

In-vivo Safety Assessment of a Polyherbal Formulation (Stress-WIN) Proposed for Clinical Hypertension Management

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Abstract

Herbal treatments are nowadays considered dietary supplements and nutraceuticals for health maintenance and alternative/complementary therapy. A large range of herbal medications worldwide can easily be accessible in the local market. The polyherbal formulation (Stress-WIN) containing *Ashwagandha* (*Withania somnifera* L. Dunal), *Pushkarmoola* (*Inula racemosa* Hook. F.), and *Jatamansi* (*Nardostachys jatamansi* (D. Don) DC.) root has been used empirically in the management of hypertension. However, toxicity data for the Stress-WIN is not available yet. The present study aims to evaluate the Stress-WIN's safety profile by 28 days of repeated dose oral toxicity in Wistar rats. Rats received different doses (500 mg/kg/day, 1000 mg/kg/day, and 2000 mg/kg/day) of Stress-WIN, for 28 days. The study was conducted in compliance with the OECD guideline 423. No significant differences were observed in hematological and biochemical parameters as compared to the control group ($p > 0.05$). Histopathological studies revealed no significant alterations in the macroscopic and microscopic architecture of the liver, kidney, heart, and spleen of the Stress-WIN treated groups in comparison to the control group ($p > 0.05$). Findings from the present study suggest that Stress-WIN supplementation has not shown any side effects in terms of sub-acute toxicity in both male and female rats. These findings indicated no observed adverse effect level (NOAEL) of Stress-WIN was higher than 2000 mg/kg/day.

1. Introduction

Hypertension is the most prevalent disease of cardiovascular disease family and its occurrence is rising in the elderly as well as young individuals and this requires long-term medical treatment. At the OPD (Outpatient Department) level, we have noted/observed that anti-hypertensive drugs symptomatically lower blood pressure. Long-term use of any antihypertensive drugs has adverse side effects on vital organs. A lot of reports indicate that hypertension could be maintained by diet regulation, exercise, and nutraceutical supplementation. Thus, in the future, we may be able to treat hypertension using medications derived from natural plants that have no side effects and greater absorption.

There are a wide range of plants which have been investigated to have anti-hypertensive properties. *Agathosma betulina* supplementation increases urine output (diuretic) and reduces the inflammation (Simpson D. Buchu. 1998). In the case of hyperlipidemia, *Allium sativum* supplementation is beneficial in variety of cardiovascular conditions (Reinhart KM et al. 2008). *Annona muricata* leaf extract supplementation reduces the blood pressure by reducing the peripheral vascular resistance however *Apium graveolens* lowers the blood pressure by acting on the liver (J A Hasrat et al. 2004; Somanadhan B et al. 1999). The crude extract of *Cassia absus* supplementation shows a dose-dependent impact on the regulation of blood pressure however, its high-dose supplementation decreases the heart rate (Cheema Ma et al. 1965). The antihypertensive effect/mechanism of some of these drugs has been investigated and reported that they act via Ca^{2+} ion channels blocking like *Saptaparna* (*Alstonia scholaris*) reduces blood pressure by blocking these channels (Bello I et al. 2015).

In the present study, we have analysed the safety analysis of Stress-WIN powder for clinical hypertension management that contains *Ashwagandha* (*Withania somnifera* L. Dunal), *Pushkarmoola* (*Inula racemosa* Hook. F.) root powder, and *Phanta* (Hot infusion) of *Jatamansi* (*Nardostachys jatamansi* (D. Don) DC.) root. Stress-WIN supplementation has been indicated to treat essential hypertension.

Most Indian Vaidya (Physicians) who practice to treat cardiovascular disorders including hypertension have used different ingredients/components of Stress-WIN alone and in combination. Reports also indicates that the pharmacological actions of each plant extract used in this formulation have anti-hypertensive effects via different actions.

2. Materials and methods

2.1. Plant material

Plant collection

All the plants were procured from renowned local market Gola-Dina Nath, Varanasi. The identification and authentication of the materials were analyzed Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences. A voucher specimen (Ref. no. DG/23–24/804) is conserved in airtight glass bottles in the Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi.

