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Research Article

PROTECTIVE ROLE OF SILIBININ AGAINST BISPHENOL A INDUCED SUB-ACUTE TOXICITY IN WISTAR RATS

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SUMMARY

Bisphenol A (BPA) is a widely used industrial chemical compound with recognized endocrine disrupting property, whereas, Silibinin is a flavonolignan component of plant *Silybum marianum* shown to have protective antioxidant potential in various toxicological animal models. This study aimed to evaluate the ameliorative efficacy of silibinin against BPA-induced systemic toxicity in male Wistar rats. A total of 50 male Wistar rats were randomly divided into experimental groups, with 10 animals per group. All animals were administered treatments via oral gavage once daily for 28 days. Clinical signs were monitored and scored daily using a standardized scale. Body weight was recorded at weekly intervals. BPA exposure led to distinct clinical signs including hyporexia, piloerection, hunched posture and pasty faeces, along with a significant reduction in body weight gain. Haematological analysis revealed elevated haemoglobin, TEC and PCV, suggestive of haemoconcentration, while serum biochemistry showed significantly increased creatinine and reduced cholesterol levels, indicating renal stress and altered lipid metabolism. Co-treatment with silibinin, particularly at 200 mg/kg, mitigated of these alterations, reflected by reduced clinical severity scores, improved weight gain, stabilization of haematological parameters to normal levels and partial restoration of serum creatinine and cholesterol levels.

Keywords: Bisphenol A, Silibinin, Sub-acute toxicity, Wistar rats

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Bisphenola (BPA) is recognized as an endocrine-disrupting chemical (EDC) due to its ability to bind to estrogen receptors and interfere with hormonal balance (Iwamoto *et al.*, 2021). Silibinin, a major flavonolignan component of *Silybum marianum* (milk thistle), is well-documented for its distinct hepatoprotective and some other pharmacological activities i.e. neuroprotective, anti-cancerous, antioxidant, anti-inflammatory etc. (Kittur *et al.*, 2002; Polyak *et al.*, 2010; Polachi *et al.*, 2016; Tuli *et al.*, 2021; Liu *et al.*, 2023). Some recent studies have described silibinin as a potent natural polyphenol with strong anti-inflammatory and antioxidant properties due to its five hydroxyl (OH) groups, three of which (5− OH, 7− OH and 20− OH) exhibit phenolic properties and can scavenge free radicals and reactive oxygen species (ROS) (Tuli *et al.*, 2021). Psotová *et al.* (2002) studied the iron chelator activity of silibinin. Owing to its strong free radical scavenging activity via multiple hydroxyl groups, silibinin has shown potential in mitigating oxidative stress and cellular apoptosis (Kim *et al.*, 2009; Liu *et al.*, 2023). However, its potential role in protecting against BPA-induced systemic toxicity, particularly in relation to growth performance and hematobiochemical alterations, remains underexplored. Therefore, the present study was undertaken to evaluate the protective effect of silibinin against BPA-induced subacute toxicity in male Wistar rats, with emphasis on relative body weight gain, clinical signs, relative organ weight changes, haematological and biochemical parameters.

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MATERIALS AND METHODS

The study was carried out at Department of Veterinary Pharmacology and Toxicology, College of Veterinary Sciences, LUVAS, Hisar from the period between July 2024 to April 2025.

1. Chemicals

Bisphenol A (BPA, purity 97%) and silibinin (purity >98%) were procured from Sigma-Aldrich (Merck Co., USA). Corn oil (Korn Health, India) was used as the vehicle for oral administration.

2. Animals

The experiment was conducted on 50 healthy male Wistar rats (6-8 weeks old, 130-170 g) obtained from the Disease-Free Small Animal House (DFSAH), LUVAS, Hisar. All procedures were approved by the Institutional Animal Ethics Committee (IAEC) (Protocol No. IAEC/LUVAS/31/01, Letter No. VCC/IAEC/2024/480-99 dated 06/07/2024) and the study adhered to CPCSEA guidelines.

