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Preliminary Investigation on the Effect of Aqueous Seed Extract of Soybean (*Glycine max)* on the Placenta of Wistar rats

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ABSTRACT

Background: *Glycine max* is a type of legume that is extensively cultivated for its versatile edible bean and has significant concentrations of nutritional minerals. However, high intakes of soy isoflavones have been linked to potential adverse effects. This study investigated the effects of aqueous seed extract of Soybean (*Glycine max*) on the placenta of Wistar rats.
Materials and method: Twenty-four (24) pregnant animals were allotted to three groups with eight (8) rats each. Group A served as the control, Group B and C were orally administered with 250mg/kg and 500mg/kg body weight of soybean respectively. On day 19 of pregnancy the rats were sacrificed. The placenta was harvested, weighed and diameter was measured. Likewise, each fetus was weighed and the crown rump length (CRL) was measured. Placenta tissues were fixed in formal-saline, processed and stained with H&E.
Result: There was a significantly lower fetal weight in the group administered with 250mg/kg and 500mg/kg of soybean, whereas, no significance was observed in placental weights compared to control. Fetal-placenta ratio was significantly lower in the group 500mg/kg compared to control. The placenta major and minor diameters were significantly lower in the group 500mg/kg while the 250mg/kg group was significantly lower in placenta minor diameter only compared to control. The CRL was significantly lower in the 500mg/kg group compared to control. Histological results showed that *Glycine max* affected the cystic degeneration of glycogen cell islands of the placenta in a dose-dependent manner.
Conclusion: Ultimately, increased consumption of soybean impaired intrauterine growth and was also a source of distress to the fetus. It is therefore advised that pregnant females consume it with caution.

Keywords: soybean, pregnancy, placenta, oxidative stress, fetus

INTRODUCTION

Soybean (*Glycine max*) is a globally cultivated leguminous crop of significant agricultural, nutritional, and economic importance. It serves as one of the most valuable plant-based protein sources in both human and animal nutrition, contributing to food security across various regions (1, 2). It is particularly rich in essential amino acids, polyunsaturated fatty acids, dietary fiber, and numerous micronutrients, making it a central component of vegetarian and vegan diets (3). In recent decades, the promotion of soy-based products as healthful alternatives to animal protein has led to their widespread consumption, especially among pregnant women seeking plant-based sources of nutrition (4).

Beyond its macronutrient composition, *Glycine max* is uniquely abundant in isoflavones—especially genistein, daidzein, and glycitein (5). These bioactive compounds are structurally similar to 17β-estradiol and are thus classified as phytoestrogens (6). Isoflavones can bind to estrogen receptors (ERα and ERβ), where they exert weak estrogenic or anti-estrogenic effects depending on their concentration, target tissues, and the hormonal milieu (7, 8). These properties have made isoflavones the focus of intense scientific inquiry, particularly regarding their influence on hormone-sensitive organs, reproductive function, and developmental biology (9).

Given the central role of hormones in pregnancy, there is growing interest in the effects of phytoestrogens on gestational processes, especially placental development (10, 11). The placenta is a complex, transient organ that facilitates maternal-fetal nutrient exchange, gas diffusion, hormone production, and immune tolerance throughout pregnancy (12). In rodents, the placenta is anatomically divided into regions such as the labyrinth zone, which is responsible for maternal-fetal exchange, and the junctional zone, which performs endocrine and regulatory functions (13). Since phytoestrogens have the capacity to influence cell proliferation, angiogenesis, and hormone signaling, there is legitimate concern that maternal consumption of soy during pregnancy could impair placental structure and function (10).

Several experimental studies have attempted to elucidate the reproductive effects of soy and its isoflavones. One of such studies demonstrated that neonatal and perinatal exposure to

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genistein resulted in persistent reproductive tract abnormalities and altered gene expression in mice (14, 15). Similarly, there has been a report of changes in placental gene profiles and fetal development following maternal genistein consumption (16). Conversely, other studies found no adverse effects on reproductive outcomes with moderate soy intake, highlighting the role of dose, timing, and preparation methods in modulating outcomes (17). Studies on isolated soy isoflavones showed that soy isoflavones can alter placental angiogenesis and cytokine profiles *in vitro* and *in vivo*, although these findings are often based on isolated isoflavones rather than whole-plant extracts (18, 19).

Notably, the majority of studies to date have either employed isolated isoflavones or used soy-based diets without detailed analysis of placental histomorphology. The placenta is frequently treated as a secondary organ of interest, with most studies emphasizing fetal weight or hormonal endpoints rather than direct structural examination of placental tissue (20). Moreover, the variation in experimental models, doses, extraction methods, and exposure durations contributes to inconsistent findings across the literature (21). There remains a lack of consensus on the threshold at which soy constituent transition from being nutritionally beneficial to biologically disruptive.

In light of these gaps, the current study aims to provide a focused investigation into the dose-dependent effects of aqueous soybean (*Glycine max*) extract on placental morphology in adult pregnant Wistar rats. By combining morphometric, histological, and biochemical assessments—particularly oxidative stress markers—this study seeks to offer a comprehensive evaluation of how soy affects placental structure and function during gestation. Unlike previous research, this study examines the whole extract of soybean rather than isolated isoflavones, thus better mimicking real-world dietary exposure. This focus on direct histomorphological outcomes of the placenta presents an important step toward clarifying the reproductive safety profile of soy consumption during pregnancy, with implications for both dietary guidance and public health policy.