2.2. Preparation of Stress-WIN formulation (SOP)

All the plants were powdered and dried for preparation formulation. Table 1 summarizes the parts of plants and their ratio in preparation for the formulation. The poly herbal formulation was prepared by taking specific doses form of roots of all parts and preparation was divided into two phases, Phase-I & Phase-II.

Phase I: Formation of fine root powder (20 mesh size) of *Withania somnifera* L. Dunal and, *Inula racemosa* Hook. F.

Phase II: Mixing and trituration with the hot infusion of coarse root powder of *Nardostachys jatamansi* (D. Don) DC. into fine root powder (20 mesh size) of *Ashwagandha* and, *Pushkarmoola*. Oral polyherbal Ayurvedic formulation (Stress-WIN) is prepared from the equal part of *Ashwagandha* (*Withania somnifera* L. Dunal), *Pushkarmoola* (*Inula racemosa* Hook. F.), root powder, and hot infusion of *Jatamansi* (*Nardostachys jatamansi* (D. Don) DC.) root. The formulation was made by trituration of powder of *Ashwagandha* and *Pushkarmoola* with hot infusion *Jatamansi* and then dried. The trituration process was used as a levigation or wet grinding for preparing a preparation made in mortar and pestle.

2.3. Animals

Male and female Wistar rats of body weight ranging from 160–180 gm were procured from the animal facility of Central Animal House Institute of Medical Science, Banaras Hindu University, Varanasi.

Animals were housed and maintained on a standard 12 h light and 12 h dark cycle in temperature temperature-controlled ($25^{\circ}\text{C} \pm 2$) room and provided *ad libitum* water and food. Central Animal Ethical Committee, Banaras Hindu University as per the guidelines of CPCSEA (Reg. No, approved the study. Dean/2021/IAEC/3040).

2.4. Sub-acute oral toxicity

Sub-acute toxicity study of the Stress-WIN extract was carried out on male and female rats as per OECD guideline 407 (OECD, 2008). A total of 32 animals were divided into four groups, each with four males and four female animals. For 28 days, Group 1 (control group) received saline as a vehicle, whereas Groups 2, 3, and 4 received Stress-WIN formulation (daily) at the dose of 500 mg/kg, 1000 mg/kg, and 2000 mg/kg of body weight respectively by oral administration. Body weight, food, and water consumption, and the development of any toxic and side effects were all observed during the study. Weekly weight, food, and water consumption were compared. Blood samples were taken at the termination of the study for hematological and biochemical analysis. Blood was collected from rats via retro-orbital sinus puncture in EDTA and heparinized tubes for hematological examination and biochemical examination respectively. After blood collection, animals were humanely sacrificed using a high dose of anesthesia, and organs (liver, kidney, spleen, and heart) were collected and weighed immediately to estimate organ weight and a part of the organs was preserved in a 4% paraformaldehyde (PFA) solution at 4°C for overnight for histological analysis.

2.5. Hematological and Biochemical Analysis

Post completion of oral supplementation of Stress-WIN formulation for 28 days, at day 29, animals were anesthetized using ketamine (80 mg/kg) and Xylazine (20 mg/kg) before sacrifice, blood samples were taken from each animal of each group, and collected in with and without EDTA containing blood sampling tubes. K_2EDTA tubes were used to collect the blood for hematological analysis. haemoglobin (Hb) (g/dL), reticulocyte count (%), neutrophils (%), lymphocytes (%), monocytes (%), eosinophils (%), basophils (%), and platelets ($10^5/\text{cm}^3$) were estimated in hematological analysis by using an automatic blood cell analyzer (Arkray's auto hematology analyzer). Biochemical analysis was performed using serum samples. These biochemical parameters were analyzed: urea, creatinine, alkaline phosphatase (ALP), cholesterol, triglycerides, serum glutamic-oxaloacetic-transaminase (SGOT), serum glutamic-pyruvic-transaminase (SGPT) and bilirubin.

