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Assessment of Some Biochemical Changes in Wistar Rats Following Administration of Three Polyherbal Preparations Repurposed for COVID-19

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Authors' contributions

This work was carried out in collaboration among all authors. Author KIA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NNW and BBB managed the analyses of the study. Author KDF managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background and Aims: The current pandemic known as Coronavirus Disease-19 (COVID-19) is a significant public health menace. Currently, there is no specific treatment for this disease, although some medicinal agents are under investigation. Some published articles have reported Severe Acute Respiratory Distress Syndrome Coronavirus-2 (SARS CoV-2) involvement in other organs, including the liver and kidneys, which can impair the metabolism and excretion of the medications taken to treat the disease. This study evaluated the effects of 3 herbal mixtures on some biochemical parameters of the liver and kidney of laboratory animals.
Methods: Liver enzymes such as serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, albumin and total serum protein were quantified. Urea, creatinine and bilirubin (total and direct) were also evaluated. These parameters were determined for all three polyherbal combinations (CoV Pla-1, CoV Pla-2, CoV Pla-3).
Results: The extracts did not produce any mortality up to 5000 mg/kg *per oral*. Results showed that the polyherbal extracts (CoV Pla-1, CoV Pla-2 CoV Pla-3) did not cause any significant changes in ALT, AST, ALP, compared to control. The mean values of urea showed an insignificant ($P>0.05$) decrease across all test doses in the three extracts, compared to the control. In the current study, the mean creatinine concentration showed slight decrease across all three extracts. Total protein was observed to be lower in CoV Pla-1 and CoV Pla-2, and increased in CoV Pla-3, though these changes were not statistically significant. Serum albumin decreased at the low and intermediate doses for the three polyherbal extracts in an insignificant manner compared to control.
Conclusion: The results obtained in this study indicate that the extracts did not cause significant changes in biochemical parameters evaluated and support their use in the treatment of Covid-19.

Keywords: Covid-19; polyherbal combinations; SARS CoV-2; serum enzymes.

1. INTRODUCTION

COVID-19 is a human infectious disease of SARS-CoV-2 origin that has affected many countries around the world. COVID-19 has now spread worldwide, affecting a sizable population that it was declared as a pandemic by the World Health Organization (WHO).

Information currently available in some research articles indicate that the liver and kidneys can be damaged in patients with COVID-19, and more than half of patients with COVID-19 showed varying levels of liver disease [1]. A recent study showed that the SARS-CoV-2 virus can bind to angiotensin-converting enzyme 2 (ACE2) on cholangiocytes, resulting in cholangiocyte dysfunction and inducing a systemic inflammatory response leading to liver injury [2] There have also been recent reports of nonelderly adults infected with COVID-19 who have developed a sudden loss of kidney function.

The liver and kidney damage in patients with COVID-19 may make it difficult in attaining the therapeutic dose of currently available medicines. Consequently, use of medicinal agents from orthodox or herbal sources would minimize injury or damage to these organs and ultimately improve therapeutic outcomes.

Salvia officinalis has been reported to have protective effects following injury to the liver and kidney of laboratory animals [3]. In addition to having antiviral properties [4,5], *Cucumis metuliferus* showed protective effects in the liver and kidney [6]. *Securidaca longepedunculata* is known to possess a broad spectrum of medicinal, pharmacological and therapeutic properties [7,8]. A search of literature showed that *Garcinia kola* has no deleterious effect on kidney function but was found to be protective

[9]. Results of a study demonstrated that *Nigella sativa* prevented liver and renal tissue damage due to its antioxidant and anti-inflammatory effects [10]. In Nigeria, researchers have established that bitter leaf (*Vernonia amygdalina*) is capable of preventing kidney and liver damage [11]. *Carissa edulis* is also reported to have protective effects in the liver [12]. *Zingiber officinale*, another plant in the herbal mixtures has been reported in several studies to alleviate liver and kidney dysfunctions [13]. In addition to its popular antimalarial function, *Artemisia annua* has also been reported anti-inflammatory and immunoregulatory properties, as well as the ability to regulate oxidative stress [14]. A search of literature showed that the administration of *Syzygium aromaticum* significantly decreased elevated levels of ALT, AST and ALP [15].