MATERIALS AND METHOD

Preparation of Extract

The seed was purchased from a local market in Benin City, Nigeria and extraction was carried out in the University of Benin (22). It was identified and assigned herbarium number UBH-G470 in the Department of Plant Biology and Biotechnology, University of Benin, Benin City. Initially, the Soy bean was air dried for about four hours. Next, the dry soy beans pulverized to powder form. The powdered *Glycine max* weighing 1.5kg was extracted thrice in distilled water (41.25 L) at room temperature on shaker for 48 hours (Stuart Scientific Orbital Shaker, UK). The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate of aqueous extract obtained was quickly frozen at -40°C and dried for 48 h using a freeze dryer and the eventual yield was 299g. The resulting extract was reconstituted with distilled water to give desired concentrations used in this study.

Research Design

A total of 24 female Wistar rats were randomly divided into three groups (n = 8 per group). Group A (control) received vital feed and distilled water, while Groups B and C were orally administered aqueous *Glycine max* extract at doses of 250 mg/kg and 500 mg/kg body weight, respectively, using an orogastric tube throughout gestation (Gestational day GD 0-19). Doses were determined from the LD50 of the plant which was greater than 5000mg/kg bodyweight. Animals were weighed on GD0 (initial bodyweight) and on GD19 (final bodyweight) to determine changes in bodyweight of dams.

Mating of Animals

To monitor the reproductive cycle and confirm regular cycling before mating, vaginal smear cytology was performed daily between 8:00 am and 9:00 am. Vaginal secretions were collected with a pipette containing normal saline, smeared on microscope slides, and examined under a light microscope (10× objective). The estrous cycle phase was determined based on the proportion of epithelial cells, cornified cells, and leukocytes observed. Presence of sperm cells in the vaginal smear the morning after mating is taken as GD0.

Sacrifice and Sample Collection

At the end of the treatment period (GD19), the animals were sacrificed through cervical dislocation, and a total of 45 placentas were harvested from the pregnant rats. Harvested tissues were used for histological investigation and others for oxidative stress analysis.

Oxidative Stress Analysis

Antioxidant enzyme activity and lipid peroxidation levels were assessed in tissue samples using standard biochemical protocols. Catalase (CAT) activity based on the decomposition of hydrogen peroxide, and the absorbance was measured at 480 nm. The result was expressed as micromoles of H_2O_2 decomposed per minute per milligram of protein (23).

Superoxide dismutase (SOD) activity was estimated by the method which relies on the inhibition of adrenaline auto-oxidation to adrenochrome. The reaction was read at 420 nm, and percentage inhibition was calculated to quantify enzyme concentration (24).

Glutathione peroxidase (GPx) activity was measured using the method based on the peroxidase-catalyzed oxidation of pyrogallol to purpurogallin, producing a brown coloration read at 420 nm. Enzyme activity was expressed in units per milligram of protein (25).

Malondialdehyde (MDA), a marker of lipid peroxidation, was quantified using the thiobarbituric acid reactive substances (TBARS). Plasma samples were reacted with TBA in acidic conditions and heated, after which the resulting pink complex was measured spectrophotometrically. MDA levels were calculated and expressed as nanomoles per milligram of protein (26).

Histological Analysis

Placental tissues were fixed in 10% formalin for approximately 72 hours to ensure optimal fixation. Dehydration was performed through a graded ethanol series (70% to absolute alcohol), followed by clearing with xylene to remove residual alcohol. Tissues were then infiltrated with molten paraffin wax in a heated oven (65–70°C), with three successive changes. Embedding was carried out using metal moulds, and the tissues were oriented longitudinally before allowing the wax to solidify into blocks. Sections were cut at 5 µm thickness using a rotary microtome.

Tissue sections were floated on a 30°C water bath and mounted on glass slides. After air drying, sections were deparaffinized with xylene and rehydrated through descending alcohol concentrations (100%, 90%, 70%) before being immersed in water. Hematoxylin staining was applied for 10 minutes, followed by water rinsing and blueing. Counterstaining was done with 1% eosin for 5–10 minutes. Sections were then dehydrated using ascending alcohol grades, cleared in xylene, and mounted with DPX mountant and coverslips for microscopic examination.

Data Analysis

The IBM Statistical Package for Social Science (SPSS) version 20 was used to analyze the data. One-way analysis of variance (ANOVA) and post hoc LSD were used to examine the various data obtained such as body weight of dams, placenta and fetal weights, placenta diameters and oxidative stress results.

RESULTS

Figure 1 illustrates the changes in body weight across the Control and *Glycine max*-treated groups (250 mg/kg and 500 mg/kg). The Control group recorded the highest weight gain, while both treatment groups exhibited a significantly lower weight gain in a dose-dependent manner. Notably, the decreases observed in the 250 mg/kg and 500 mg/kg groups were significantly lower ($P< 0.05$) compared to the Control group, with the 500 mg/kg group showing the greatest reduction.

Figure 2 presents the placenta weight (g) across the Control and *Glycine max*-treated groups (250 mg/kg and 500 mg/kg). The 250 mg/kg treatment group exhibited the highest placenta weight, slightly exceeding that of the Control group, while the 500 mg/kg group showed the lowest placenta weight. However, these differences were not significant ($P> 0.05$).