3. Experimental Design

Rats were acclimatized for seven days under standard laboratory conditions. Animals were randomly divided into 5 groups (n = 10 per group) and treated once daily by oral gavage for 28 days as follows:

- Group 1 (Control): Corn oil @ 1ml/100 g b.wt.
- Group 2 (BPA): BPA @ 325 mg/kg b.wt.
- Group 3 (SLB): Silibinin @ 200 mg/kg b.wt.
- Group 4 (BPA + SLB low): BPA @ 325 mg/kg + SLB @ 100 mg/kg b.wt.
- Group 5 (BPA + SLB high): BPA @ 325 mg/kg + SLB @ 200 mg/kg b.wt.

Silibinin was administered 30 minutes prior to BPA in co-treatment groups i.e. group 4 and 5. Doses were prepared freshly each day in corn oil and administered at 1 ml/100 g body weight using an 18G metal gavage needle.

4. Clinical Observations, Body Weight and organ weight

Animals were observed daily for clinical signs of toxicity, behavioural changes and mortality. A semi-quantitative scoring system (0: absent, ±: rare, +: occasional, ++: frequent, +++: persistent) was used to assess clinical signs. Body weight was recorded weekly and relative body weight gain and organ weights were calculated by using following formula:

$$\text{Relative body weight gain} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

$$\text{Relative organ weight} = \frac{\text{Organ weight (g)}}{\text{Body weight (g)}} \times 100$$

5. Blood Collection

On day 29, animals were anaesthetized with thiopental sodium (200 mg/kg b.wt.) and blood was collected via retro-orbital plexus using heparinized capillary tubes. Blood was collected in separate vials for haematology (EDTA-coated vials) and serum biochemistry (clot activator coated vials) and sent immediately for analysis.

6. Haematological Analysis

Parameters such as haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, total leukocyte count (TLC)and differential leukocyte count (DLC) were analysed using an automatic haematology analyzer (MS4Se, Melet Schloesing Laboratories, France).

7. Serum Biochemistry

Serum levels of ALT, AST, ALP, total protein, triglycerides, cholesterol, BUN and creatinine were estimated using commercial diagnostic kits (Erba Mannheim,

Table 1. Clinical signs and behavioral changes due to 28-days oral dosing of Bisphenol A (BPA), silibinin and their co-treatment

Clinical Signs & Symptoms	G-1	G-2	G-3	G-4	G-5
Hyporexia	0	+++	0	+	±
Ruffled & Dull Hair coat	0	+++	0	++	+
Pasty Faeces	0	+++	0	++	±
Piloerection	0	+++	0	++	+
Hunched Posture	0	+++	0	±	0
Perinasal Erythema	0	++	0	+	±
Foul Odour & Bedding Discoloration	0	++	0	±	0
Bradypnea (Open-Mouth Breathing)	0	++	0	±	0
Mortality	0	0	0	0	0

G-1: Vehicle Control, G-2: BPA (325 mg/kg), G-3: SLB (200 mg/kg), G-4: BPA + Low dose SLB (100 mg/kg), G-5: BPA + High dose SLB (200 mg/kg).

Table 2. Effect of 28-days oral dosing of Bisphenol A (BPA), silibinin and their co-treatment on relative organ weights (g/100g b. wt.) in male Wistar rats