The search for the treatment and cure for the Coronavirus led the Government of Plateau State, Nigeria to set up a Committee to exploit on the natural flora and fauna and search for the possible cure for this disease. *In-silico* studies and using past literature, the Team came up with three herbal preparations. Combinatorial Chemistry showed that the candidate drugs are useful in the treatment of microbes, including viruses. The aim of this study evaluated the effects of 3 herbal mixtures (Cov Pla-1, Cov Pla-2 and Cov Pla-3) on some biochemical parameters of the liver and kidney of laboratory animals [15].

2. METHODS

2.1 Experimental Animals

Three-week old albino rats (100-160 g) of both sexes were purchased from the Animal Experimental Unit, Department of Pharmacology and Toxicology, University of Jos, Nigeria. The animals were kept at room temperature (27 ± 2°C; 70-80% humidity; 12 h light/dark cycle) in the Animal Experimental Unit for at least 48 h prior to the procedure. Commercial food pellets and water were supplied *ad libitum*. Animal experimental protocols were in accordance with the current guidelines for the care of laboratory animals by the U.S. National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research [16].

2.2 Materials

Distilled water, oven, needle and syringes, forceps, metal cages, examination gloves, refrigerator.

2.3 Collection/Preparation and Extraction of Plant Materials

The plants were identified by Mr. Joseph Ajila, of the Federal College of Forestry, Jos, Nigeria. Voucher specimens were deposited at the herbarium of the institute. A ratio of (4:2:2:1:1) of the dried parts of the different plants were pulverized, mixed together and macerated in 10 L of hydroethanol (3 litres water to 7 liters ethanol) for 24 h. The mixture was thereafter decanted, filtered and the residue was remacerated in 1000 mL hydroethanol for 24 h to ensure exhaustive extraction. At the end of the extraction process, the combined filtrate was evaporated to dryness under reduced pressure at 40°C. A dark brownish solid extract with a yield of 15.4-15.8% was obtained. The solid extract was reconstituted in distilled water to give appropriate concentrations before administration to experimental animals. The same procedure was followed for the plants in the other two polyherbal combinations. The three herbal preparations (Cov Pla-1, Cov Pla-2 and Cov Pla-3) were kept in the refrigerator until used.

2.4 Drug Administration

Twenty albino rats weighing 100-160 g were divided into 4 groups of 5 each and administered the three extracts (Cov Pla-1, Cov Pla-2 and Cov Pla-3) respectively for fourteen (14) days as follows: For Cov Pla-1, the test animals (groups 1-3) were administered 100, 200 and 400 mg/kg of the extract, while group 4 animals had 0.1 mL of distilled water *per oral.* For Cov Pla-2, animals in the test groups (1, 2, 3) received 100, 200 and 400 mg/kg of the extract respectively via the oral route, while animals in group 4 (control) had 0.1 mL distilled water throughout the period of the experiment. For Cov Pla-3, the test animals (groups 1-3) were administered 100, 200 and 400 mg/kg of the extract, while group 4 animals had 0.1 mL of distilled water orally.

2.5 Biochemical Analysis

For the serum biochemical analysis, blood samples were collected into vacutainers without anticoagulant, and serum was separated within two hours of collection after centrifugation at 10,000 rpm for 3 minutes and stored in a freezer at -20°C until use.

Total proteins were measured using the Biuret method with albumin levels determined using agarose gel electrophoresis on a Helena Laboratories Rapid Electrophoresis analyzer

according to manufacturer's guidelines (Helena Laboratories, Texas). All other biochemical parameters (AST, ALT, ALP, urea, creatinine) were analyzed using a Randox Imola clinical chemistry analyzer according to manufacturer's guidelines (Randox Laboratories Ltd. London).

2.6 Statistical Analysis

Data was analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test using a commercially available statistics software package (SPSS for Windows, V. 15.0) program. Results were presented as means ± SD. $P<0.05$ were considered statistically significant.