Figure 3 illustrates fetal weight (g) in the Control and *Glycine max*-treated groups (250 mg/kg and 500 mg/kg). The Control group recorded the highest fetal weight, closely followed by the 250 mg/kg group. The 500 mg/kg group exhibited a significantly lower fetal weight ($P< 0.05$) compared to the Control group.

Figure 4 presents the fetal-to-placenta weight ratio across the Control and *Glycine max*-treated groups (250 mg/kg and 500 mg/kg). The Control and 250 mg/kg groups exhibited comparable fetal-placenta ratios, both higher than the 500 mg/kg group. The 500 mg/kg group showed a significantly lower ($P< 0.05$) fetal-placenta ratio compared to the Control group.

Figure 5 illustrates the effect of *Glycine max* on the major and minor diameters of the placenta. Significantly lower values were observed in both placental diameters in the group administered 500 mg/kg ($P< 0.05$). In contrast, the 250 mg/kg group showed significantly lower values ($P< 0.05$) only in the minor diameter compared to the Control group.

Figure 6 depicts the crown-rump length (CRL) across the experimental groups. The control group exhibited the highest CRL values. The 250 mg/kg group showed values comparable to the Control, while significantly lower values ($P< 0.05$) in CRL was observed in the 500 mg/kg group compared to Control.

Figure 7 illustrates the serum superoxide dismutase (SOD) activity across the control, 250 mg/kg, and 500 mg/kg treatment groups. Although the 250 mg/kg group exhibited the highest mean SOD activity, followed closely by the 500 mg/kg group, and the control group showed the lowest value, these variations were not significant ($P> 0.05$). This suggests that administration of *Glycine max* at either dosage did not significantly alter serum SOD activity compared to the control.

Figure 8 illustrates the catalase (CAT) activity in the serum of experimental animals across three groups: control, 250 mg/kg, and 500 mg/kg. The control group showed the lowest CAT activity, while both the 250 mg/kg and 500 mg/kg treatment groups exhibited elevated levels, with the 250 mg/kg group having the highest mean value. However, all results were not significant ($P> 0.05$) compared to Control.

Figure 9 displays the levels of glutathione peroxidase (GPx) activity in the serum of experimental animals across three groups: control, 250 mg/kg, and 500 mg/kg. The control group exhibited the lowest GPx activity. Administration of 250 mg/kg extract resulted in the highest GPx levels, while the 500 mg/kg group showed slightly reduced activity compared to the 250 mg/kg group but remained elevated relative to control. Result showed no significant difference ($P> 0.05$) across treatment groups compared to Control.

Figure 10 presents the serum levels of malondialdehyde (MDA), a marker of lipid peroxidation, in experimental

animals across three groups: control, 250 mg/kg, and 500 mg/kg. The control group showed the highest MDA concentration. Treatment with 250 mg/kg extract reduced MDA levels moderately, while the 500 mg/kg group exhibited the lowest MDA concentration among all groups. However, no significant difference among all groups *(P> 0.05).*

Histological findings

Histologically, the Control showed normal architecture of the placenta showing the cells of the junctional zone, the fetal capillary and maternal sinusoids (plate i and iv). 250mg/kg group extract showed cystic degeneration of glycogen cell islands in the junctional zone as well as sinusoids been infiltrated with inflammatory cells (plate ii and v). 500mg/kg of the extract showed severe cystic degeneration of glycogen cell islands in the junctional zone as well as sinusoidal dilatation and congestion (plate iii and vi).

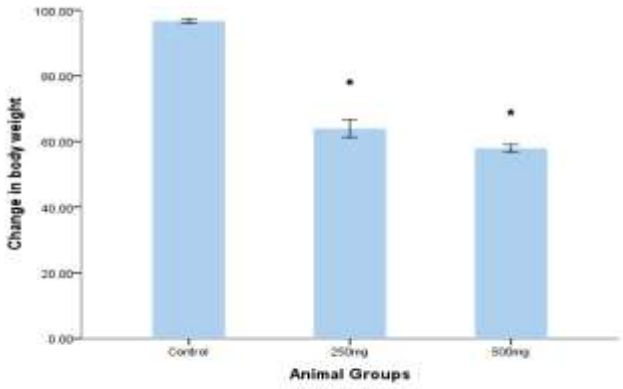


Figure 1: Change in body weight
* indicates *P*<0.05 compared to control

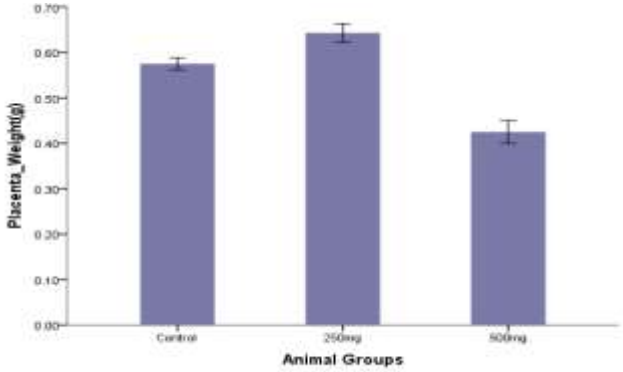
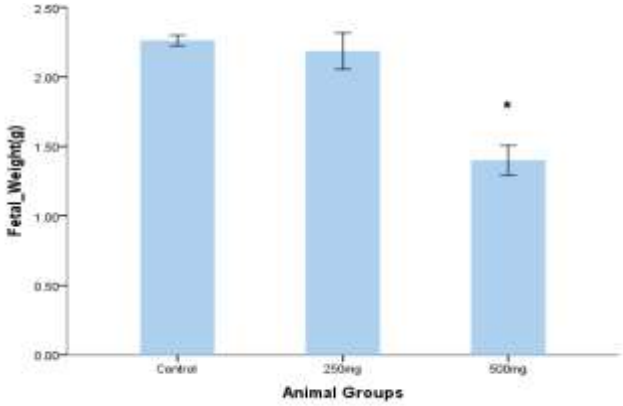