2.6 Histological examinations

The organs of the animals such as liver, kidney, heart and spleen were collected for histological assessment. The organs were chopped and kept in 4% paraformaldehyde (PFA) for 24 hours at 4°C . Further, the next day tissues were washed in phosphate-buffered saline (PBS) and followed by dehydration through a graded series of alcohol (30% ethanol to absolute ethanol) and then in xylene for 2 minutes and subjected to xylene + paraffin wax (1:1) solution in an oven at 64°C for 40 minutes. Then tissues were kept in 3 consecutive wax solutions each for 40 minutes in the oven. After that, tissues were embedded in paraffin by MEDIMEAS MTC-TE tissue embedding centre. Tissue blocks were subjected to

cut 5 µm thin section for staining procedure by using Rm2245 Leica microtome. Tissue sections were stained with haematoxylin and eosin to see histological changes. Slides were first deparaffinised in xylene for 15 minutes then hydrated through a graded series of alcohol (absolute to 50% ethanol) and distilled water each for 10 minutes. Then slides were stained in haematoxylin for 5 minutes and kept under running tap water for 15 minutes for blueing. Then sections were counterstained with eosin for 1 minute. Slides were then dehydrated through 3 changes of 70%, 90% and absolute ethanol each for 5 minutes. Slides were cleared with a single change in xylene and then mounted with DPX (Dibutylphthalate Polystyrene Xylene). Afterwards, slides were examined under a bright field microscope (Nikon Eclipse E200) at 10X to observe the histoarchitectural changes in the tissue.

2.7 Statistical analysis

One-way ANOVA followed by Dunnett's multiple comparisons test was used for statistical comparison between different groups. Results were expressed as mean ± SEM (standard error of the mean). The values were considered to be statistically significantly different when a P-value of ≤ 0.05 . Data analysis was performed by using Graph Pad Prism 8.0.2 software.

3. Results

3.1. Body weight gain, food and water consumption of rats treated orally with stress-WIN

During the entire duration of the study, we did not observe any mortality in the animals treated with the Stress-WIN extract. There were no behavioral changes seen in either the Stress-WIN treated or control groups. As shown in Tables 2A & 2B, the body weight of all animals increased in the first and second weeks, but no significant changes were observed. Food and water consumption did not alter considerably.

3.2 Hematological examination

All hematological parameters were estimated and found to be within normal limits. The result showed that there was no significant difference ($p > 0.05$) in the levels of haemoglobin, platelets, reticulocytes, neutrophils, lymphocytes, eosinophil, basophils and monocytes levels of groups treated with Stress-WIN for 28 days as compared to control groups (Table 3A & 3B). Thus, Stress-WIN formulation considered as safe at the dose of 2000mg/kg.

3.3. Serum biochemical analysis

The results of the biochemical analysis of all stress-WIN treatment groups (500 mg/kg, 1000 mg/kg, and 2000 mg/kg) were compared to the control group (Table.4A & 4B), and there was no significant difference was observed ($p > 0.05$) (Graph 3 & 4). In male Wistar rats subjected to subacute administrations, the enzyme responsible for hepatic function, alkaline phosphatase, remained unchanged ($p > 0.05$) across various doses of Stress-WIN (500, 1000, and 2000 mg/kg). Comparable results were observed in female

rats when compared to the control group. Also, the kidney function markers, including creatinine and urea levels, remained unchanged ($p > 0.05$) across various doses of Stress-WIN (500, 1000, and 2000 mg/kg). Similarly, when comparing female rats to the control group, we observed no significant alterations in these parameters. SGOT, SGPT, and bilirubin levels can be indicative of liver dysfunction or hepatotoxicity. The results of the liver profile show no significant alterations of these markers in male and female rats, suggesting that treatment with Stress-WIN had no noticeable adverse effect which was further confirmed by the histological examination. Assessing lipid profiles (cholesterol & triglycerides) is essential for understanding the overall health status of the rats, particularly their cardiovascular health and metabolic function. These measurements are useful for the detection of any potential toxic effects on the liver and other organs. With treatment doses of 500, 1000, and 2000 mg/kg of Stress-WIN, the triglyceride and cholesterol levels didn't show any significant alteration as compared to healthy control groups.