Group	Left Testes	Right Testes	Liver	Left Kidney	Right Kidney	Spleen	Brain	Left Epididymis	Right Epididymis
G-1	$0.73^{a} \pm 0.03$	$0.75^{a} \pm 0.03$	$4.30^{a} \pm 0.12$	$0.35^{a} \pm 0.01$	$0.36^{a} \pm 0.01$	$0.24^{a} \pm 0.01$	$1.06^{a} \pm 0.04$	$0.31^{a} \pm 0.01$	$0.32^{a} \pm 0.01$
G-2	$0.73^{a} \pm 0.04$	$0.74^{a} \pm 0.04$	$4.67^{a} \pm 0.14$	$0.34^{a} \pm 0.02$	$0.32^{a} \pm 0.01$	$0.20^{a} \pm 0.01$	$1.02^{a} \pm 0.03$	$0.28^{a} \pm 0.01$	$0.29^{a} \pm 0.01$
G-3	$0.74^{a} \pm 0.03$	$0.76^{a} \pm 0.03$	$4.56^{a} \pm 0.10$	$0.42^{a} \pm 0.03$	$0.41^{a} \pm 0.02$	$0.25^{a} \pm 0.01$	$1.28^{a} \pm 0.07$	$0.32^{a} \pm 0.03$	$0.33^{a} \pm 0.03$
G-4	$0.74^{a} \pm 0.03$	$0.76^{a} \pm 0.03$	$4.61^{a} \pm 0.35$	$0.39^{a} \pm 0.02$	$0.40^{a} \pm 0.02$	$0.23^{a} \pm 0.01$	$1.17^{a} \pm 0.04$	$0.31^{a} \pm 0.02$	$0.31^{a} \pm 0.02$
G-5	$0.70^{a} \pm 0.04$	$0.72^{a} \pm 0.04$	$4.37^{a} \pm 0.11$	$0.36^{a} \pm 0.01$	$0.37^{a} \pm 0.01$	$0.26^{a} \pm 0.02$	$1.07^{a} \pm 0.04$	$0.32^{a} \pm 0.03$	$0.33^{a} \pm 0.03$

Values are expressed as Mean ± SE (n = 10/group). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Different superscripts (a, b, c, etc.) within a column indicate significant ($p \leq 0.05$) differences.

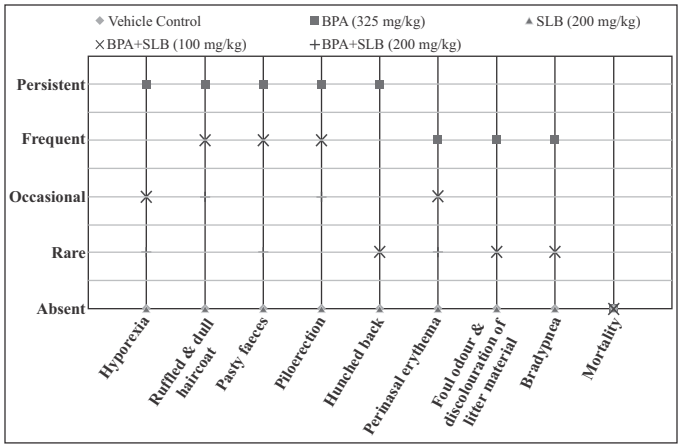

Fig. 1. Scatter plot for clinical signs and behavioral changes due to 28-days oral dosing of Bisphenol A (BPA), silibinin and their co-treatment

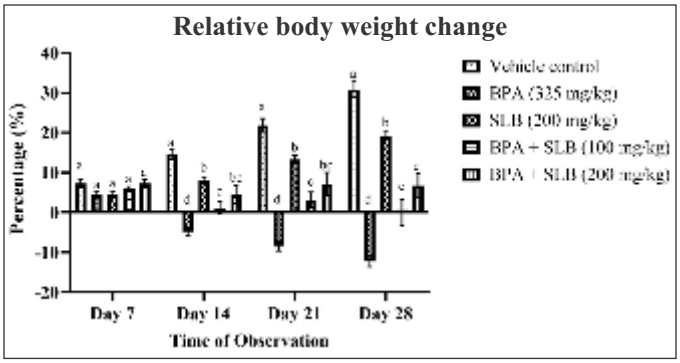

Fig. 2. Effect of 28-days oral dosing of Bisphenol A (BPA), silibinin and their co-treatment on relative body weight change (%) in male Wistar rats. Values are expressed as mean ± SEM (n=10/group). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Different superscripts (a, b, c, etc.) on bars indicate significant ($p \leq 0.05$) difference between groups.

