Indonesian Journal of Pharmacy

VOL 33 (4) 2022: 641–652 | RESEARCH ARTICLE

Acute and Subchronic Oral Toxicities Study of *Channa striata*

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Submitted: 30-01-2022
Revised: 26-04-2022
Accepted: 16-11-2022

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ABSTRACT

Food and drug control authorities in each country have issued warnings about the toxic effect potential of using commonly consumed traditional medicines and food supplements. The safety assurance, standardization, and market regulation of herbal medicines being sold need to be determined. Snakehead fish (*Channa striata*) are a kind of fish that is often used as a dietary supplement, and there is no comprehensive evidence of its safety. This study aims to investigate the acute and subchronic toxicity of cold-processed snakehead fish flesh. *In-vivo* rats models were given dosages of 350, 1400, and 5600 mg/kg processed snakehead fish (SF) and were monitored for toxic symptoms and mortality for 14 days. Meanwhile, regarding subchronic toxicity, doses of 350, 700, and 1400 mg/kg SF were administered orally for 28 days. Afterward, the animal subjects were sacrificed for histopathological, hematological, and biochemical examinations. There was no evidence of toxicity or mortality in rats during the acute investigation, which lasted 14 days. The subchronic toxicity results showed no significant changes in most of the organs' hematological, biochemical, and histological profiles. Some changes observed in blood biochemistry and relative organ weight were assumed as a temporary effect and not a sign of toxicity. The overall results showed that the SF was non-toxic, up to 1400 mg/kg, which can be considered a safe dose for the application of health supplement raw materials.

Keywords: acute toxicity, subchronic toxicity, *Channa striata*, fatty acid, fish

INTRODUCTION

The use of animals or animal components in medical treatment is well established. Fish is one of the possible resources for medication and health supplements (Karsli, 2021). Snakehead (SF) is a freshwater fish that is famous in Asia and often used in the health sector (Virginia *et al*., 2016, Sahid *et al*., 2018) because of the protein content in the form of amino acids (Truong *et al*., 2021), (Pratama *et al*., 2020), and fatty acids (Sasongko *et al*., 2018). Farmed snakeheads have become economically important in developing countries such as Indonesia, Malaysia, Thailand, Bangladesh, India, and Vietnam (Bich *et al*., 2020). In 2016, the world produced 92.523 tons of SF (FAO, 2019). Indonesian snakehead fish output increased from 39,030 tons in 2014 to 97,795.25 tons in 2018 (MMAF, 2020).

Many studies have been developed using this fish species. Snakehead extract supplementation in tuberculosis patients is known to significantly increase Body Mass Index (BMI) with minimal adverse effects (Ma'rufi *et al*., 2020). Furthermore, its fillet extract is known to exert a hepatoprotective effect in a rat model induced with oxidative stress (Suhartono *et al*., 2013). The crude extracts of SF fillet and all of its fractions contained proteins with varying inhibitory effects against Angiotensin Converting Enzyme (ACE) (Budiari *et al*., 2018). The content of fatty acids such as omega-3 and the derivatives has been widely characterized (Irnawati *et al*., 2021,

Rohman *et al*., 2021), making the fish liable to be developed for treatment purposes in the future (Mohd & Abdul Manan, 2012). Therefore, in Indonesia and other Asian countries, numerous medicines claimed to be traditional contain SF ingredients. Generally, the basis for discovering drugs, including herbs, is several procedures believed to possess safety value. The currently agreed method has been established with various regulations, including preclinical toxicity tests to determine a drug's safety (BPOM RI, 2020).

Materials' physiological and pharmacological effects, namely animals and vegetables, are associated with their contained chemical compounds. Modern synthetic pharmacological agents have traditionally been created as raw materials, such as tinctures, teas, powders, various herbal formulations, and certain active medicines obtained directly from plant or animal sources (Osagie-Eweka *et al*., 2021). There is no comprehensive toxicity test data regarding the safety level for snakehead fish consumption. However, reports of injuries or deaths arising from adverse reactions to traditional medicines are fewer than synthetic drugs (Zhu *et al*., 2021). In silico studies stated that fatty acids such as myristic (C14:0), stearic (C18:0), caproidic (C6:0), caprylic (C8:0), pentadecylic (C15:0), and palmitoleic (C16:1) received a toxic hazard warning (Elharafi *et al*., 2021), wherein the free form of these metabolites initiate different harmful activities in cells, particularly on mitochondrial phosphorylation (Schönfeld & Reiser, 2021). Because of the protein content in the form of amino acids (Truong *et al*., 2021), (Pratama *et al*., 2020) and fatty acids (Sasongko *et al*., 2018), some amino acid compounds have been reported for dangerous toxic effects in certain doses. They are methionine, tryptophan, DL-aspartate, histidine, tyrosine, phenylalanine, cystine, leucine, valine, isoleucine, glycine, asparagine, arginine, L-aspartate, lysine, threonine, and glutamate (Samuels, 2020). This finding highlights the necessity to assess SF's acute and subchronic oral toxicity to safeguard the community. The processed snakehead fillet was then given orally for 28 days, and parameters such as body weight, relative organ weight, hematological, clinical, biochemical profile, and organ histology were assessed.