3.4. Histological examination

The liver, heart, kidney, and spleen were sectioned for histological study to observe the changes in organs at the dose (stress-WIN) of 300 mg, 1000 mg, and 2000 mg/kg. Observation by the microscope showed no remarkable histological changes. The liver, heart, and spleen were structurally similar to those of healthy control animals. Both hepatocytes and cardio-myocytes displayed their normal morphologies, with no indications of apoptosis or the appearance of neutrophil and lymphocyte clusters, demonstrating that Stress-WIN administration had no negative effects on these critical organs. During the sub-acute toxicity studies, normal interstitium, glomeruli, and tubules were detected in the kidney, confirming that the usage of Stress-WIN had no discernible adverse effects on renal tissue. Microscopic investigations revealed no significant differences in spleen between normal and treated rats. (Figure. 1A & 1B).

Discussion

Polyherbal formulations are the combination of two or more herbal extracts, these formulations are more commonly utilized in developed countries than allopathic medicine for the treatment of various disorders. The possible health risk that plant extracts pose to humans due to unintended inherent effects is assessed through studies of toxicity in animals. These side effects may manifest as changes in anthropometric, biochemical, hematological, or histological parameters (Kamyab et al., 2021; Sharma et al., 2023). Our main focus of the study was to examine the subacute toxicity of the polyherbal ayurvedic oral formulation (Stress-WIN), which contains *W. somnifera*, *I. racemosa*, and *N. jatamansi*. The World Health Organisation advises the advancement of scientific research on the hazardous side effects of traditional herbs used to treat ailments (World Health Organization (WHO), 2013). Although the plants used for the formulation of Stress-WIN, have historically been used in folk medicine for the prevention of inflammatory, neurological, nephrological, and cardiovascular disorders, to date, there are no reports on its toxicity assessment (Chukwuma et al., 2019; Jeyanthi and Subramanian, 2009; Kumar et al., 2023; Sharma et al., 2023; Wangchuk and Jamtsho, 2023).

A 28-day repeated dosing, subacute toxicity study provides evidence of potential adverse effects and the safety profile of the formulation (OECD, 2008). In the present study, a dose of 500 mg/kg, 1000 mg/kg, and 2000 mg/kg of Stress-WIN was administered for 28 days and no toxic effects, mortality, or abnormal clinical signs, nor changes in weight, food and water consumption were observed in the rats treated with Stress-WIN. Such events indicate that this formulation displays no toxicity. Earlier it was reported that in animals administered with root extract of *Withania somnifera* at the dose of 3000 mg/kg for 7 days, no mortality, evident clinical symptoms of toxicity, stress, or negative effects on food and water intake were observed (Ilayperuma et al., 2002). Thus, Stress-WIN can be considered relatively safe for subacute exposure. Hematopoietic parameters are viewed as the most sensitive markers to evaluate the harmful effects of drugs on the physiological and pathological state of humans and animals (Mukinda and Syce, 2007). If the information from animal studies is applied, it is recognized that alterations in this system are a sensitive index for human toxicity. There were no discernible changes haematological parameters in the Stress-WIN subacute toxicity study as well as in liver and kidney function assessment parameters and also lipid profiles of both male and female rats were mostly non-significant when compared to normal control in the present study.

When assessing the hazardous potential of any test compound, the measurement of biochemical parameters is crucial. Indeed, biochemical markers would be a great source of knowledge regarding particular organs like the liver and kidney (Nfozon et al., 2019). No significant differences in the level of biochemical markers are found in the present study. Haematological and biochemical analyses are supported by histopathological studies. There are no substantial alterations were seen in histological investigations of the heart, liver, kidney, and spleen, which is consistent with biochemical and haematological assessments. Similarly, an earlier toxicity study of aqueous root extract of *Withania somnifera* on rats for 28 days at a dose of 2000 mg/kg had no adverse effects (Patel et al., 2016). The present study provides information on the subacute toxicity profile of the polyherbal formulation (Stress-WIN) ought to be very helpful for any future in-vivo and clinical study. Stress-WIN was found to be non-toxic when oral sub-acute toxicity in rats was performed. These results signified that the Stress-WIN is safe and further clinical trials can be done to establish its toxicity clinically.