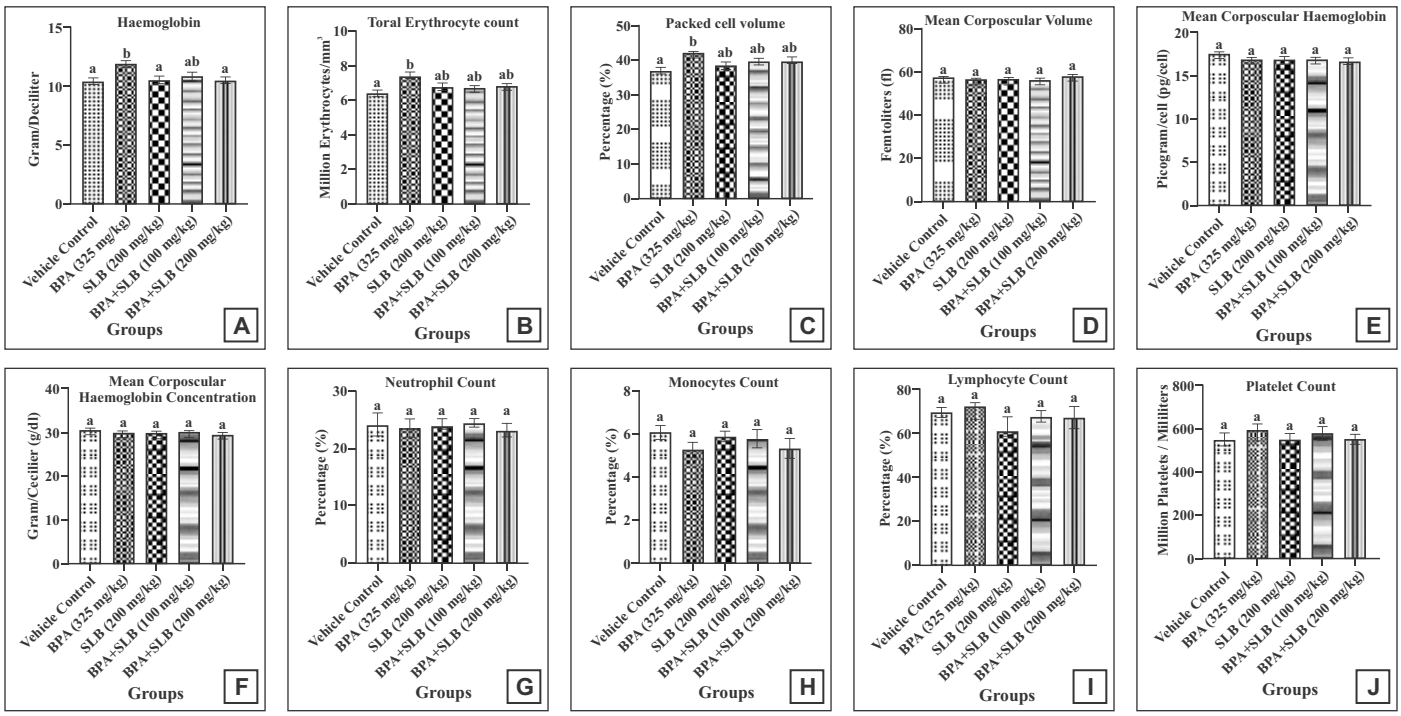

Fig. 3. (a-k): Effect of 28-days oral dosing of Bisphenol A (BPA), silibinin and their co-treatment on various haematological parameters in male Wistar rats **(a)** Haemoglobin (g/dl); **(b)** Total erythrocyte count (Mil/mm3); **(c)** Packed cell volume (%); **(d)** Mean corpuscular volume (fl); **(e)** Mean corpuscular haemoglobin (pg/cell); **(f)** Mean corpuscular haemoglobin concentration (g/dl); **(g)** Neutrophil count (%); **(h)** Monocyte count (%); **(i)** Lymphocyte count (%); **(j)** Platelet count (Mil/ml); **(k)** Total leucocyte count (Mil/mm^3). Values are expressed as Mean ± SE (n=10/group). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Different superscripts (a, b, c etc.) on bars indicate significant ($p \leq 0.05$) difference between groups.