MATERIALS AND METHODS

The snakehead fish used were obtained from a farm in Kendal Regency, Central Java, Indonesia, with an average weight of 600-700 grams/head. All species were identified and confirmed by the Biology Laboratory at the Faculty of Mathematics and Natural Sciences, Sebelas Maret University, Indonesia. Voucher specimens were stored at the Sebelas Maret University Laboratory (008/UN27.9.6.4/Lab/2020).

Sample Preparation

Samples were processed by a cold drying method using a freeze dryer. In addition, they were cleaned and separated from the bones, stomach, scales, fins, tail, head, and other impurities, then filleted with a thickness of ± 0.5 cm. The fillets were frozen at -20^{0}C for a day & a night before being subjected to vacuum freeze-drying for 72 hours to obtain dried SF that were mashed and stored in a desiccator until further testing.

Experimental Animals

Acute toxicity was tested in normal male Wistar rats, whereas subchronic investigations were conducted on additional normal males and females (28 days). The experiment used rats measuring 180-200 grams and aged 6-8 weeks. A 12-hour light/12-hour dark cycle was used to acclimate them at room temperature with a normal pellet diet. An experimental and control group was formed during acclimation. The Health Research Ethics Committee of Moewardi Hospital, affiliated with Sebelas Maret University, Surakarta, Indonesia, authorized all protocols with the number: 11/II/HREC/2020.

Acute Oral Toxicity Investigation

The Indonesian Food and Drug Supervisory Agency's non-clinical toxicity testing guidelines were followed for acute oral toxicity testing (BPOM RI, 2020). Twenty male rats were used and divided into four groups consisting of 1 control & 3 treatments at a dose of 350 mg/kg, 1400 mg/kg, and 5600 mg/kg SF suspended in 0.25 % cellulose. The lowest dosage was chosen based on previous studies on the hypoglycemic effect of SF (Muhtadi *et al* 2021), and was increased in multiples until the maximum dose was administered in the rat (BPOM RI, 2020). The suspension was given orally only once in a single dose. All of the animals were given unrestricted access to food and water, and symptoms of acute toxicity were looked for, such as changes in autonomic effects (lacrimation & salivation), alterations in the central nervous system (tremors, convulsions, & lethargy), hair loss, weight loss, diarrhea, and increase or decrease in food & drink consumption for 24 hours with

extra care in the first four hours and once a day for the next 14 days

Oral Subchronic (28-day) Toxicity Investigation

A sub-chronic oral toxicity study was performed following the non-clinical toxicity testing guidelines of the Indonesian Food and Drug Supervisory Agency (BPOM RI, 2020). The forty rats used, consisting of males and females, were randomly divided into Group 1 (control), Group 2 (low dose 350 mg/kg SF), Group 3 (moderate dose 700 mg/kg SF), and Group 4 (highest dose 1400 mg/kg SF) where each group consisted of 5 animals/gender. Based on the oral subchronic toxicity test protocol from BPOM RI (2020), the dose determination in this toxicity test is based on the lowest dose to the therapeutic dose carried out in the previous study (Muhtadi *et al* 2021). After the 28-day intervention, they were fasted overnight and then anesthetized using ketamine to draw blood. Following that, relative organ weights, as well as hematological and biochemical profiles, were determined.

Body Weight and Clinical Monitoring

All rats' body weight was recorded before testing and on a weekly during the research. Furthermore, all of the animals were monitored daily for mortality and their overall health for toxic signs.

Organ index observation

The rats' livers, spleens, kidneys, and stomachs were removed and weighed. As a consequence, the following formula for calculating relative organ weight:

Relative organ weight = (organ weight (g) / body weight of the animal on sacrifice day (g)) × 100 (Sutrisni *et al*., 2019).

Hematological Analysis

Hematological parameters including hemoglobin (HGB), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), lymphocytes (LYM), neutrophils (NEUT), red cell distribution width (RDW), platelet distribution width (PDW), mean platelet volume (MPV), platelet large cell ratio (P-LCR), and hematocrit (HCT) were evaluated using the Sysmex KX-21 Hematology Analyzer.

