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ORIGINAL ARTICLE

Nephroprotective and anti-inflammatory effects of resveratrol topical ointment in albino rats following full-thickness cutaneous burn wound

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ABSTRACT

Background: Recent studies on resveratrol (RSV) have generated great interest, owing to its pleiotropic, health-promoting properties that have been documented not only in animals but also in humans to exhibit anti-neoplastic and alleviate oxidative stress and inflammation, anti-diabetic, protective role in cardiac diseases, and anti-ulcerative properties, among others.
Aims: This study is aimed at evaluating the effects of topical RSV ointment on hematology, serum biochemistry, and serum vascular endothelial growth factor (VEGF), following full-thickness cutaneous burn wound (BW).
Methods: Four groups of 15 rats were arranged in groups A (negative control), B (positive drug control; BW + 1% silver sulphadiazine [SSD] cream), C (experimental group; BW + 5% RSV topical application), and D (positive wound control; BW with no topical application of ointment). The dorsum was shaved using a clipper, and 23.5 mm of BW was inflicted in groups B–D. Rats from groups B and C were treated twice daily for 21 days. Five rats from each group were anesthetized on days 5, 8, and 21, and blood samples were collected post-wounding (PW).
Results: A statistically significant reduction in neutrophil and monocyte counts in the RSV-treated group was recorded ($P < 0.05$). Increased aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities on day 5 were due to acute hepatic injury associated with burns but were normalized on days 8 and 21. Serum levels of urea and creatinine were lower in the RSV-treated group than in the SSD-treated group on days 5 and 8 post-treatment (PT). The RSV-treated group had a lower VEGF concentration in comparison to other groups.
Conclusion: The study demonstrated that RSV suppresses neutrophil and monocyte counts in the peripheral circulation, thus acting as an anti-inflammatory compound. Similarly, RSV exhibited a nephroprotective effect by suppressing creatinine and urea levels. RSV reportedly suppressed the serum activity of VEGF, making it a good antineoplastic agent.
Relevance for Patients: RSV formulation can be used to enhance BW healing in human patients through its anti-inflammatory effect. RSV can also ameliorate kidney dysfunction associated with BW in human patients.

1. Introduction

A wound is defined as an injury to the skin caused by physical, chemical, thermal, microbial, or immunological factors. Accidental exposure to chemicals, wildfires, irradiation, electricity, or sunburn causes burn wounds (BWs) [1]. Burns are categorized by skin depth: first-degree burns only involve the superficial epidermis; second-degree burns involve both the epidermis and dermis; and full-thickness burns involve the three layers of the skin and the underlying blood vessels and muscles [2]. BWs are a broad category of cutaneous injuries with different healing outcomes compared to penetrating

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or excisional wounds. BWs often result in more significant fibrosis than excisional wounds [3]. Burn injuries produce significantly more transforming growth factor-β, which is a chemoattractant for fibroblast activation and differentiation into myofibroblast [3]. The repair of cutaneous injury follows a defined biological sequence aimed at wound closure, tissue repair, and remodeling [4].

Resveratrol (RSV) is a naturally occurring polyphenol and a phytoalexin that is abundant in different plant species. The bark of the plant or fruit is the most abundant site of RSV synthesis. Most plants, like knotweeds, cocoa bushes, peanut plants, pine trees, grape vines, turmeric, and *Vaccinium* shrubs, are all rich sources of RSV [5]. It is produced as a defense against bacterial or fungal attacks [5]. In addition, RSV regulates vascular endothelial growth factor (VEGF) expression, promoting angiogenesis in incisional wounds [6]. RSV also plays an antineoplastic role by directly inhibiting endothelial cells of the capillary through VEGF suppression [7]. Likewise, RSV reduces steatosis and protects the liver from fluoride damage by lowering liver enzyme levels and activities, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [8,9].