Figure 2: Placenta weight (g)



.Figure 3: Fetal weight (g)
* indicates *P*<0.05 compared to control

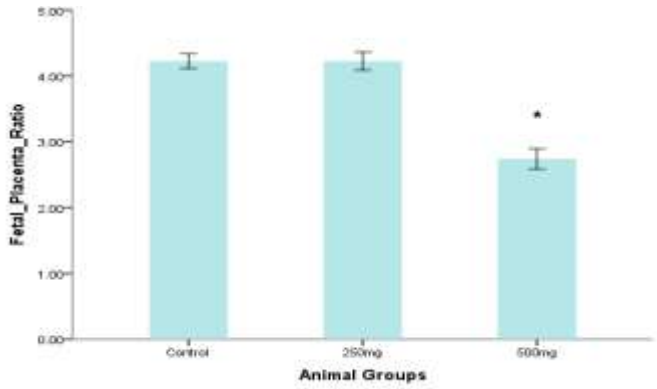


Figure 4: Fetal/Placenta-ratio
* indicates *P*<0.05 compared to control

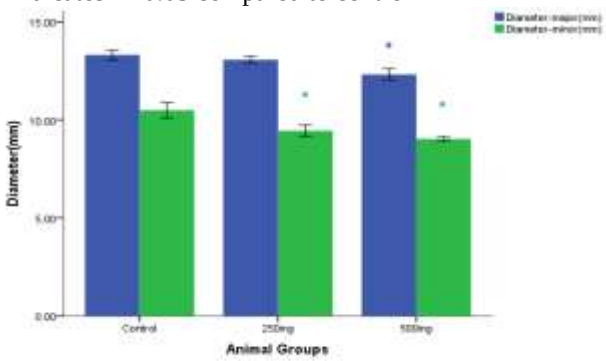


Figure 5: Placenta major and minor diameter among groups
* indicates *P*<0.05 compared to control