Biochemical Analysis

Immediately the rats were euthanized with high doses of ketamine, blood was drawn from the heart (intracardiac) and put into non-heparin effendrof (anticoagulants) tubes, then centrifuged at 4,000 rpm for 10 minutes to separate the serum. The biochemical measurements based on enzymatic colorimetric tests included serum glucose levels, lipid profiles (total cholesterol (TC), triacylglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very high-density lipoprotein (VHDL), as well as parameters of renal function (urea & creatinine) and liver function (alanine transaminase (ALT) & (AST). The above parameters were measured with a commercial kit (Biosystems, Spain) using a Biosystem Semi analyzer (BTS350).

Histopathology

The liver, heart, and kidney were carefully removed and kept in 10% buffered formalin fixation solution for histopathology, while organ paraffin slices were produced, stained with hematoxylin and eosin, and processed for light microscopy using an optilab viewer (Elangovan *et al*., 2019).

Statistical Analysis

The data were analyzed statistically using one-way Analysis of Variance (ANOVA) and presented as mean standard error of the mean (SEM). Tukey's post hoc test was also used to compare the results to the control group using statistics, and $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Acute Oral Toxicity Investigation

The investigation result during 14 days of observation, no rats died after oral administration of SF up to the highest dose of 5600 mg/kg. Bodyweight measurement led to an average of 180.00 ± 13.11; 187.00 ± 8.87; 191.00 ± 8.79 & 191.00 ± 8.52 g on the last day of observation. This parameter steadily increased within the typical weight growth range. All test animals were euthanized after 14 days of treatment and macroscopically examined for organ abnormalities. As a result, it was determined that SF was not toxic after short-term exposure.

Subchronic Oral (28-day) Toxicity Investigation

Observation on body weight and clinical condition of test animals

The weighing of the test animals was carried out once every seven days, then the average of the value obtained was calculated weekly to determine changes in their body weight and health (Figure 1 and 2). Based on the graph plotted, the average body weight of male and female rats in the first to

the fourth week experienced an increase in all groups. This shows that the SF from a dose of 350 to 1400 mg/kg did not reduce the test animals' body weight during 28 days of administration.

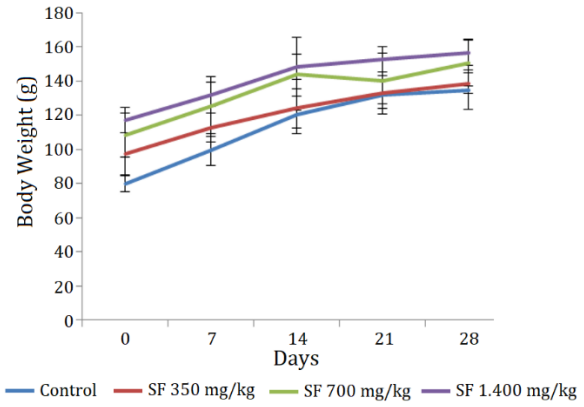

Figure 1. Effect of snakehead fish (SF) on body weight changes in subchronic oral (28-day) toxicity in female rats. Data are expressed as Mean ± SEM (n = 5).

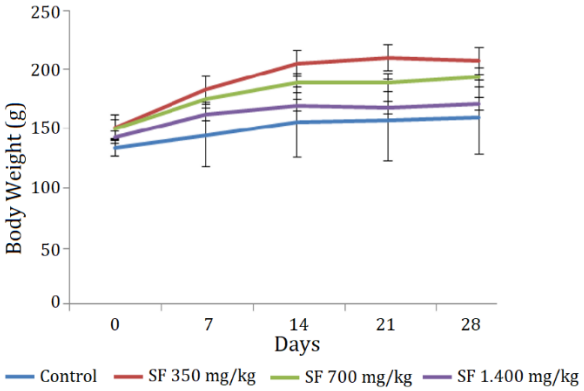

Figure 2. Effect of snakehead fish (SF) on body weight changes in subchronic oral (28-day) toxicity in male rats. Data are expressed as Mean ± SEM (n = 5).

Organ index observation

The relative organ weights of male and female rats after 28 days of SF treatment (Table I). The SF dosages of 350, 700, and 1400 mg/kg did not show a significant difference (P≥0.05) in the organ index of female rats compared to the control group. The male rat kidney organ index findings demonstrated substantial differences between the treatment and control groups.

Hematological profile observation

Male and female rats' hematological profiles after 28 days of oral SF administration, which produced no significant effect (Tables II and III). Several parameters, such as WBC, HGB, & HCT of male rats, showed differences with the control group (P 0.05), but the levels were still in the normal range. The results showed that most of the hematological parameters were not significantly different between all the tested groups and the control.

Blood biochemical observation

The biochemical profiles of rats following 28 days of oral SF treatment (Table IV). Male rats treated with doses of 350, 700, and 1400 mg/kg showed significantly lower total cholesterol (TC) and low-density lipoprotein (LDL) concentrations than the control group. Furthermore, the 700 mg/kg caused significantly increased serum creatinine. Meanwhile, in female rats treated with 700 and 1400 mg/kg, fasting blood glucose concentrations increased significantly compared to the control group. However, the increase or decrease in blood biochemical levels was still within the normal range.