Silver sulphadiazine (SSD) ointment is used widely as an antimicrobial agent to alleviate partial- and full-thickness BWs due to its wide spectrum of activity against microorganisms [10]. However, its main disadvantage in burn treatment is its tendency to delay healing [11]. This study aims to evaluate the efficacy of topical RSV on the hematological and serum biochemical profiles in albino rats with full-thickness cutaneous BW.

2. Methods

2.1. Study animals

In this study, 60 albino rats (200–250 g) were used. The rats were purchased from the Laboratory Animal House, Department of Biochemistry, University of Maiduguri, Nigeria. Clean water and feed were supplied throughout the period of acclimatization.

2.2. Experimental design

The rats were categorized randomly into four groups: A (negative control), B (positive drug control; BW + 1% SSD cream), C (experimental group; BW + 5% RSV topical application), and D (positive wound control; BW with no topical application of ointment).

2.3. Infliction of injury

Following proper restraint, a 4 × 4 cm area on the dorsum of 15 rats from categories B–D was shaved using a clipper [12]. An angle of 45° was meticulously created between the clipper and the skin to reduce injury to the site [13]. Intraperitoneal anesthesia was induced using a combination of ketamine and xylazine at a dosage of 60 and 7 mg/kg body weight (b.w.), respectively. The toe-pinch test was used to determine the depth of anesthesia on the limbs prior to wound creation [13]. A 23.5-mm stainless steel metal was heated to 100°C using a hot plate (DB-3; Yancheng Yukai Equipment Co. Ltd., China) for 10 min and placed on the dorsum for 10 min. A third-degree burn was created following 300 g pressure exertion [14]. Analgesia was performed using acetaminophen injection into each of the injured rats [12].

2.4. Experimental RSV ointment preparation

Vaseline®, a petroleum jelly, was melted upon mild heating, and 20 mL of the jelly was used to dissolve 1 g of RSV (Chromadex, United States of America [USA]) with vigorous stirring in a vial. The resulting homogenous mixture was composed of 5% RSV (50 mg/mL) and 95% petroleum base.

2.5. Treatment of wound

Treatment was administered twice daily topically in groups B–D for 21 days. Specifically, the rats in group B were topically applied with 1% SSD ointment (Dermazin®; Salutas Pharma, Germany); group C was topically applied with 5% RSV ointment; and group D was topically applied with the petroleum base only.

2.6. Collection of blood sample

Five rats from each of the four groups were anesthetized using ketamine/xylazine at days 5, 8, and 21 post-BW infliction. Cardiac puncture was used for blood collection, and the syringe was emptied into both plain and EDTA bottles for biochemical and hematological analyses, respectively.

2.7. Sample processing

Hematological parameters, such as packed cell volume (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), total white blood cell count (tWBC), and differential leukocyte count (DLC), were assessed following standard laboratory techniques, as described by Thrall *et al.* [15]. Serum activities of ALT, AST, and ALP, as well as the serum levels of creatinine and urea, were determined using Randox test kits (Randox Laboratories, United Kingdom [UK]) [16]. Serum concentration of VEGF was determined using an ELISA kit (Abbkine Laboratories, China).

2.8. Statistical analysis

Data generated from the study is presented as the mean ± standard deviation. Hematological results and serum VEGF data were compared using one-way analysis of variance (ANOVA) with least significant difference (LSD) comparison after statistics. Serum biochemistry was subjected to two-way ANOVA using SPSS version 16 (IBM, USA). Values with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Effects of burn wound treatment on red blood cell parameters

The effect of BW treatment on erythrocyte parameters is summarized in Table 1 as the mean ± standard deviation. The

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Table 1. Effects of burn wound treatment on red blood cell parameters in albino rats treated with 1% silver sulphadiazine (SSD) or 5% resveratrol (RSV) topical ointments or no treatment (NT)

