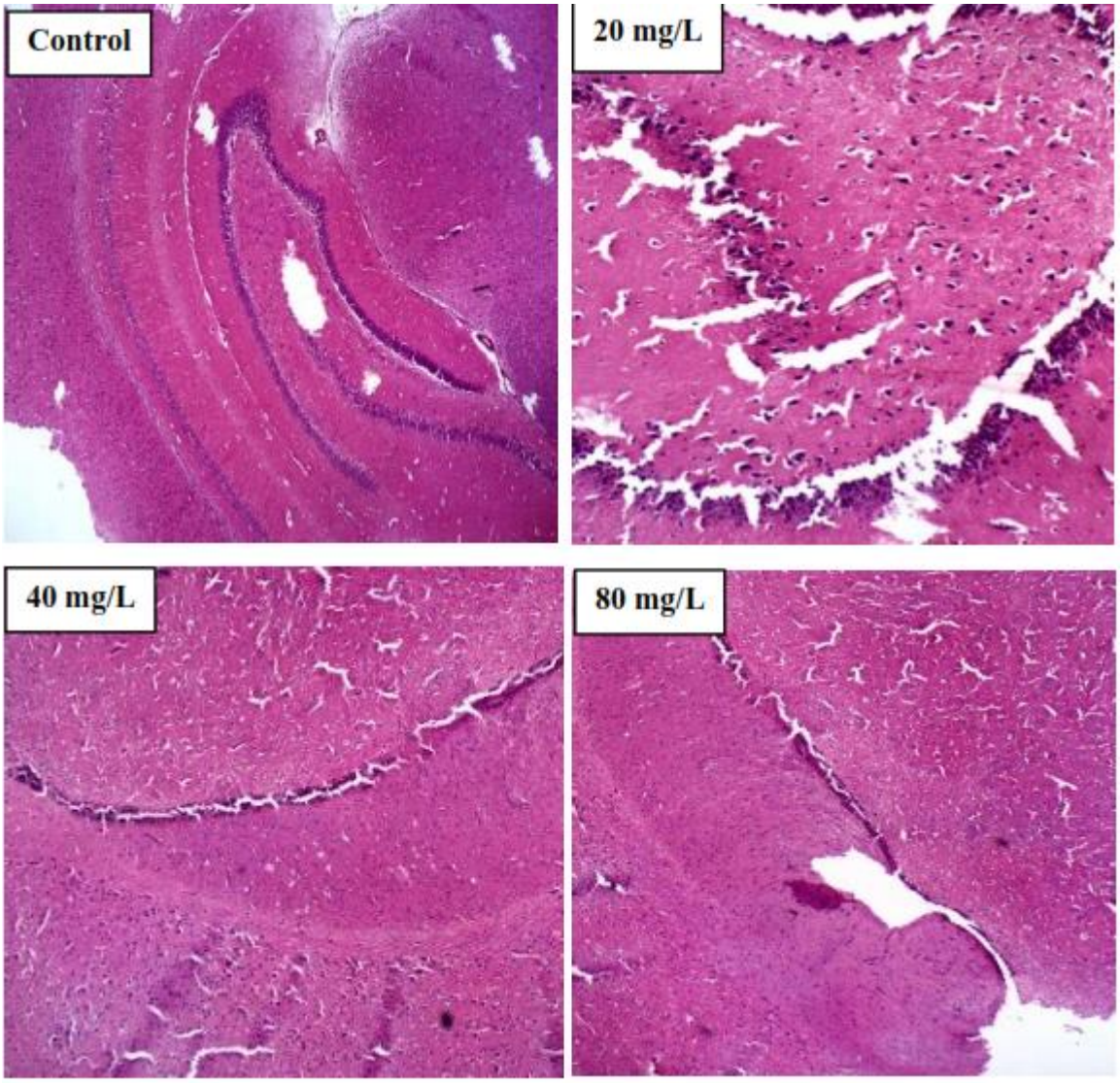

Plate 3: Photomicrograph of brain in rats administered *Covicure* herbal tea. **Control, 20 mg/L, 40 mg/L and 80 mg/L:** Normal brain tissue composed of preserved neuronal bodies and ganglion layers surrounded by glial matrix in the cerebral cortex. The hippocampus shows distinct molecular, granular and polymorphic layer. H&E (x400).