Teratogenic Potential of *Solenstemma Argel* Extract in Wistar Albino Rats

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Abstract

Background:

The majority of people in Africa receive their basic healthcare through herbal treatments. Herbal medicine may negatively impact fetal development irreparably.

Methods:

This study examined teratogenic potential of ***Solenstemma argel*** **extract** in pregnant rats. pregnant rats were treated with ***Solenstemma argel*** from 7th to 16th day of gestation. Dosage used was 500g/kg, intraperitoneal.

Results:

Solenstemma argel extract treated group showed fetal abnormalities appeared as body clots, limbs abnormalities and Resorption of fetuses that appears in 25% of the fetuses (P-value = 0.01) which is significantly differ from control group. furthermore, Histopathological findings of liver sections from fetuses of *Solenstemma argel* - treated mothers showed loose liver texture and hepatocyte hemorrhage.

Conclusions:

In this study we explore the teratogenic potential of *S. argel* extract during the organogenesis period in pregnant rats.

Background

In Africa, nearly 80% of the population relies largely on herbal medicine to provide basic healthcare (1). Insufficient quality control and safety are the most common problems with using herbal medicine (2). Consequently, herbal remedies need to be standardized to ensure their safety and effectiveness(3).

Solenstemma argel is found in tropical Africa in the desert area of Mali, Niger, Chad and Sudan. It is also widely distributed in Algeria, Libya, Egypt and Saudi Arabia.

In Sudan, *Solenstemma argel* is cultivated under irrigation for the production of leaves. In northern and central Sudan, the flowering aerial parts are sold in the local markets for medicinal use.

Major chemical constituents of Solenstemma argel:

Murwan and Murwa reported that *S. argel* contains various percentages of minerals, carbohydrates and proteins(4), together with a number of organic compounds including flavonoids, kaempferaol, quercetin, rutin, flavonols, flavanones, chalcones and alkaloids (5).

Phytochemical studies of the leaves, stems and flowers showed the existence of -amyrin and -amyrin, -sitosterol, 7- methoxy-3 -22 -dihydroxy-stigmastene, ethoxy derivative of vangurolic acid, an unidentified sterol.

the presence of flavonoids and saponins in different organs, alkaloids and/or nitrogenous bases in the leaves, stems and flowers have been reported (6). Also, proteins, sugars, fiber, and vitamins are present with minerals Na^{+}, K^{+}, Ca^{++}, Ni^{+3}, Mg^{+2} and P^{+3} (7).

Traditional uses of *Solenstemma argel*

Solenstemma argel was used as antispasmodic (8), anti-inflammatory (8,9) and anti- oxidant(10).

The plant was used for the treatment of diabetes mellitus(11), and cancer (12,13).

Infusion of leaves of the plant was used for the treatment of gastrointestinal cramps, jaundice and urinary tract infections (10). It is also used as anti-colic and anti-syphilitic when used for prolonged period of 40 to 80 days (14,15).

Leaves of the plant possess purgative properties (14) and in the crushed form used to treat suppurating wounds(10).

The smoke of the plant is also considered useful for nasal congestion of common cold(14).

1. *argel* was reported to reduce aluminum toxicity(16).

Pregnane glycosides isolated from this plant were reported to reduce cell proliferation(17).

The ethanolic extract of *S.argel* plant demonstrated presence of antibiotic substances. It was reported to have antibacterial and antioxidant activity (10).

Toxicity of Solenstemma argel

Toxicological study conducted by Shyoub *et al* to evaluate the acute toxicity of *S. argel* and to determine the lethal doses of *S. argel* in albino Canadian rats and local species of rabbits using Intra-peritoneal doses. The results showed that the mean lethal dose was 6.35g/kg for the rabbits and 5.49g/kg for the rats. Another toxicological parameter determined was the median lethal dose (LD50) was 5.0g/kg in albino rats (6).

Histopathological findings of some of the vital organs of Nubian goats treated with 5g/kg dose of *S. argel* syrup once daily for 45 days showed some tissue abnormalities: congested heart; hyperemia of the intestinal tissues revealing catarrhal inflammation with lymphocyte infiltration; liver tissue necrosis of centrilobular hepatocytes, fatty cytoplasm vaculation and slight congestion of the sinusoids; kidney tissue necrosis of the renal tubules, pyknosis, karyolysis of tubular epithelial cells, and interstitial mononuclear cells infiltration.(6)

To the best of our knowledge, no studies have been conducted on the teratogenic potential of *Solenstemma argel* extract and among its wide range of traditional uses as herbal medicine, it has a high probability of being used by pregnant women. Therefore, our study aimed to investigate adverse effect on fetuses.