Parameter	Groups (n=5)	Day		
		5	8	21
PCV (%)	Control	46.40±4.39[a]	44.60±3.21[a]	45.60±3.97[a]
	1% SSD	46.80±2.39[a]	46.20±4.15[a]	47.60±4.15[a]
	5% RSV	45.40±3.78[a]	43.40±3.97[a]	45.40±3.36[a]
	NT	44.60±3.50[a]	43.40±3.97[a]	45.20±3.03[a]
Hb (g/dL)	Control	17.06±2.72[a]	16.40±1.39[a]	16.30±0.84[a]
	1% SSD	15.10±1.19[a]	16.00±1.66[a]	16.20±1.68[a]
	5% RSV	16.58±1.52[a]	15.90±1.24[a]	16.00±1.00[a]
	NT	16.42±0.89[a]	14.00±2.72[a]	16.12±0.38[a]
RBC (× 10^6 µL)	Control	7.53±0.85[a]	7.30±0.31[a]	7.37±0.51[a]
	1% SSD	7.93±0.86[a]	7.62±0.44[a]	7.85±0.59[a]
	5% RSV	7.26±0.54[ab]	7.10±0.82[a]	7.33±0.59[a]
	NT	6.80±1.25[b]	5.48±1.42[b]	7.15±0.61[a]
MCV (fL)	Control	61.76±1.41[a]	61.01±2.28[a]	61.84±1.99[a]
	1% SSD	59.32±3.58[a]	60.56±2.80[a]	60.59±2.31[a]
	5% RSV	62.55±2.52[ab]	61.39±4.10[a]	62.01±4.15[a]
	NT	65.64±1.25[b]	84.39±27.23[b]	63.37±4.16[a]
MCH (pg)	Control	22.86±5.15[a]	22.49±2.29[a]	22.16±1.12[a]
	1% SSD	19.18±2.20[a]	20.96±1.09[a]	20.61±0.72[a]
	5% RSV	22.99±3.13[ab]	22.74±4.06[a]	21.97±2.50[a]
	NT	24.21±1.05[b]	25.96±3.43[a]	22.65±1.77[a]
MCHC (g/dL)	Control	36.94±6.08[a]	37.00±5.20[a]	35.91±3.00[a]
	1% SSD	32.29±5.52[a]	34.63±1.53[b]	34.02±1.40[a]
	5% RSV	36.81±5.18[a]	36.94±5.07[a]	35.41±3.66[a]
	NT	36.89±1.60[a]	32.76±8.09[a]	35.77±2.06[a]

Note: Superscripts ([a] and [b]) define the significance of differences in the mean (for each parameter) between groups (row) for each day (column). The same superscript applied down a column indicates non-significant differences (P>0.05), while a different superscript in the same column indicates a significant difference (P<0.05). For instance, on day 8 (RBC), the means for the control, 1% SSD, and 5% RSV are not significantly different from each other (denoted by [a]), but they are significantly different from the mean of the NT group (denoted by [b]). Abbreviations: PCV: Packed cell volume; Hb: Hemoglobin; RBC: Red blood cell; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration.

mean packed cell volume and hemoglobin concentration across all the groups are not significantly different ($P > 0.05$) at 5, 8, and 21 days PT (DPT). The mean total red blood cell count (tRBC) is significantly lower in the 5% RSV group compared to the control and 1% SSD group at 5 DPT ($P < 0.05$), but significant differences were not observed at 8 and 21 DPT ($P > 0.05$). The mean value of MCV in the 5% RSV group was significantly lower ($P < 0.05$) compared to the no treatment (NT) group at 5 and 8 DPT, but no differences were observed at 21 DPT ($P > 0.05$). The mean MCH was significantly different in 5% RSV-treated and NT rats at 5 and 8 DPT ($P < 0.05$), but no significance was observed at 21 DPT. Similarly, no significant difference was observed for mean MCHC in all groups at 5, 8, and 21 DPT ($P > 0.05$).