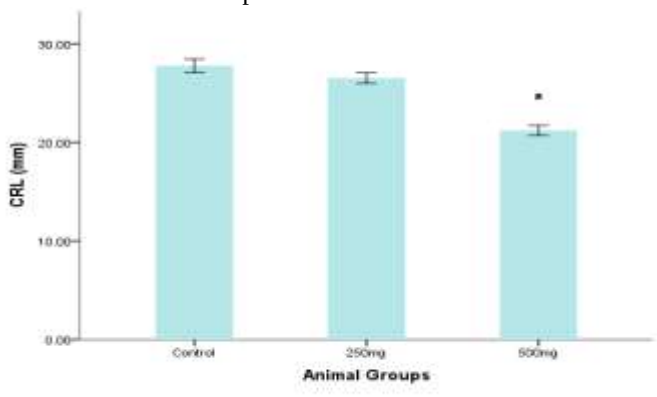


Figure 6: Crown-Rump-Length (mm)
* indicates p<0.05 compared to control

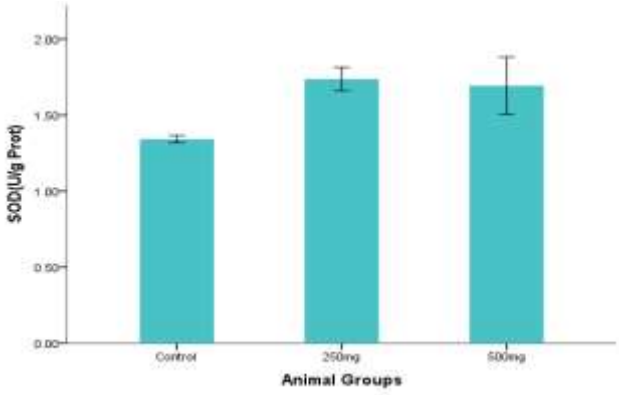


Figure 7: Superoxide dismutase level

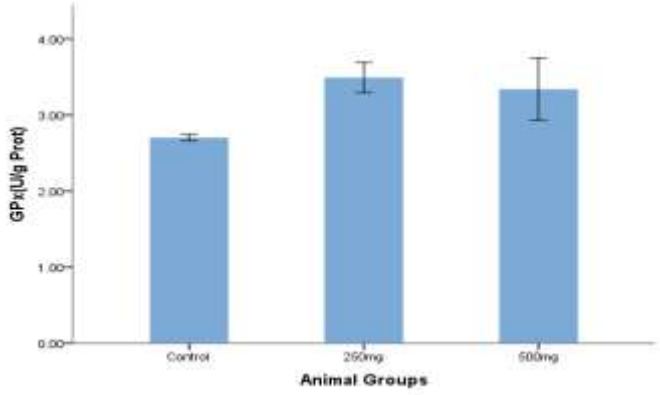


Figure 9: Glutathione peroxidase level

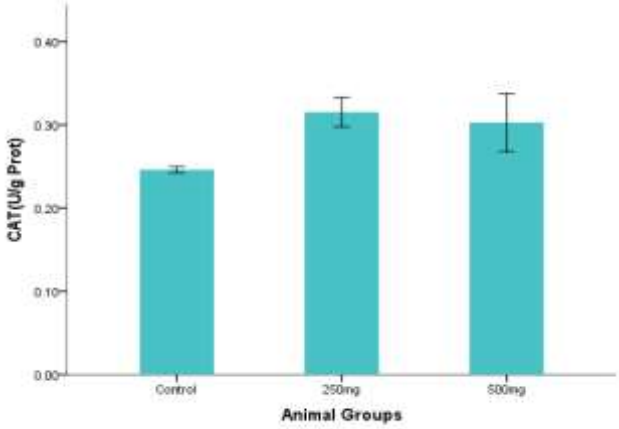


Figure 8: Catalase level

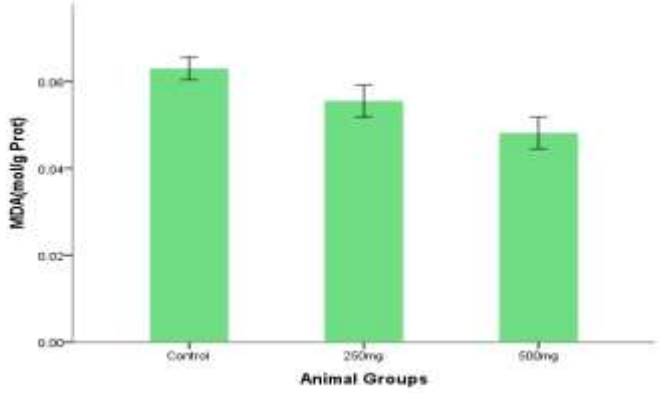


Figure 10: Malondiadehyde level

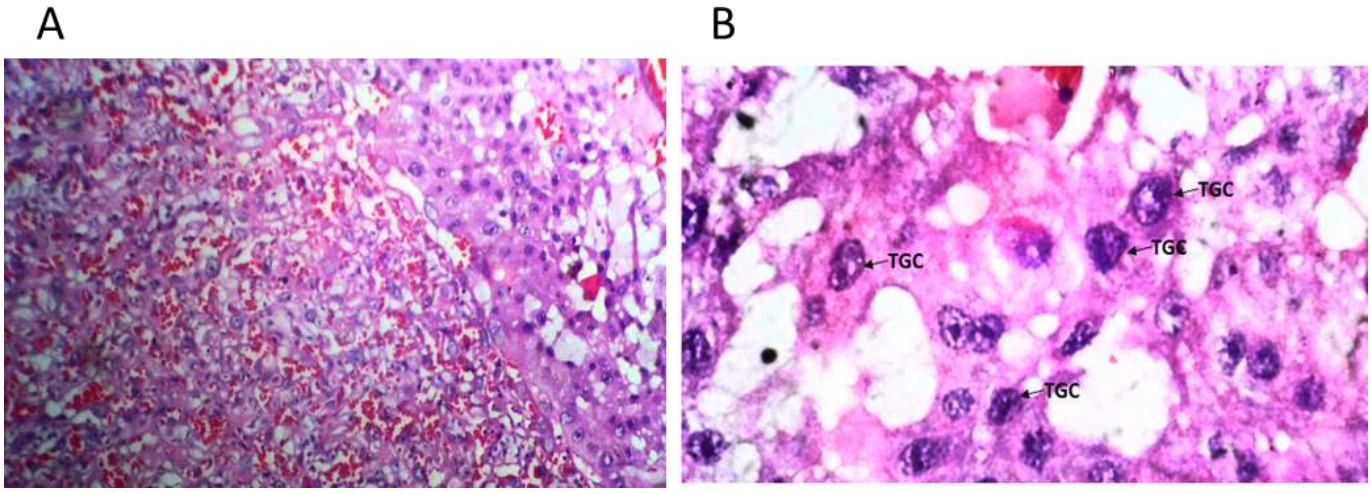


Plate i: illustrates the placental junctional zone in the control group. At low magnification (40×) (Plate iA), the tissue architecture appears well-organized, while high magnification (100×) (Plate iB) highlights distinct trophoblastic giant cells (TGC) and spongiotrophoblasts, demonstrating normal histology.