Conclusion

The subacute toxicity study of Stress-WIN was performed on both male and female rats. The outcome demonstrated that repeated 28-day administration of Stress-WIN at doses of 500, 1000, and 2000 mg/Kg did not result in mortality or signs of toxicity in rats. There was no discernible variation between the biochemical and hematological markers, which were all within the normal range. Histopathological analysis of the heart, liver, kidney, and spleen revealed that there was no significant differences were found as all the organs exhibit normal architecture. Therefore, it can be said that repeated administration with Stress-WIN did not result in appreciable toxicity. Based on these results, no observed adverse effect level (NOAEL) of Stress-WIN was higher than 2000 mg/kg/day, suggesting a potential for safe use.

Declarations

Credit author statement

SA, AR, VT, PP, KA, AKS, and SK were principal investigators of the study and drafted the manuscript. SK, VT, and AKS were advisors of the study. SA, AR, VT, and AKS performed the statistical analysis. SK, PP, and KA collected the raw drug and formed the polyherbal formulation. Experimental analysis was performed under the supervision of VT and AKS. All authors contributed to the design and data analysis and assisted in the preparation of the final version of the manuscript. All authors read and approved the final version of the manuscript.

Disclosure Statement

The authors reported no potential conflict of interest.

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Ethical Approval

The behavioural investigations carried out in this study followed the guidelines outlined by the Committee for Control and Supervision of Experiments on Animals (CCSEA), Government of India. The experimental procedures employed and received ethical clearance from the Institute Animal Ethics Committee of Institute of Medical Sciences, Banaras Hindu University in Varanasi, Uttar Pradesh, India, bearing the approval reference (Reg. No, approved the study. Dean/2021/IAEC/3040).

Conflict of Interest

This manuscript has no conflicts of interest, and all authors have given their approval for publication. We really appreciate you taking the time to consider our work, and we eagerly await the reviewers' feedback.

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Data availability statements

Data of this study will be available upon reasonable request to the corresponding author

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Tables

Table 1: Summary of selected plants of Stress-WIN

S.no.	Biological Name	Family	Sanskrit Name	Part selected	Ratio of the ingredient
• 1.	<i>Withania somnifera</i> L. Dunal	Solanaceae	<i>Ashwagandha</i>	Root	1 Part (Powder)
• 2.	<i>Inula racemosa</i> Hook. F.	Asteraceae	<i>Pushkarmoola</i>	Root	1 Part (Powder)
• 3.	<i>Nardostachys jatamansi</i> (D.Don) DC.	Valerianaceae	<i>Jatamansi</i>	Root	1 Part (hot infusion)

Table 2A: Body weight, food, and water consumption of male rats treated orally with Stress-WIN in subacute toxicity study

Parameters	Control	Stress-WIN		
		500 mg/kg	1000 mg/kg	2000 mg/kg
Initial weight (g)	178.0±2.33	166±5.04	179±1.84	185.7±3.71
First week (g)	181.1±2.31	169±4.88	183±4.42	190.6±3.88
Second week (g)	183.3±2.88	172.5±4.99	187±4.02	192.8±3.01
Third week (g)	187.0±2.95	176.3±5.22	192±4.02	197.8±3.27
Final weight (g)	189.9±3.0	180±5.45	196.6±3.57	202.7±2.63
Food intake (g/day)	14.7±0.62	14.9±0.69	15.1±0.67*	14.6±0.23
Water intake (ml/day)	14.8±0.46	15.9±0.54	16.6±0.58	15.4±0.31

Table 2B: In the subacute toxicity study, the body weight, food, and water consumption of female rats were treated orally with Stress-WIN.