3.2. Effects of burn wound treatment on white blood cell parameters

The effect of RSV topical ointment on leukocyte parameters is summarized in Table 2 as the mean ± standard deviation. The mean tWBC count is not significantly different at 5 and 8 DPT ($P > 0.05$). At 21 DPT, the 5% RSV group displayed a non-significant decrease compared to the 1% SSD group ($P > 0.05$). The mean absolute neutrophil count in the 5% RSV group decreased significantly compared to the control, 1% SSD, and NT group at 5, 8, and 21 DPT, suggesting neutropenia induced by 5% RSV treatment. There was slight monocytopenia in rats treated with 5% RSV compared to the 1% SSD group at 8 DPT ($P < 0.05$). At 5 and 21 DPT, no statistical differences were recorded in all groups. No significant differences were observed in mean absolute lymphocyte count at 5, 8, and 21 DPT between groups ($P > 0.05$).

3.3. Effects of burn wound treatment on the activities of serum ALT, AST, and ALP

The effects of BW treatment on liver function are summarized in Table 3. Elevated serum ALT level was observed in the 5% RSV group at 5 DPT ($P < 0.05$), suggesting that 5% RSV did not prevent hepatocellular damage at the early stages of BW. As the wound healed, a significant decrease in mean serum ALT activity was observed in the 5% RSV group from 5–21 DPT ($P < 0.05$). Serum AST activity was elevated in the 1% SSD, 5% RSV, and NT groups compared to the control group at 5 and

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Table 2. Effects of burn wound treatment on leukocyte indices in albino rats treated with 1% silver sulphadiazine (SSD) or 5% resveratrol (RSV) topical ointments or no treatment (NT)

Parameter	Groups (*n*=5)	Day		
		5	8	21
WBC ($\times 10^3/\mu L$)	Control	10.78 ± 1.57^{a}	11.60 ± 1.29^{a}	11.20 ± 1.44^{a}
	1% SSD	12.20 ± 1.68^{a}	12.00 ± 1.37^{a}	12.50 ± 1.32^{a}
	5% RSV	10.00 ± 2.18^{a}	8.90 ± 1.08^{a}	10.40 ± 1.64^{a}
	NT	11.00 ± 1.73^{a}	8.60 ± 2.46^{a}	11.20 ± 1.44^{a}
Neutrophils (N/µL)	Control	2262 ± 136^{ab}	3355 ± 557^{a}	2879 ± 999^{a}
	1% SSD	3936 ± 2086^{a}	3395 ± 336^{a}	3274 ± 1047^{a}
	5% RSV	1556 ± 620^{b}	1081 ± 692^{b}	1688 ± 507^{b}
	NT	2980 ± 1456^{ab}	2727 ± 908^{a}	2789 ± 559^{a}
Monocytes (M/µL)	Control	408 ± 311^{a}	510 ± 203^{a}	366 ± 118^{a}
	1% SSD	154 ± 142^{b}	411 ± 152^{ab}	322 ± 246^{a}
	5% RSV	232 ± 168^{ab}	230 ± 161^{b}	206 ± 118^{a}
	NT	302 ± 129^{a}	206 ± 119^{c}	247 ± 161^{a}
Lymphocytes (L/µL)	Control	8218 ± 665^{a}	7685 ± 1049^{a}	7915 ± 1615^{a}
	1% SSD	8110 ± 1233^{a}	8194 ± 1054^{a}	8852 ± 1001^{a}
	5% RSV	8212 ± 1681^{a}	5696 ± 3271^{a}	8506 ± 1173^{a}
	NT	7718 ± 1572^{a}	5624 ± 1650^{a}	8164 ± 975^{a}

Note: Superscripts (^{a}, ^{b}, and ^{c}) define the significance of differences in the mean (for each parameter) between groups (row) for each day (column). The same superscript applied down a column indicates non-significant differences (P>0.05), while a different superscript in the same column indicates a significant difference (P<0.05). For instance, on day 8 (neutrophils), the means for the control, 1% SSD, and NT are not significantly different from each other (denoted by ^{a}), and they are significantly different from the mean of the 5% RSV group (denoted by ^{b}). Abbreviation: WBC: White blood cell.