Histopathology Study

Male and female rats received SF for 28 days. The kidney, liver, and heart of rats from the control group and those treated with 350, 700, and 1400 mg/kg showed normal histology (Figure 3-5).

Snakehead fillets are processed using freeze-drying to minimize protein denaturation due to heat. The toxicity assessment dosages of the test sample were validated by making a suspension of SF in a cellulose solution. The acute oral toxicity of SF suspension was examined as a preliminary step, and the results showed that it was well tolerated. The maximum dosage of SF suspension (5600 mg/kg) showed no signs of acute toxicity, death, or abnormal behavior in rats for 14 days. According to the Generally Recognized As Safe (GRAS) table and classification of acute systemic toxicity for traditional medicines, SF suspension is non-toxic, with an oral LD50 of 5000–15000 mg/kg (Hodge & Sterner, 1949). The samples were then tested for subchronic toxicity in rats for 28 days.

Administration of SF samples for 28 days had no adverse effects on behavior or death in rats. Based on the graph plotted (**Figures 1** and **2**), the average body weight of male and female rats in the first to the fourth week experienced an increase in all groups, indicating the samples' provision does not reduce appetite or interfere with the digestive system of the test animals. Furthermore, organ index measurements were also conducted in this subchronic toxicity test to determine the test animals' organ-to-body weight ratio or comparison.

Table I. Data on the Measurement Results of the Liver, Heart, Kidney, Spleen, and Stomach Organ Index

Male Rat Organ Index (Mean ± SEM)				
Organ	**Control**	**SF 350 mg/kg**	**SF 700 mg/kg**	**SF 1.400 mg/kg**
Liver	4.09 ± 0.31	3.87 ± 0.23	3.93 ± 0.21	3.46 ± 0.60
Heart	0.41 ± 0.03	0.39 ± 0.03	0.38 ± 0.02	0.39 ± 0.01
Kidney	0.86 ± 0.04	0.69 ± 0.04*	0.63 ± 0.09*	0.75 ± 0.03
Spleen	0,63 ± 0.06	0.63 ± 0.05	0.69 ± 0.13	0.70 ± 0.07
Stomach	1.37 ± 0.11	1.32 ± 0.09	1.41 ± 0.12	1.37 ± 0.12
Female Rat Organ Index (Mean ± SEM)				
Organ	**Control**	**SF 350 mg/kg**	**SF 700 mg/kg**	**SF 1.400 mg/kg**
Liver	3.94 ± 0.20	3.02 ± 0.17	3.61 ± 0.28	3.93 ± 0.37
Heart	0.34 ± 0.02	0.28 ± 0.03	0.3 ± 0.02	0.36 ± 0.03
Kidney	0.75 ± 0.02	0.6 ± 0.05	0.68 ± 0.04	0.76 ± 0.06
Spleen	0.53 ± 0.11	0.44 ± 0.07	0.40 ± 0.04	0.59 ± 0.09
Stomach	1.54 ± 0.17	1.56 ± 0.18	1.24 ± 0.14	1.38 ± 0.20

Note: The data shows the mean ± SEM (n=5). Testing with ANOVA, compared with the control group where * there is a significant difference ($p < 0.05$).

Table II. Effect of SF on Hematological Profile in Male Rats

Parameter	**Male Rat Data (Mean ± SEM)**			
	Control	**SF 350 mg/kg**	**SF 700 mg/kg**	**SF 1.400 mg/kg**
WBC ($x10^3/\mu L$)	16.64 ± 2.46	18.04 ± 3.13	13.92 ± 1.39	18.18 ± 2.40
RBC ($x10^6/\mu L$)	7.27 ± 0.34	7.99 ± 0.15	7.60 ± 0.41	6.71 ± 0.49
HGB (g/dL)	12.94 ± 0.48	14.38 ± 0.29*	13.30 ± 0.74	11.78 ± 0.72*
HCT (%)	44.00 ± 1.63	47.96 ± 1.18*	44.68 ± 2.20	38.68 ± 2.98*
MCV (fL)	60.66 ± 1.26	60.08 ± 1.72	58.88 ± 1.42	57.62 ± 0.51
MCH (pg)	17.86 ± 0.44	18.00 ± 0.40	17.50 ± 0.46	17.64 ± 0.41
MCHC (g/dL)	29.46 ± 0.85	30.00 ± 0.35	29.74 ± 0.34	30.66 ± 0.83
PLT ($x10^3/\mu L$)	695.60 ± 110.40	764.00 ± 96.26	801.60 ± 61.69	746.00 ± 91.95
LYM ($x10^3/\mu L$)	12.84 ± 2.01	14.16 ± 2.95	11.18 ± 1.14	13.20 ± 1.56
NEUT ($x10^3/\mu L$)	3.82 ± 0.50	3.88 ± 0.56	2.74 ± 0.35	4.98 ± 1.28
RDW (fL)	44.38 ± 5.67	34.66 ± 2.62	34.86 ± 2.02	36.64 ± 1.65
PDW (fL)	8.60 ± 0.39	9.30 ± 0.34	9.48 ± 0.64	9.32 ± 0.55
MPV (fL)	6.74 ± 0.17	7.38 ± 0.20	7.42 ± 0.25	7.20 ± 0.26
P-LCR (%)	6.12 ± 0.82	9.58 ± 0.94	9.74 ± 1.54	9.04 ± 1.25