Parameters	Control	Stress-WIN		
		500 mg/kg	1000 mg/kg	2000 mg/kg
Initial weight (g)	175.5±1.71	162.5±4.05	170.3±2.32	174.3±3.64
First week (g)	177±2.12	166.8±3.59	172.3±2.56	176.3±3.50
Second week (g)	179.3±2.32	169±4.14	175±1.87	179±3.74
Third week (g)	182.3±2.17	170.5±3.12	177.8±1.25	182.5±3.38
Final weight (g)	183.8±2.56	173.5±3.01	181±2.12	185.8±3.28*
Food intake (g/day)	13.7±0.51	13.1±0.23	13.7±0.36	13.0±0.29
Water intake (ml/day)	14.9±0.30	15.8±0.61	16.1±0.45	15.7±0.51

Table 3A: Hematological analysis of male rats treated Stress-WIN for 28 days.

Parameters	Control	Stress-WIN		
		500 mg/kg	1000 mg/kg	2000 mg/kg
Hb (g/dL)	14.45±0.60	14.50±0.47	14.03±0.25	13.73±1.33
Platelets (10 ⁵ /cm)	7.56±0.25	8.61±1.37	7.78±1.72	7.40±1.02
ESR (mm/1 st hour)	3.50±0.50	6.00±1.41	7.25±2.06	10.75±3.20
Reticulocyte count (%)	0.53±0.03	0.58±0.05	0.80±0.10	0.53±0.14
Differential Leukocyte Count				
Neutrophils (%)	10.0±4.73	12.25±2.87	8.25±1.44	11.0±3.81*
Lymphocytes (%)	90.0±4.73	87.75±2.87	91.75±1.44	89.00±3.81
Eosinophils (%)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Basophils (%)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Monocytes (%)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Table 3B: Hematological analysis of female rats treated Stress-WIN for 28 days.

Parameters	Control	Stress-WIN		
		500 mg/kg	1000 mg/kg	2000 mg/kg
Hb (g/dL)	13.85±0.37	14.40±0.53	14.33±0.55	14.23±0.33
Platelets (10 ⁵ /cm)	8.06±1.12	6.77±1.86	7.66±1.16	6.70±0.74
ESR (mm/1 st hour)	7.25±1.60	6.00±1.86	7.50±1.26	7.75±2.59
Reticulocyte count (%)	0.53±0.03	0.60±0.10	0.63±0.09	0.65±0.10
Differential Leukocyte Count				
Neutrophils (%)	11.25±3.73	6.75±1.25	16.25±3.75	7.00±1.08*
Lymphocytes (%)	91.25±1.49	93.25±1.25	83.75±3.75	93.00±1.08
Eosinophils (%)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Basophils (%)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Monocytes (%)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Table 4A: Biochemical parameters of male rats treated Stress-WIN for 28 days.

Parameters	Control	Stress-WIN		
		500 mg/kg	1000 mg/kg	2000 mg/kg
Serum urea (mg/dL)	36.55±5.78	48.63±4.96	42.99±5.09	36.22±5.37
Serum creatinine (mg/dL)	1.04±0.03	1.36±0.20	1.03±0.06	0.94±0.10
Alkaline Phosphatase (IU/L)	245.15±24.82	186.90±2.74	138.09±4.60	77.32±26.02
Liver profile				
Serum bilirubin (mg/dL)	0.86±0.05	0.72±0.05	0.77±0.05	0.85±0.05
SGPT (IU/L)	67.32±19.84	113.41±28.57	65.76±5.36	82.66±17.66
SGOT (IU/L)	261.53±115.69	149.28±28.14	117.08±19.24	131.11±22.87
Lipid profile				
Cholesterol (mg/dL)	55.68±11.01	34.30±6.15	39.53±3.28	46.08±3.72
Triglycerides (mg/dL)	91.48±6.43	69.81±5.84	78.04±5.23	84.21±5.36

Table 4B: Biochemical parameters of female rats treated Stress-WIN for 28 days.