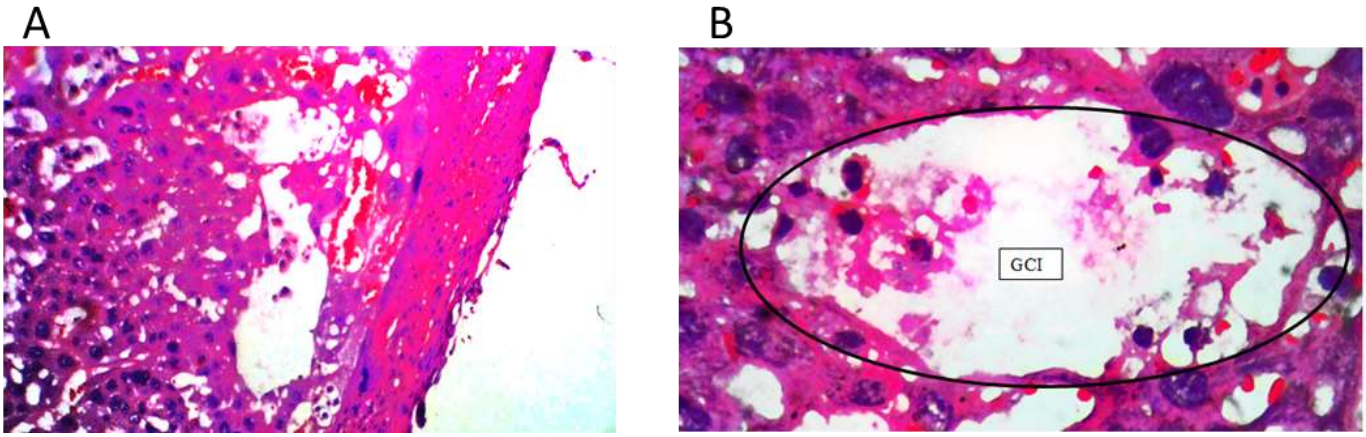


Plate ii: illustrates the placental junctional zone in the 250 mg/kg treatment group. Low magnification (40×) (plate iiA) reveals cystic degeneration within glycogen cell islands (GCI), and high magnification (100×) (plate iiB) provides a clearer view of these degenerated areas, which are distinctly encircled.

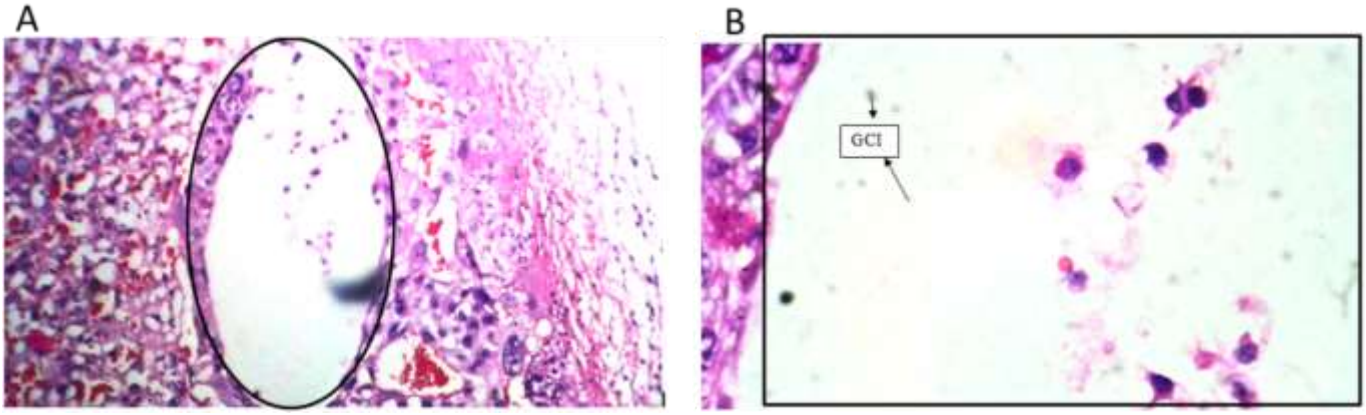


Plate iii: illustrates the placental junctional zone in the 500 mg/kg treatment group, showing more extensive cystic degeneration of glycogen cell islands (GCI) at low magnification (40×) (Plate iiiA). The high magnification image (100×) (Plate iiiB) offers detailed visualization of these degenerated areas.

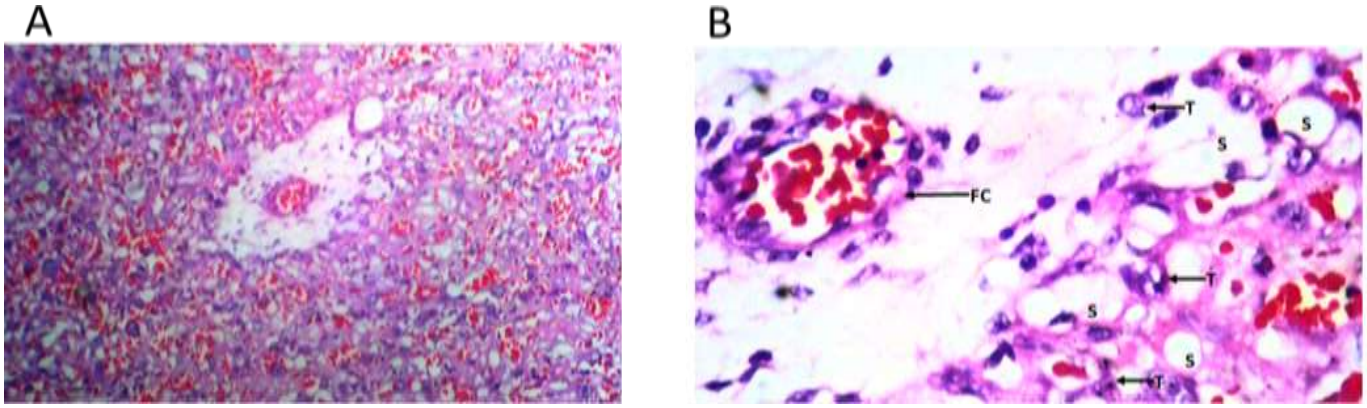


Plate iv: illustrates the placental labyrinth zone in the control group. At 40× magnification (Plate ivA), fetal capillaries (FC), maternal sinusoids (S), and trophoblast cells (T) are clearly visible, while 100× magnification (Plate ivB) emphasizes the detailed cellular structures.