Note: The data shows the mean ± SEM (n=5). Testing with ANOVA, compared with the control group where * there is a significant difference ($p < 0.05$). WBC = White Blood Cell, RBC = Red Blood Cell, HGB = Hemoglobin, HCT = Hematocrit, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration, PLT = Platelet, LYM = Lymphocyte, NEUT = Neutrophil, RDW = Red cell Distribution Width, PDW = Platelet Distribution Width, MPV = Mean Platelet Volume, P-LCR = Platelet Large Cell Ratio.

Moreover, the heart, liver, kidneys, spleen, and stomach were observed for subchronic toxicity, as their index profiles are very important and are widely used to interpret the effects of test substances in toxicity studies (Sutrisni *et al*., 2019). Alterations in renal weight (indicating renal toxicity connected with the test substance's action) and hepatocellular hypertrophy (e.g., enzyme induction or peroxisome proliferation) are all signs of treatment-related changes (Sellers *et al*., 2007). The test results showed that only the kidney organs of male rats produced statistical differences compared to the control, which various metabolic factors can cause in each test animal.

Table III. Effect of SF on Hematological Profile in Female Rats

Parameter	Female Rat Data (Mean ± SEM)			
	Control	SF 350 mg/kg	SF 700 mg/kg	SF 1.400 mg/kg
WBC ($x10^3/\mu L$)	12.00 ± 1.03	10.46 ± 2.08	13.46 ± 2.93	9.80 ± 1.81
RBC ($x10^6/\mu L$)	6.15 ± 0.31	5.76 ± 0.34	6.00 ± 0.49	5.60 ± 0.67
HGB (g/dL)	11.46 ± 0.65	11.08 ± 0.38	11.78 ± 1.01	10.70 ± 1.23
HCT (%)	39.14 ± 2.14	36.86 ± 1.41	39.00 ± 3.84	34.80 ± 4.02
MCV (fL)	63.76 ± 2.15	64.18 ± 2.79	64.78 ± 3.44	62.46 ± 1.38
MCH (pg)	18.64 ± 0.61	19.36 ± 0.66	19.58 ± 0.61	19.24 ± 0.60
MCHC (g/dL)	29.32 ± 1.10	30.10 ± 0.47	30.54 ± 1.53	30.78 ± 0.44
PLT ($x10^3/\mu L$)	421.80 ± 84.45	423.00 ± 114.40	522.00 ± 128.33	515.20 ± 128.44
LYM ($x10^3/\mu L$)	9.54 ± 0.92	7.88 ± 1.52	11.00 ± 2.60	7.48 ± 1.50
NEUT ($x10^3/\mu L$)	2.46 ± 0.57	2.58 ± 0.59	2.46 ± 0.41	2.32 ± 0.55
RDW (fL)	43.58 ± 4.84	42.62 ± 3.48	42.80 ± 7.28	40.24 ± 2.59
PDW (fL)	8.80 ± 0.24	8.80 ± 0.36	9.60 ± 0.87	8.86 ± 0.32
MPV (fL)	7.06 ± 0.14	6.86 ± 0.20	7.50 ± 0.48	6.96 ± 0.08
P-LCR (%)	7.66 ± 0.75	7.42 ± 0.87	10.86 ± 3.17	6.94 ± 0.47

Note: The data shows the mean ± SEM (n=5). Testing with ANOVA, compared with the control group where * there is a significant difference ($p < 0.05$). WBC = White Blood Cell, RBC = Red Blood Cell, HGB = Hemoglobin, HCT = Hematocrit, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration, PLT = Platelet, LYM = Lymphocyte, NEUT = Neutrophil, RDW = Red cell Distribution Width, PDW = Platelet Distribution Width, MPV = Mean Platelet Volume, P-LCR = Platelet Large Cell Ratio.