Table 3. Effects of burn wound treatment on the serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in albino rats treated with 1% silver sulphadiazine (SSD) or 5% resveratrol (RSV) topical ointments or no treatment (NT)

Parameter	Groups (*n*=5)	Day		
		5	8	21
ALT (U/L)	Control	$72.40\pm9.50^{a,x}$	$72.00\pm8.51^{a,x}$	$73.60\pm4.39^{a,x}$
	1% SSD	$79.80\pm1.92^{a,x}$	$79.20\pm3.65^{a,x}$	$67.80\pm4.15^{a,y}$
	5% RSV	$108.60\pm2.97^{b,x}$	$79.00\pm16.46^{a,y}$	$72.60\pm4.56^{a,y}$
	NT	$107.20\pm6.06^{b,x}$	$77.40\pm3.85^{a,y}$	$67.80\pm5.54^{a,y}$
AST (U/L)	Control	$100.40\pm8.26^{a,x}$	$86.40\pm14.72^{a,x}$	$90.80\pm44.63^{a,x}$
	1% SSD	$115.20\pm1.92^{bc,x}$	$123.40\pm10.71^{bc,x}$	$110.40\pm2.19^{a,y}$
	5% RSV	$128.00\pm12.12^{ac,x}$	$129.40\pm24.30^{c,x}$	$110.20\pm1.10^{a,x}$
	NT	$114.60\pm5.07^{c,x}$	$118.60\pm12.93^{bc,y}$	$105.80\pm4.92^{a,x}$
ALP (U/L)	Control	$14.40\pm1.14^{a,x}$	$11.80\pm1.92^{a,x}$	$13.60\pm1.14^{a,y}$
	1% SSD	$18.00\pm1.87^{b,x}$	$13.80\pm2.17^{ab,y}$	$13.20\pm1.30^{a,z}$
	5% RSV	$20.80\pm0.83^{c,x}$	$16.60\pm3.65^{b,y}$	$14.00\pm1.22^{a,y}$
	NT	$15.80\pm1.30^{a,x}$	$14.60\pm2.07^{ab,y}$	$11.00\pm2.23^{b,z}$

Note: Superscripts (^{a}, ^{b}, and ^{c}) define the significance of differences in mean (for each parameter) between groups (row) for each day (column); superscripts (^{x}, ^{y}, and ^{z}) define the significance of differences in mean (for each parameter) between days (column) for each group (row). The same superscript applied across a row (or down a column) indicates non-significant differences (P>0.05), while a different superscript in the same row (or column) indicates a significant difference (P<0.05). For instance, on day 5 (ALT), the means for the control and 1% SSD are not significantly different from each other (denoted by ^{a}), but they are significantly different from the means of the 5% RSV and NT groups (denoted by ^{b}).

8 DPT ($P < 0.05$). However, at 21 DPT, there was a decrease in serum AST activity in all treatment groups, relative to 5 and 8 DPT ($P > 0.05$). Serum ALP activity was significantly elevated in the 5% RSV group at 5 DPT but decreased gradually on 8 and 21 DPT ($P < 0.05$).

3.4. Effects of burn wound treatment on the serum concentrations of creatinine and urea

The serum concentrations of creatinine and urea following BW treatment are summarized in Table 4. A decrease in the serum urea concentration was observed in the 5% RSV group at 5 DPT. At 8 DPT, the mean serum urea concentration was lower in the 5% RSV-treated group compared to the 1% SSD group ($P < 0.05$). At 21 DPT, the concentration of urea was comparable in all treated and NT groups compared to the control group ($P > 0.05$). Creatinine concentration was lower ($P < 0.05$) in the 5% RSV group at 5 DPT. At 8 DPT, all treatment groups and the NT group had a comparable mean creatinine concentration ($P > 0.05$), which was higher than the control group ($P < 0.05$), except for the 5% RSV group which had a comparable concentration with the control group. At 21 DPT, there was no significant difference in the concentration of creatinine between all groups. Within all treatment groups, mean creatinine concentrations declined gradually from 5–21 DPT ($P < 0.05$).