Methods

Experimental Animals:

Healthy Female Wistar rats weighing 150–200g were used. The rats were housed in groups, under controlled conditions of temperature (22 ± 1 °C) and relative humidity (~ 50 %) as well as 12-hour light / dark cycle. Animals were allowed food (standard laboratory rodent's chow) and water *ad libitum*.

Plant material

Solenstemma argel was brought from Omdurman market, the plant then taxonomically authenticated at the herbarium of medical and aromatic plant research institute (MAPRI).

Preparation of the Solenstemma argel extract

Solenstemma argel plant sample was grounded using mortar and pestle and successively extracted by soaking 96 % ethanol for about seventy-two hours with daily filtration and evaporation. Solvents were evaporated under reduced pressure to dryness using rotary evaporator apparatus and the extracts were combined together. Extracts allowed to air till complete dryness(18).

Phytochemical screening tests:

Test for unsaturated Sterols and Triterpenes

Ten milliliters of *Solenstemma argel* extract were evaporated to dryness on a water bath and the cooled residue was stirred several times with petroleum ether to remove most of the coloring materials. The residue was then extracted with 20ml chloroform. The chloroform solution was dehydrated over Sodium Sulphate anhydrous. Portion of 5 ml of the chloroform solution was mixed with 0.5 ml of Acetic Anhydride followed by two drops of concentrated Sulphuric acid. The gradual appearance of green, blue pink to purple color was taken as an evidence of

the presence of sterols (green to blue) and or triterpenes (pink to purple) in the sample.

Test for Alkaloids

About 7.5ml of the *Solenstemma argel* extract were evaporated to dryness on a water bath. 5 ml of 2N HCl were added and stirred while heating on the water bath for 10 minutes, cooled, filtered and divided into two test tubes.

Few drops of Mayer's reagent were added to one test tube while to the other tube few drops of Valser's reagent were added. A slight turbidity or heavy precipitate in either of the two test tubes was taken as presumptive evidence for the presence of alkaloids.

Tests for Flavonoids

A volume of 17.5 ml of the *Solenstemma argel* extract was evaporated to dryness on water bath, cooled and the residue was defatted by several extractions with petroleum ether. The defatted residue was dissolved in 30 ml of 80% ethanol and filtered. The filtrate was then used for following tests:

To 3 ml of the filtrate, 1 ml of 1% Aluminum Chloride solution was in methanol was added. Formation of a yellow color indicated the presence of flavonoids, flavones or and chalcone.

To 3 ml of the filtrate, 1ml of 1% Potassium Hydroxide solution was added. A dark yellow color indicated the presence of flavonoids compounds (flavones or flavonenes), chalcone and or flavonols.

Tests for Tannins

Seven milliliters the *Solenstemma argel* extract were evaporated to dryness on water bath. The residue was extracted several times with N-hexane and filtered. The insoluble residue was stirred with 10 ml of hot saline solution. The mixture was cooled, filtered and the volume of the filtrate was adjusted to 10 ml with more saline solution. The solution was then used for following tests:

To 5 ml of the solution, few drops of gelatin salt reagent were added. Immediate formation of a precipitate was taken as an evidence for the presence of tannins in the plant sample.

To 5 ml of the solution, few drops of Ferric Chloride test reagent were added. The formation of blue, black or green was taken as an evidence for the presence of tannins.

Test for Saponins

One gram of the original dried powder *Solenstemma argel* leaves was placed in a clean test tube. 10 ml of distilled water were added and the tube was stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of honeycomb. The appearance of honeycomb, which persisted at least for an hour, was taken as an evidence for the presence of Saponins.

Test for Anthraquinone glycosides

Ten grams of the powdered *Solenstemma argel* leaves were boiled with 10 ml of 0.5N KOH and 1 ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene. 5 ml of the solution were shaken with 3ml of 10% Ammonium Hydroxide solution and the two layers were allowed to separate. The presence of Anthraquinone was indicated if the alkaline layer was found to have assumed pink or red color.

Test for Cumarins

Three grams of the original powdered plant sample were boiled with 20 ml distilled water in test tube. A filter paper spotted with 0.5 N KOH was attached to the test tube. The filter paper was allowed to be saturated with vapor and then inspected under UV light. Absorption of UV light indicate the presence of cumarins.

Induction of pregnancy

Adult female Wistar rats weighing 150–200g were allowed to mate with proven male rats in the ratio of 2 females to 1 male/cage. Vaginal smear for sperm detection was taken as evidence for copulation and that day was designated as day 1 of pregnancy. We adopted that method which described by Al-Harbi *et al* and (19) and ElTahir *et al* (20), with slight modifications.