Table IV. Effect of SF on serum biochemical parameters

Male				
Parameter	**Control**	**SF 350 mg/kg**	**SF 700 mg/kg**	**SF 1.400 mg/kg**
ALT (U/L)	52.52 ± 2.24	57.16 ± 1.07	54.14 ± 1.55	64.64 ± 1.61
AST (U/L)	124.78 ± 12.81	123.68 ± 9.62	127.90 ± 11.64	141.48 ± 12.25
Glucose (mg/dL)	104.6 ± 4.57	95.8 ± 2.94	108 ± 12.4	110.8 ± 5.26
TC (mg/dL)	76.78 ± 5.51	62.14 ± 2.52*	62.82 ± 3.50*	60 ± 4.33*
TG (mg/dL)	84.88 ± 11.00	90.64 ± 5.98	94.4 ± 22.11	96.84 ± 11.36
LDL (mg/dL)	18.92 ± 1.97	11.86 ± 1.15*	12.58 ± 1.29*	11.47 ± 0.95*
Creatinine (mg/dL)	0.25 ± 0.03	0.25 ± 0.01	0.34 ± 0.03*	0.31 ± 0.03
Urea (mg/dL)	21.38 ± 2.09	18.58 ± 1.68	21.96 ± 0.87	31.32 ± 9.18
Female				
Parameter	**Control**	**SF 350 mg/kg**	**SF 700 mg/kg**	**SF 1.400 mg/kg**
ALT (U/L)	68.82 ± 1.54	87.36 ± 3.99	82.04 ± 1.53	65.26 ± 2.18
AST (U/L)	132. 56 ± 5.49	166.68 ± 17.47	130.58 ± 10.96	138.20 ± 12.25
Glucose (mg/dL)	78.8 ± 8.53	94,2 ± 9.29	123.2 ± 3.86*	106.2 ± 6.59*
TC (mg/dL)	87.3 ± 7.15	84.18 ± 6.27	83.14 ± 5.56	78.32 ± 8.23
TG (mg/dL)	101.68 ± 6.88	129.88 ± 25.32	114.66 ± 19.57	91 ± 9.16
LDL (mg/dL)	22.1 ± 1.64	21.88 ± 5.16	17.2 ± 0.74	16.84 ± 1.61
Creatinine (mg/dL)	0.28 ± 0.023	0.30 ± 0.013	0.26 ± 0.018	0.26 ± 0.021
Urea (mg/dL)	29.24 ± 6.43	29.96 ± 1.25	20.9 ± 1.36	26.12 ± 3.13

The difference in the organ index was still in the normal range, which was supported by clinical and biochemical data, such as creatinine and urea.

Blood is a connective tissue in the form of a solution, flows in the circulatory system, and has the functions of transportation, regulation, and body defense. Furthermore, changes in its composition permit the toxicity caused by decreased blood function (Rastogi, 2007). Analysis of hematological parameters is important in determining the toxic effects of a substance and the body's physiological and pathological status

because changes in these parameters can be linked to a variety of diseases and conditions, such as anemia, leukemia, inflammation, and infections (Olson *et al*., 2000, Sutrisni *et al*., 2019). Blood cells have a limited life span, hence their formation process, called hemopoiesis is carried out continuously (Ebdon *et al*., 2013) and is very sensitive to toxic compounds (Adeneye *et al*., 2006). Hematological profile testing is important since the morphology, number, and comparison of various blood cell types are indicators of different pathological changes in the body (Garbus *et al*., 2019). Hematological profiles provide information about the presence of disease & its severity and show the body's physiological conditions related to health (Birhanu *et al*., 2017). Toxic effects are observed from test animals' functional, structural, or biochemical changes. An important factor affecting the safety potential of a substance for consumption is the relationship between the dose and the effects (Arthaud & Loomis, 1978). This study showed that most of the hematological profiles had no significant difference compared to the control group. Some significant differences occur but are still within normal ranges, such as hemoglobin (HGB) and hematocrit (HCT). The experimental results showed that HGB and HCT levels were 14.38 ± 0.29 and 47.96 ± 1.18. The normal range of HGB is 10.4-16.5 g/dL (Delwatta *et al*.,2018), and HCT is 39.1-48.5 % (Wolford *et al*., 1986).

The liver is the main organ for drugs and environmental chemicals' metabolism & detoxification (Sutrisni *et al*., 2019). Serum ALT and AST activities have historically been frequently used as major biomarkers for liver injury in preclinical studies (Ozer *et al*., 2010). Liver damage causes the contained enzymes to be released into the bloodstream, hence their levels in the blood increase and indicate an impaired organ function (Meganathan *et al*., 2011, Sasongko *et al*., 2019). The measurement results of ALT and AST levels in male & female rats showed there was no significant difference between the three-dose groups compared to the control. ALT levels (54.14 – 64.64 U/L) in male rats and (65.26 – 87.36 U/L) in female rats did not fulfill the diagnostic criteria for liver damage, compared with the control group (52.52 U/L male, 68.82 female). Normal ALT levels in Wistar rats were 33-80 U/L (extreme normal value: 30-91 U/L) in males and 20-72 U/L (extreme normal value: 18-177 U/L) in females (Tucker, 1997). The same thing was also shown in the AST levels of male & female rats which were still in the normal range.