3.5. Effect of burn wound treatment on the serum concentration of VEGF

The concentrations of serum VEGF are summarized in Table 5 as the mean ± standard error. No significant difference was observed at 5 DPT in the treated groups ($P > 0.05$). At 8 and 21 DPT, a significant difference in VEGF concentration between the 1%SSD and 5% RSV groups was observed ($P < 0.05$).

Table 4. Effects of burn wound treatment on the serum concentrations of urea and creatinine in albino rats treated with 1% silver sulphadiazine (SSD) or 5% resveratrol (RSV) topical ointments or no treatment (NT)

Parameter	Groups (*n*=5)	Day		
		5	8	21
Urea (mmol/L)	Control	$9.12\pm0.46^{a,x}$	$9.62\pm0.62^{a,x}$	$7.62\pm0.41^{a,y}$
	1% SSD	$9.08\pm0.91^{a,x}$	$11.94\pm1.19^{b,y}$	$6.12\pm1.31^{b,z}$
	5% RSV	$7.00\pm0.38^{b,x}$	$10.42\pm1.51^{a,y}$	$6.82\pm0.73^{ab,x}$
	NT	$8.16\pm1.15^{a,x}$	$11.26\pm0.97^{bc,y}$	$6.52\pm0.58^{ab,z}$
Creatinine (µmol/L)	Control	$179.60\pm6.77^{a,x}$	$200.20\pm9.68^{a,y}$	$134.40\pm9.86^{a,z}$
	1% SSD	$202.20\pm14.41^{ac,x}$	$287.80\pm51.93^{b,y}$	$115.00\pm18.71^{a,z}$
	5% RSV	$147.60\pm22.68^{b,x}$	$232.60\pm52.82^{ab,y}$	$118.20\pm12.30^{a,x}$
	NT	$220.60\pm33.56^{c,x}$	$271.00\pm37.36^{b,x}$	$149.80\pm51.07^{a,y}$

Note: Superscripts (^{a}, ^{b}, and ^{c}) define the significance of differences in mean (for each parameter) between groups (row) for each day (column); superscripts (^{x}, ^{y}, and ^{z}) define the significance of differences in mean between days (column) for each group (row). The same superscript applied across a row (or down a column) indicates non-significant differences (P>0.05), while a different superscript in the same row (or column) indicates a significant difference (P<0.05). For instance, on day 5 (urea), the means for control, 1% SSD, and NT are not significantly different from each other (denoted by ^{a}), but they are significantly different from the mean of the 5% RSV group (denoted by ^{b}).

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Table 5. Effects of burn wound treatment on the serum concentration of vascular endothelial growth factor (VEGF) in albino rats treated with 1% silver sulphadiazine (SSD) or 5% resveratrol (RSV) topical ointments or no treatment (NT)

Day	Concentration of VEGF (pg/mL)				*P*
	Control	1% SSD	5% RSV	NT	
5	20.12±11.27[a]	42.47±26.57[a]	20.80±5.52[a]	39.20±26.48[a]	0.784
8	106.60±41.33[ab]	212.73±87.89[a]	0.81±0.81[b]	139.71±75.42[ab]	0.210
21	101.80±44.23[a]	150.09±64.03[a]	4.63±4.15[b]	9.95±7.83[b]	0.046

Note: Superscripts ([a] and [b]) define the significance of differences in mean between groups (column) for each day (row). The same superscript applied across a row indicates non-significant differences ($PS>0.05$), while a different superscript across a row indicates a significant difference ($P<0.05$). For instance, on day 21, the means for control and 1% SSD are not significantly different (denoted by [a]), but they are significantly different from the means of the 5% RSV and NT groups (denoted by [b]).