Germany) on an automated biochemistry analyzer (EM 200, Transasia Bio-Medicals Ltd., India).

8. Statistical analysis

The data obtained from various experimental parameters were subjected to statistical analysis using GraphPad Prism version 10.4.2 (San Diego, CA, USA). One-way ANOVA was applied and Tukey's post-hoc test was used to test the differences between the means and significance level was set at $P<0.05$.

RESULTS AND DISCUSSION

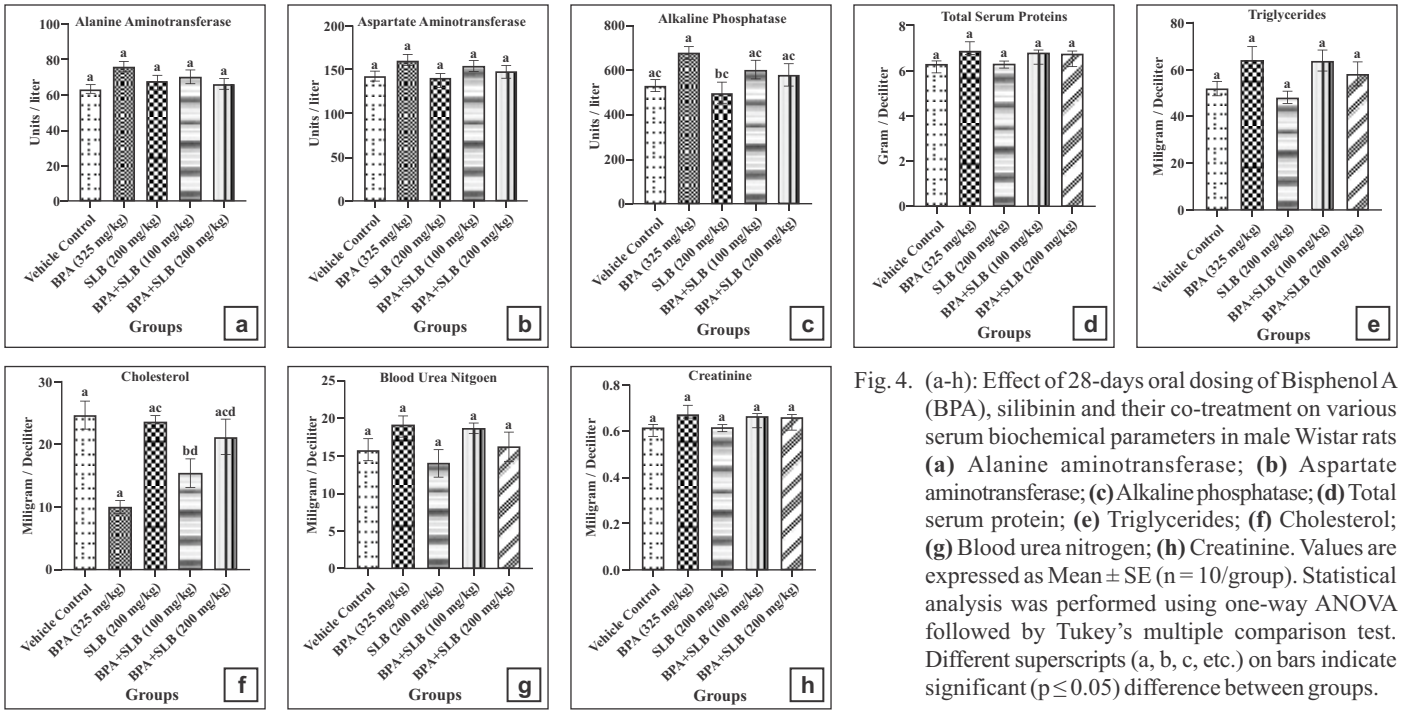

Fig. 4. (a-h): Effect of 28-days oral dosing of Bisphenol A (BPA), silibinin and their co-treatment on various serum biochemical parameters in male Wistar rats **(a)** Alanine aminotransferase; **(b)** Aspartate aminotransferase; **(c)** Alkaline phosphatase; **(d)** Total serum protein; **(e)** Triglycerides; **(f)** Cholesterol; **(g)** Blood urea nitrogen; **(h)** Creatinine. Values are expressed as Mean ± SE (n = 10/group). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Different superscripts (a, b, c, etc.) on bars indicate significant ($p \leq 0.05$) difference between groups.

1. Clinical Signs

BPA-treated rats exhibited persistent clinical signs including hyporexia, piloerection and hunched posture, whereas control and silibinin-alone groups remained asymptomatic. Co-treatment with silibinin reduced the severity and frequency of these signs in a dose-dependent manner, with minimal manifestations at 200 mg/kg. No mortality was observed (table 1; Fig. 1). These signs are indicative of systemic toxicity and are consistant with the findings of Karnam (2003) and Prakash (2010), who reported a similar spectrum of BPA induced behavioural and physical changes in rats.

2. Relative body weight gain

BPA caused significant suppression of body weight gain, whereas silibinin co-treatment, particularly at 200 mg/kg, significantly restored growth trends toward normal, indicating mitigation of BPA-induced metabolic stress (Fig. 2). These findings are in line with the reports of Molayousefian et al. (2024) and Grami et al. (2020). who observed similar body weight reductions following BPA exposure.

3. Relative organ weight

No significant differences were observed in the relative weights of liver, kidney, brain, spleen, testes or epididymis among the experimental groups, indicating absence of gross organ hypertrophy or atrophy (table 2).