The toxicity of the substance allows for a drastic increase or decrease in lipid levels. Lipid profiles identify the primary and secondary diagnoses of hyperlipoproteinemia, triglycerides, hepatic obstruction, and fatty liver disease (Matteoni *et al*., 1999). High levels of triglycerides & cholesterol are associated with atherosclerosis and predispose a person to cardiovascular disease (Shen, 2007). This study carried out quantitative measurements of serum cholesterol, triglyceride, and low-density lipoprotein (LDL) levels. The results showed SF administration in the animal subjects reduced serum cholesterol and LDL levels compared to the control group. The triglyceride levels, which is an ester of glycerol and fatty acid stored in adipose tissue, tended to increase compared to the control but were not significantly different (Rader & Hobbs, 2014). Excessive intake of fat and carbohydrates increases triglyceride levels in the blood (Goff Jr *et al*., 2006). Normal triglyceride levels in rats are 25-145 mg/dL (Suckow & Stewart, 2016). The content of omega 3 in SF as unsaturated fat helps lower cholesterol levels in the blood. Unsaturated fatty acids have a hypocholesterolemic effect by lowering LDL cholesterol levels in the blood and increasing high-density lipoproteins cholesterol levels thereby reducing the risk of atherosclerosis & cardiovascular disease (Adkins & Kelley, 2010).

The impact of the SF on the kidneys was studied using serum urea and creatinine as specific markers of renal function. Alteration to working nephrons can be detected by gradual changes in these parameters (Lamiere *et al*., 2005). Except for increased creatinine levels in the male group at a dosage of 700 mg/kg, no significant changes in serum urea and creatinine levels were detected, which were still considered normal. The relative weight of the kidneys and histological examinations of renal tissue corroborated this finding, indicating that the kidneys were safe and had non-toxic symptoms.

Histopathological examination of the kidneys showed normal-looking tubules and glomeruli, which are important parts of the body that do important things (**Figure 3**). There were no signs of necrosis, iron deposition, or bilirubin buildup in the bodies of the rats that were treated with t he SF drug.

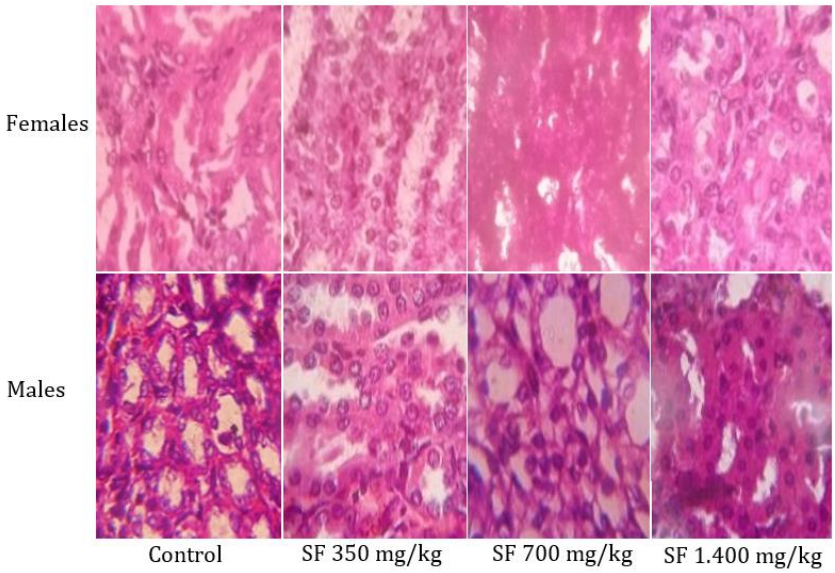

Figure 3. Histopathological evaluations of the male and female kidneys after 28 days of SF treatment orally