Parameters	Control	Stress-WIN		
		500 mg/kg	1000 mg/kg	2000 mg/kg
Serum urea (mg/dL)	30.79±3.35	34.51±2.10	29.83±1.91	35.12±1.25
Serum creatinine (mg/dL)	1.15±0.28	0.86±0.03	0.76±0.03	0.92±0.05
Alkaline Phosphatase (IU/L)	176.92±23.19	181.65±43.76	164.75±25.61	171.65±22.42
Liver profile				
Serum bilirubin (mg/dL)	0.92±0.10	0.81±0.07	0.76±0.06	0.89±0.01
SGPT (IU/L)	53.42±4.44	60.33±6.67	54.51±5.39	80.12±27.46
SGOT (IU/L)	117.33±3.46	129.50±5.33	123.68±5.33	113.33±1.15
Lipid profile				
Cholesterol (mg/dL)	72.85±10.73	57.67±6.40	52.71±2.88	43.66±3.83
Triglycerides (mg/dL)	73.50±10.96	65.26±12.92	73.89±10.96	102.38±24.30

Figures

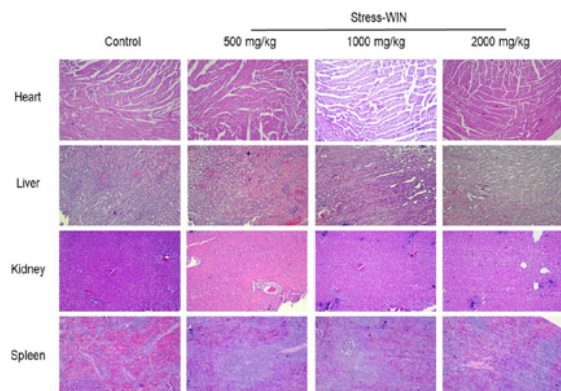


Figure 1A: Histopathological images of four organs (Heart, Liver, Kidney, and Spleen) in male rats after repeated 28-days oral administration of Stress-WIN at the doses of 500 mg, 1000 mg, 2000 mg/Kg, and control group at 10X. Apoptosis was not seen in the liver images. The kidney glomeruli and tubules were normal in histology. The spleen and heart showed normal typical standard architecture.

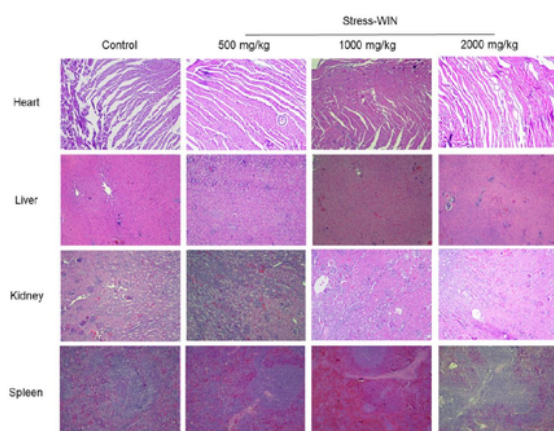


Figure 1B: Histopathological images of four organs (Heart, Liver, Kidney, and Spleen) in female rats after repeated 28-days oral administration of Stress-WIN at the doses of 500 mg, 1000 mg, 2000 mg/Kg, and control group at 10X. Apoptosis was not seen in the liver images. The kidney glomeruli and tubules were normal in histology. The spleen and heart showed normal architecture.

Figure 1

A: Histopathological images of four organs (Heart, Liver, Kidney, and Spleen) in male rats after repeated 28-days oral administration of Stress-WIN at the doses of 500 mg, 1000 mg, 2000 mg/Kg, and control group at 10X. Apoptosis was not seen in the liver images. The kidney glomeruli and tubules were normal in histology. The spleen and heart showed normal typical standard architecture.

B. Histopathological images of four organs (Heart, Liver, Kidney, and Spleen) in female rats after repeated 28-days oral administration of Stress-WIN at the doses of 500 mg, 1000 mg, 2000 mg/Kg, and control group at 10x. Apoptosis was not seen in the liver images. The kidney glomeruli and tubules were normal in histology. The spleen and heart showed normal architecture.

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