3. RESULTS

3.1 Effects of Oral Treatment of Extract on Some Biochemical Parameters in Albino Rats

Alkaline phosphatase (ALP) decreased with increase in dose (100-400 mg/kg) in Cov Pla-2 and Cov Pla-3 extracts and at 400 mg/kg for Cov Pla-1; alanine aminotransferase (ALT) was observed to be decrease as dose increased (100-400 mg/kg in Cov Pla-3) (Table 1). Aspartate aminotransferase (AST) decreased as dose increased (100-400 mg/kg) in Cov Pla-2 and Cov Pla-3 extracts. Total protein was observed to be higher at all doses (100-400 mg/kg) for Cov Pla-3, compared to the control. Albumin was higher at 400 mg/kg for Cov Pla-1 and Cov Pla-3, compared to the control (Table 1).

3.2 Effect of Extracts on Some Renal Parameters in Albino Rats

Urea and creatinine were observed to be lower in test animals, compared to control, at all doses for all extracts except at 100 mg/kg (urea) for Cov Pla-1 and 400 mg/kg (creatinine) for Cov Pla-2 (Table 2). Total bilirubin was higher in the test groups, compared to control animals, at 100-200 mg/kg for Cov Pla-1; at 200-400 mg/kg (Cov Pla-2) and at 100 and 400 mg/kg in animals administered Cov Pla-3 (Table 2).

Direct bilirubin was higher at all doses (100-400 mg/kg) of Cov Pla-1, Cov Pla-2 and Cov Pla-3, when compared to the animals in the control group. The exception was at 100 mg/kg in animals administered Cov Pla-2 (Table 2).

4. DISCUSSION

In toxicological evaluation, biochemical parameters have important roles as markers because of their response to clinical signs and symptoms produced by drugs. Evaluation of hepatic and renal function is vital in order to ascertain the toxicity of extracts and drugs [17] and also to aid disease investigation and diagnosis [18]. The abnormal elevation of the liver enzymes (ALT and AST) is usually associated with liver damage or alteration in bile flow. In the present study, all biochemical parameters did not show significant changes from the control at all doses (100-400 mg/kg). Alanine aminotransferase (ALT) is a cytoplasmic enzyme and increase in plasma is an indication of mild injuries caused by drugs to the liver. In this study, the inability of all the extracts (Cov Pla-1, Cov Pla-2 Cov Pla-3) to cause any significant changes in the liver enzymes, compared to control, is an indication of its safety at the doses administered. Liver injury is characterized as hepatocellular when there is predominant elevation of ALT, while Aspartate aminotransferase (AST) is a mitochondria enzyme whose elevated plasma level reflects severe tissue injuries [19].

Table 1. Effects of oral treatment of extract on some biochemical parameters in albino rats

Treatment	Dose(mg/kg)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	TP (g/dl)	ALB (g/dl)
Cov Pla-1	100	415.12±105.55	42.05±7.82	126.36±13.67	73.15±8.03	31.98±2.36
	200	464.66±62.50	42.62±7.77	132.56±25.15	72.71±4.86	29.13±6.51
	400	322.03±93.21	48.75±11.26	157.77±59.43	74.40±1.15	34.22±2.01
Cov Pla-2	100	364.25±73.15	42.00±8.17	149.08±18.28	65.27±15.03	31.58±1.82
	200	350.46±116.06	42.84±13.85	132.86±33.03	75.95±2.72	32.42±1.61
	400	338.04±48.77	42.71±12.11	140.68±24.63	71.81±2.52	31.84±2.09
Cov Pla-3	100	423.96±111.95	47.62±13.30	148.48±21.35	76.08±2.74	31.76±2.26
	200	345.18±88.24	29.67±11.12	137.06±16.57	74.79±7.56	31.49±1.32
	400	323.48±128.60	40.18±7.77	140.55±19.97	78.67±2.19	34.58±1.17
Control	(0.1 ml)	335.60±70.46	42.50±4.66	133.52±20.94	73.11±3.03	32.93±2.99

Values are expressed as mean ± SD (n=5)