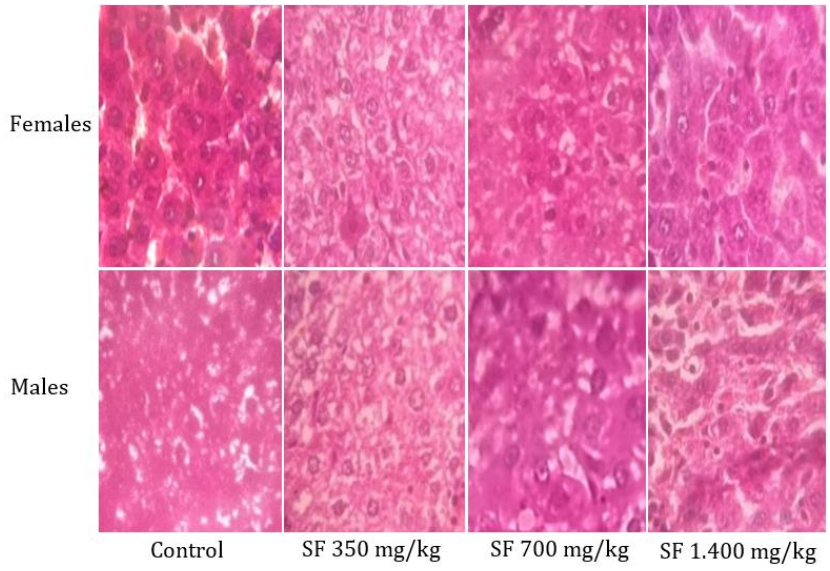

Figure 4. Histopathological evaluations of the male and femaleliver after 28 days of SF treatment orally

All Wistar rats who were given SF had normal liver architecture based on major histological structures like hepatocytes, the portal triad, and the central vein, which are all important parts of the liver (Figure 4**)**. Also, necrosis, fibrosis, and iron and bilirubin deposition were not found in any of the people who had their bodies checked out. In histopathological tests of the heart, there were no differences from those in the control group (Figure 5). Even from a histological point of view, the kidney, liver, and heart were still the same size and shape Test substance didn't have an effect on these organs or cells, and the organs and cells functioned well. This means the substance didn't affect these organs or cells. There were no changes in the kidney, liver, or heart in the control after SF-administered groups.

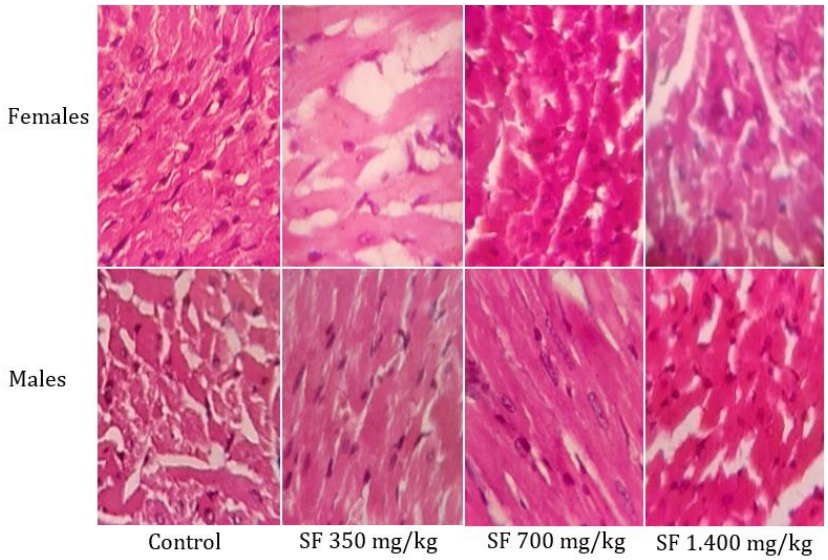

Figure 5. Histopathological evaluations of the male and female heart after 28 days of SF treatment orally.

Blood glucose concentrations reflect intestinal and tissue glucose uptake and hepatic glucose production (Hall & Everds, 2001). Normal blood glucose levels prevent the emergence of disorders that trigger cell damage. The examination results of blood glucose levels showed no significant difference in the male test animal group but a significant difference in the female group. Blood glucose levels in female and male rat groups were still in the normal range of 78.8-123.2 mg/dL. Therefore, administering a dose of snakehead meat for 28 days in the current study can be said not to have a subchronic toxic effect on the blood glucose levels of Wistar white rats because no increase in this parameter exceeded normal limits.

CONCLUSIONS

The findings indicate that SF processed through cold drying was well tolerated when given orally to Wistar rats at 1400 mg/kg. Changes in biochemical profiles such as blood glucose, cholesterol, LDL, and creatinine were regarded as a transient impact of the sample supplied since they remained within the normal range in test animals. While these findings indicate that SF has a low potential risk to health, more research on its chronic toxicity is necessary to determine the consequences of prolonged exposure to the sample.

FUNDING

This study was funded by Sebelas Maret University, Surakarta, through non-State Budget funds with a flagship research scheme in 2021 (No. 260/UN27.22/HK.07.00/2021) with a contract on behalf of Heru Sasongko.

ACKNOWLEDGMENTS

The authors are grateful to the pharmacology and nutraceutical study group of the Diploma 3 pharmacy study program, Vocational School, Sebelas Maret University, for providing equipment support.

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