Tests to investigate fetal abnormalities

Fetuses were removed on 21st day of pregnancy by caesarian section under light diethyl ether anesthesia. Litter size, alive and dead fetus number of resorption were recorded. Fetal and placental weights were also recorded.

The fetuses were examined for external gross malformation and histopathological examination of the liver.

Histopathological method

Slices of the liver were immediately fixed in 10% neutral buffered formalin, and then embedded in paraffin wax, sectioned at 5µm and stained routinely with hematoxylin and eosin (H&E).

Experimental design and treatment protocol:

The selected pregnant rats were divided into two groups of 7 animals each. The first group received normal saline and served as control. Rats in second group received *Solenstemma argel* extract intraperitoneal from day 7 to day 16 of pregnancy [period of organogenesis in rats(20)].

Statistical work:

The results were analyzed using IBM SPSS Statistics version 24 and box plots using R-Statistics. Results summaries were presented as mean ± Standard Error of the Mean (S.E.M.). Differed inferential statistical tests (t-test, one-way ANOVA, Chi square test, etc.) were performed. Difference between groups was considered statistically significant at P-value < 0.05.

Results

Table 1 phytochemical screening of *Solenstemma argel* extract

Phytochemical component	Result
Alkaloids	+
Anthraquenone glycosides	-
Cumarinns	+
Flavonoids	+++
Saponins	++
Taninns	++
Triterpens	+
Steroids	++

(-) = Negative (+) = Trace (++) = Moderate (+++) = High

Table 2 Effect of *Solenstemma argel* extract on maternal weight between first & last day of gestation:

	Mean of Maternal weight in grams			
	Control group		*Solenstemma argel* group	
	Day 1	Day 21	Day 1	Day 21
Mean	119.71	157.36	164.71	200.07
SEM	5.99	10.24	3.37	8.96
P-value	0.010*		0.007**	

*The mean difference is significance at P value< 0.05 level.

Discussion

Herbal medicines are widely used as traditional remedies that have been consumed more frequently in recent decades(21). Herbal medicines can have negative impacts on the health of the pregnancy that may extent to teratogenicity in a dose related manner during specific gestational period (22). Due to these concerns, researchers have been more cautious when studying the effect of herbal extracts during pregnancy(22, 23).

In the present study the we investigate the teratogenic potential of *S. argel* extract in pregnant rats and the Phytochemical screening results showed high content of flavonoids and moderate contents of

Saponins. The results are consistent with the findings of (5, 6). High contents of tannins and steroids were detected with traces of triterpenes and cumarins, where Anthraquinone glycosides are completely absent.

Regarding maternal weight during pregnancy, in control group and S. *argel* treated group, a significant increase occurred (P-value = 0.010* and 0.007*) respectively, from day1 to day 21of gestation as shown on Table (2) and Figure (1). This increase was much more in S. *argel* treated group compared to the control group according to the p-value.

Figure (2) showed that *S. argel* extract did not exert significant change in pregnancy index. Similarly, there was no significant effect of S. *argel* extract on maternal litter size (Fig. 3). These results are in harmony with the findings by Weidner *et al.* (24)who investigated the teratogenic potential of *Zingiber officinale* extract in rats that share the phytochemical components of Saponins and flavonoids with *S.argel* (25).

In *S. argel* treated group, 25% of fetuses were abnormal. Figures (4), *S. argel* extract in the dose of 500g/kg. (I.P) revealed a significant teratogenic potential in treated pregnant rats in the form of upper or lower limb abnormalities or absent limbs, and body clots and subcutaneous bleeding. Image (1). Resorbtion of fetuses was seen in the treated group as seen in image (2).

A study on *Melia azedarach L* Leaves extract on mice that share most of the active components of our studied plant such as alkaloid, flavonoid, Saponins, and steroid have identical traits in teratogens(26).

There was no significant difference in fetal and placental weights between control and *S. argel* group Figure (5) and (6).

Histopathological examination of the fetal liver sections in contrast to control where liver texture appeared dense (compact) with high erythropoietic activity and numerous megakaryocytes, whereas liver sections from fetuses belongs to *S. argel* treated mothers showed loose liver texture, hepatocyte vaculation, nuclear vesiculations, hepatocyte hemorrhage and erythropoietic activity observed but megakaryocytes were not easily recognized. Also, the liver texture appeared loose with dilated sinusoids and central veins were obvious as shown on images 5, 6, 7 and 8. This histopathological results agree with findings of the study by Shyoub *et al*. (2013)(6) for evaluation of acute toxicity of *S.argel* on Nubian goats.