Key: TP = Total Protein, ALB = Albumin, AST = Aspartate Aminotransferase, ALT = Alanine Aminotransferase, AP = Alkaline Phosphate; Grp= Group

Table 2. Effect of extracts on some renal parameters in albino rats

Treatment	Dose (mg/kg)	Urea (mmol/L)	Creatinine (µmol/L)	Total Bilirubin (µmol/L)	Direct Bilirubin (µmol/L)
Cov Pla-1	100	5.78±1.32	64.14±10.69	34.93±6.04	12.30±7.52
	200	4.08±1.16	55.96±6.48	41.00±35.60	19.29±19.61
	400	4.23±1.20	52.45±8.43	17.77±6.39	10.65±5.50
Cov Pla-2	100	3.99±1.01	48.98±8.47	22.86±4.24	8.61±2.58
	200	5.33±1.22	59.09±16.58	33.16±20.02	23.70±14.90
	400	5.20±2.59	66.78±21.93	35.14±11.80	17.23±6.64
Cov Pla-3	100	4.38±0.50	51.40±5.32	43.44±39.08	16.89±14.48
	200	3.90±1.54	54.32±9.33	27.00±16.99	15.02±9.45
	400	4.94±0.64	56.55±8.16	40.50±19.57	17.98±7.95
Control	(0.1 ml)	5.65±0.87	64.82±15.28	28.43±8.04	10.36±3.97

All the values are expressed as mean ± SD (n=5)

COVID-19 is generally associated with mild-to-moderate elevations of aspartate aminotransferase (AST) and, to a lower extent, alanine aminotransferase (ALT) levels, which are more frequent in patients with severe disease than in those with a benign outcome [20]. Total bilirubin levels can also be slightly elevated, whereas alkaline phosphatase and γ-glutamyltransferase elevations have been rarely reported [20]. Any extract or drug that would have pharmacological effects without causing serious derangement of hepatic and renal parameters would be of advantage in therapy.

Measurement of plasma urea is used as an indicator of kidney function, which is usually increased in renal diseases. Kidney failure causes urea clearance to decrease, with its resultant accumulation [21]. In the present study, the mean values of urea showed an insignificant (P>0.05) decrease across all test doses in the three extracts, compared to the control. This is an indication that the extracts may have protective effects on the kidney.

Plasma creatinine concentration has been used as a marker of the glomerular filtration rate [22]. In the current study, the mean creatinine concentration showed slight but insignificant decrease across all three extracts, though values were all within the reference range of creatinine in adult rat at about 0.2–0.8 mg/dL [23], which is equivalent to 17.7-70.7 µmol/L. This is indicative of the safety of the three polyherbal extracts.

Serum total protein change is caused by a change in the volume of plasma water and a change in the concentration of one or more specific proteins in the plasma. Elevated total protein may indicate inflammation. In the current study, the amounts of total protein were observed to be lower in Cov Pla-1 and Cov Pla-2, and increased in Cov Pla-3, though these changes were not statistically significant. The reference value of total protein in the serum of adult rat is in the range of 5.6–7.6 mg/dL [24]. The mean values of total protein in this current study were within the reference range for rats.

Albumin and total bilirubin are mixtures of molecules that can be used to evaluate the normal functioning of the liver of animals [25]. Serum albumin has many physiological functions, including anti-inflammatory and antioxidant properties [26]. The reduction in the level of serum albumin indicates diminished synthetic function of the liver [27]. A similar observation was made in this current study at the low and intermediate doses for the three polyherbal extracts, though the decrease was insignificant compared to control. Bilirubin is an important metabolic product of blood with biological and diagnostic values.

5. CONCLUSION

The results obtained in this study indicate that all three polyherbal extracts were relatively safe and did not cause significant derangement in liver and renal biomarkers evaluated. Though high doses of Cov Pla 3 showed an insignificant change, caution should be exercised when administering for a long time. However, these findings support their use in the treatment of symptoms of Covid-19 locally.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Approval to conduct the study was given by the Ethical Committee Animal Experimental Unit,

Department of Pharmacology and Toxicology, University of Jos, Nigeria, Reference number F17-00379.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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