4. Haematological parameters

BPA exposure resulted in significant increases in haemoglobin, TEC and PCV, suggestive of haemoconcentration likely due to dehydration and gastrointestinal disturbances. These changes were partially normalized by silibinin co-treatment, supporting its protective role against BPA-induced hematological imbalance (Fig. 3). Similar findings were reported by Alabi *et al.* (2021) who demonstrated the hematoxic effects of BPA in mice. Likewise, Esam *et al.* (2022) observed BPA induced sub-acute toxicity of Wister rats, characterized by alterations in hematological parameters.

5. Serum biochemistry

BPA exposure significantly elevated serum creatinine and alkaline phosphatase levels, along with a reduction in serum cholesterol, indicating renal stress, altered lipid metabolism and possible hepatobiliary disturbance. The elevated ALP levels may indicate early hepatobiliary disturbances, which is plausible given that BPA is known to be excreted through bile (Pattenger *et al.*, 2000). These results also align with those of Abdul Hassan and Hussein (2022), who reported elevated serum creatinine following BPA exposure in albino rats. The decreasing trands in serum cholesterol levels were consistent with Higashihara *et al.* (2007). Co-administration of silibinin attenuated these biochemical alterations, particularly at 200 mg/kg, suggesting a protective effect. These findings are consistent with earlier reports on BPA-induced renal and metabolic toxicity and the documented nephroprotective and lipid-modulatory properties of silibinin (Fig. 4).

CONCLUSIONS

Sub-acute oral exposure to bisphenol A induced systemic toxicity in male Wistar rats, evidenced by adverse clinical signs, impaired body weight gain, haematological

alterations and renal dysfunction. Co-administration of silibinin, particularly at 200 mg/kg body weight, significantly mitigated these toxic effects. The protective influence of silibinin is likely mediated through its antioxidant and anti-inflammatory properties. The findings support the potential use of silibinin as a natural ameliorative agent against BPA-induced systemic toxicity.

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