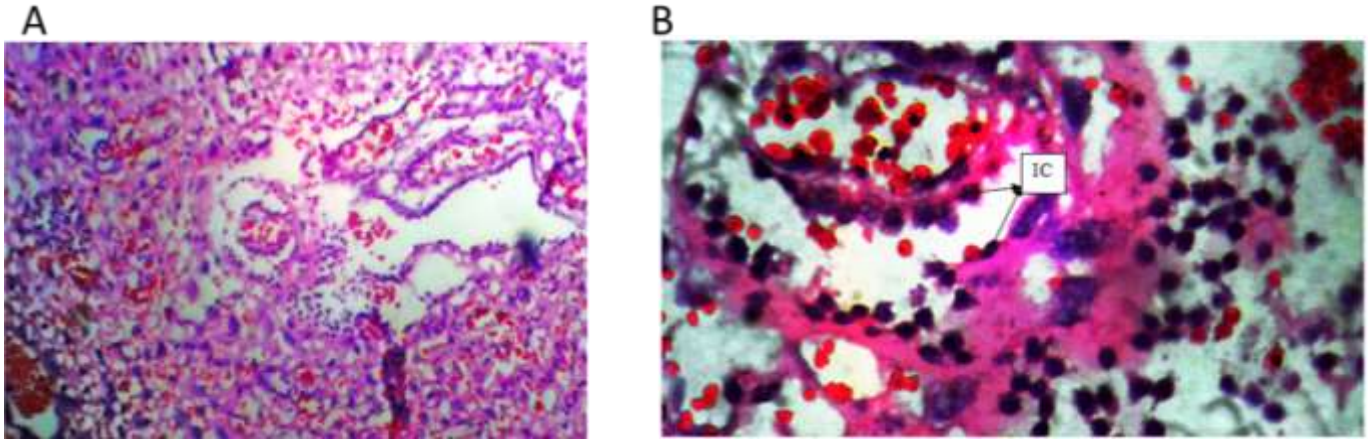


Plate v: illustrates the placental labyrinth zone in the 250 mg/kg treatment group. Low magnification (40×) (Plate vA) shows infiltration of inflammatory cells (IC) within the sinusoidal spaces, which is more clearly identified at high magnification (100×) (Plate vB).

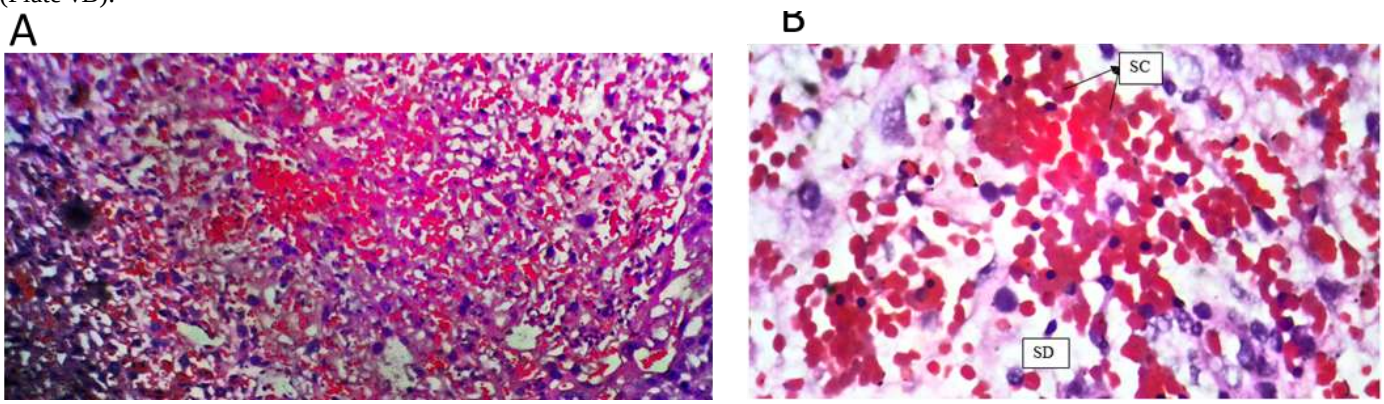


Plate vi: illustrates the placental labyrinth zone in the 500 mg/kg treatment group, where sinusoidal dilatation and congestion are evident at low magnification (40×) (Plate viA), and high magnification (100×) (Plate viB) highlights these vascular disturbances in greater detail.

DISCUSSION

Soybean (*Glycine max*), as a major component of plant-based diets globally, has attracted increasing scientific interest due to its rich nutritional profile and the presence of biologically active compounds, particularly isoflavones. These polyphenolic compounds, structurally similar to endogenous estrogens, can exert weak estrogenic or anti-estrogenic effects, raising questions about their safety, especially during sensitive periods such as gestation. While soy-based products are widely consumed and often promoted for their cardiovascular, metabolic, and postmenopausal health benefits (3, 24), their impact on reproductive physiology and fetal development remains a subject of debate.