4. Discussion

This study demonstrated that RSV did not affect RBC parameters, except for a reduction in differential leukocyte counts of monocyte and neutrophil. These findings are consistent with Atmaca *et al.* [9], where RSV reportedly restored normal RBC parameters after RSV administration secondary to fluoride toxicosis. In contrast, Juan *et al.* [17] reported that elevated doses of RSV did not change RBC and WBC parameters in rats. Decreased neutrophil and monocyte counts observed in the RSV-treated group were reported to be due to the anti-inflammatory effects of RSV [9,18-20]. In this study, the hepatic enzymatic activity of ALT, AST, and ALP increased significantly at 5 days PW (DPW), likely due to hepatocellular injury associated with burn injury [21,22]. Jeschke *et al.* [23] reported a 2–4 fold increase in AST and ALT activities immediately after burn injury, suggesting a correlation with burn-induced liver damage. Nielson *et al.* [24] also reported an increase in ALT and AST activities immediately after burn injury. In this study, RSV decreased the activities of AST and ALT as healing progressed, suggesting a protective effect of RSV on the liver [9]. ALP was found to be increased throughout the period of this study; this could be attributed to the fact that RSV reduced the rate of bile flow following hepatic injury [25]. In this study, the creatinine and urea levels in rats treated with 5% RSV were lower compared to that of the 1% SSD group. This could be due to the protective effect of RSV on the nephrons by preventing tubular injury and enhancing clearance [26]. This finding also agrees with the work of Grujić-Milanović *et al.* [27], where RSV reportedly improved the structure and function of the kidneys in malignant hypertensive rats. The process of BW healing in patients with concurrent acute kidney injury is delayed due to numerous factors, such as the inability to mobilize interstitial fluid into the intravascular compartment [28].

VEGF concentration was lower in rats treated with 5% RSV at 5, 8, and 21 DPW, and this may be attributed to RSV binding to the VEGF receptor, which results in significant displacement of VEGF and subsequent effects in angiogenesis [29]. The effect of RSV on the formation of new blood vessels in wound recovery is complex; it tends to have a positive pro-angiogenic effect in ischemic myocardial conditions, but an anti-angiogenic effect in neoplastic cells [7,30]. In a study with human adult retinal pigment epithelial (ARPE-19) cells, Lee *et al.* [30] reported the anti-VEGF activities of RSV through its potent inhibition of hypoxia-inducible factor 1 alpha (HIF-1α) through activated phosphatidylinositol 3 kinase/mammalian target of rapamycin (PI3K/Akt/mTOR; i.e., a signaling pathway that regulates cells adhesion, proliferation, apoptosis, migration, and angiogenesis). It has been reported that PI3K/Akt/mTOR mediates the effect of VEGF [31]. In a study by Gan *et al.* [32], burn ointment could facilitate BW healing through the activation of the PI3K/Akt/mTOR signaling pathway. However, RSV has been demonstrated to suppress the NF-κB transcription factor, which subsequently initiates the inflammatory pathway and deactivates the PI3K/Akt/mTOR-axis to inflict apoptosis [33].

5. Conclusion

Topical application of RSV does not affect RBC indices but suppresses the release of neutrophils and monocytes from the hematopoietic centers. Topical RSV application ameliorated the extent of liver injury but elevated serum ALP activity, possibly attributed to intra-hepatic cholestasis. RSV also ameliorated the extent of BW-induced acute kidney injury, as evidenced by decreased urea and creatinine levels. The VEGF-suppressive role of RSV makes it a potent antineoplastic agent.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Approval and Consent to Participate

All procedures performed in this experimental study were in accordance with the guidelines of the animal care and use committee of the University of Maiduguri (approval number FVM/UM/AUEC/19/003). Informed consent was obtained from all the individual participants of this experimental study.

Consent for Publication

Consent for publication was obtained for every individual's data included in this experimental study.

Availability of Data

Data are available from the corresponding author upon reasonable request.

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