Phytochemical analysis of the aqueous extract used in this study revealed the presence of bioactive compounds such as saponins and proteins in appreciable concentrations, alongside trace quantities of reducing sugars, flavonoids, phenols, eugenols, alkaloids, and terpenoids. Tannins and steroids were absent. The presence of these compounds is consistent with prior analyses of *Glycine max* and underpins many of the physiological effects observed in vivo (5). Of particular interest are isoflavones such as genistein and daidzein, which mimic estrogens and are known to interact with estrogen receptors α and β, influencing gene expression patterns related to development, angiogenesis, and endocrine signaling (14, 27).

Saponins and flavonoids, though widely recognized for their antioxidant and anti-inflammatory properties, have also been shown to modulate cellular signaling pathways in trophoblasts and endothelial cells (11). These effects may explain the subtle but important histological changes observed in the placenta following exposure to soybean extract.

In the current study, administration of *Glycine max* extract during gestation was associated with a dose-dependent reduction in several key growth indices, including fetal body weight, fetal-to-placental weight ratio, placental diameters (major and minor axes), and crown-rump length (CRL). These reductions were statistically significant when compared to the control group, particularly at the higher dosage of 500 mg/kg. Interestingly, while placental weight itself did not differ significantly across groups, the observed decrease in the fetal-placental weight ratio suggests an impaired functional efficiency of the placenta (28). Such changes imply that

although placental mass was preserved, its ability to support optimal fetal growth may have been compromised.

These findings corroborate those who reported that dietary exposure to soybean oil during gestation impaired placental development and fetal growth in rodent models (10). Likewise, another study observed suppressed fetal weight gain and reduced maternal food efficiency in animals exposed to high concentrations of soy isoflavones (29). These observations may be due to altered maternal metabolism, reduced nutrient transfer, or direct interference with placental development.

Assessment of oxidative stress markers—including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA)—showed no significant differences between treated and control groups. This suggests that, under the conditions and duration of exposure in this study, the aqueous extract of soybean did not induce oxidative stress in placental tissues. This finding is noteworthy, as oxidative damage is a common pathophysiological mechanism implicated in fetal growth restriction, preeclampsia, and placental insufficiency (30).

Previous studies have demonstrated that phytoestrogens may exhibit antioxidant properties in vitro and in vivo by scavenging reactive oxygen species (ROS) and upregulating endogenous antioxidant enzymes (31). Therefore, the unchanged oxidative profile observed may reflect a balancing act between the pro-oxidant effects of certain phytochemicals and the antioxidant potential of isoflavones.

Nevertheless, the absence of oxidative stress does not exclude other potential mechanisms of toxicity or developmental disruption. For example, changes in endocrine signaling, epigenetic regulation, or angiogenic factors may be involved and warrant further investigation.

Histological analysis provided critical insights into the structural and functional integrity of the placenta. While placentas from the control group showed well-preserved architecture, including organized labyrinthine and junctional zones, the treatment groups exhibited varying degrees of histopathological alterations.

At 250 mg/kg, the presence of cystic degeneration in glycogen-rich cell islands and mild inflammatory infiltration within the sinusoidal spaces suggests an early stage of cellular stress or disruption. At 500 mg/kg, these abnormalities were more pronounced and included sinusoidal dilatation, vascular congestion, and extensive cystic degeneration within the junctional zone. These morphological changes could impair maternal-fetal nutrient exchange and endocrine function, both of which are essential for fetal survival and development (20).

Similar observations which noted that genistein impaired trophoblast proliferation and induced structural abnormalities in placental tissues. The specific disruption of the junctional zone, a region critical for the endocrine output of the rodent placenta, raises concerns about potential downstream effects on hormonal regulation of gestation, including progesterone and placental lactogen levels (32).

The vascular congestion observed may also be indicative of impaired angiogenesis or altered hemodynamic regulation, potentially mediated by phytoestrogens. Previous research has shown that genistein can interfere with vascular endothelial growth factor (VEGF) signaling, resulting in compromised placental vascularization (18). This could explain the observed reduction in fetal growth despite the absence of overt oxidative damage.

Taken together, the results of this study provide compelling evidence that maternal exposure to *Glycine max* extract, particularly at higher doses, can negatively impact placental morphology and fetal development in Wistar rats. These effects appear to be independent of oxidative stress and are more likely attributable to structural and possibly hormonal disruptions at the placental level.

The translational relevance of these findings must be approached with caution, as rodent models differ from humans in placental structure, hormonal profiles, and metabolism of phytoestrogens. However, the data raise important questions about the unregulated use of soy supplements or high-isoflavone diets during pregnancy.

Given the growing advocacy for plant-based nutrition and soy-derived products as healthy alternatives to animal proteins, it is essential to understand the full spectrum of their biological actions during pregnancy. While moderate dietary intake may be harmless or even beneficial, concentrated extracts or supplements could pose risks during critical windows of fetal development.

Conclusion: This study demonstrates that high-dose exposure to *Glycine max* extract during pregnancy can impair placental morphology and fetal development in Wistar rats, as evidenced by histological alterations and reduced fetal growth parameters. While oxidative stress markers remained unaffected, structural disruptions in placental tissue, particularly at the higher dose, suggest potential fetal distress and compromised placental function. These findings underscore the need for further investigation into the reproductive safety of soy consumption during pregnancy, particularly regarding its dose-dependent effects on placental integrity and fetal development.

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