In our study, we used a crude extract, we cannot pinpoint components responsible for the observed effect. Hence, it is necessary to conduct further researches to test which of the phytochemical components of the plant is teratogenic.

Conclusion

In conclusion, the use of *S. argel* extract during the organogenesis period of pregnancy in rats will induce catastrophic teratogenic effects, and liver histopathological abnormalities.

Abbreviations

S. argel
Solenstemma argel
H&E
hematoxylin and eosin.
ERB
Ethical Review Board

Declarations

Authors' contributions

Nazik mohamned Elamin Suliman Mustafa carried out the experimental part and wrote the manuscript editing tabeles and figures.

Tarig Mohamed Hashim Elhadeyah conception design of the experiment revised the maniscript.

Nafisa Abo Obieda Osman carried out the statistical analysis of data.

Ahmed Abdel Rahim Gameel carried out the histopathology part of the experiment.

Shahenaz Satti wrote and revised the final manuscript.

All authors read and approved the final manuscript

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Availability of data and materials

All data analyzed in this study is available from the corresponding author on

reasonable request

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Ethics approval and consent to participate

All methods were performed in accordance with International ethical guidelines and regulations required for animal studies. The study was approved by the Ethical Review Board (ERB)- Al-Neelain University-

Sudan.

Consent for publication

All authors declare no conflict of interest.

Competing interests

The authors declare that they have no competing interests.

References

1. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z. Medicinal plants in therapy. Bull World Health Organ. 1985;63(6):965–81.
2. James PB, Wardle J, Steel A, Adams J. Traditional, complementary and alternative medicine use in Sub-Saharan Africa: A systematic review. BMJ Glob Heal. 2018;3(5).
3. Sukandar EY, Safitri D. Evaluation of teratogenic effect of tempuyung (Sonchus arvensis) extract on wistar rats. Int J Pharmacogn Phytochem Res. 2016;8(5):761–6.
4. El-Kheir MKS, Murwa AM. Chemical composition, minerals, protein fractionation, and anti-nutrition factors in leaf of hargel plant (Solenostemma Argel). Eur J Sci Res. 2010;43(3):430–4.
5. Kamel MS. Acylated phenolic glycosides from Solenostemma argel. Phytochemistry. 2003;62(8):1247–50.
6. Shyoub Elhassan Mohammed; Haj Elamin. E. Azhari; Dr. Makawy. Abd. A; Dr. M. R. Rasha;Dr.Abd. A. Mona. Determination of acute Toxicity of Solenstemma argel and it is effect on the vital organs and hematological parameters. J Pharm Sci. 2013;3(2):11–7.
7. Ridgway K, Rupp R. The mixing of powder layers on a chute: The effect of particle size and shape. Powder Technol. 1971;4(4):195–202.
8. Innocenti G, Dall'Acqua S, Minesso P, Budriesi R, Micucci M, Chiarini A. Evaluation of muscarinic M3-receptor antagonism of Solenostemma argel leaves. Planta Med. 2010;76(12):634.
9. Innocenti G, Dall'Acqua S, Sosa S, Altinier G, Della Loggia R. Topical anti-inflammatory activity of Solenostemma argel leaves. J Ethnopharmacol. 2005;102(2):307–10.
10. Shafek R, Shafik N, Michael H. Argel Anti Bactrial Activity.Pdf [Internet]. Vol. 11, Asian journal of plant sciences. 2012. p. 143–7. Available from: http://docsdrive.com/pdfs/ansinet/ajps/2012/143-147.pdf
11. Trojan-Rodrigues M, Alves TLS, Soares GLG, Ritter MR. Plants used as antidiabetics in popular medicine in Rio Grande do Sul, southern Brazil. J Ethnopharmacol [Internet]. 2012;139(1):155–63. Available from: http://dx.doi.org/10.1016/j.jep.2011.10.034
12. Nassr-Allah AA, Aboul-Enein AM, Aboul-Enein KM, Lightfoot DA, Cocchetto A, El-Shemy HA. Anti-cancer and anti-oxidant activity of some Egyptian medicinal plants. J Med Plants Res. 2009;3(10):799–808.

13. N. Hanafi and S.Z. Mansour. Antitumer efficacy of salennostemma Argel and/or -Irradiation Againist Ehrich Carcinoma. J Biol. 2010;10(6):468–79.
14. EL-KAMALI HH, KHALID SA. The most common herbal remedies in Central Sudan. Fitoterapia. 1996;67(4):301–6.
15. Floch L. Medicinal plants for North Africa. Vol. 31, Journal d'agriculture traditionnelle et de botanique appliquée. 1984. 127–127 p.
16. Osman HM, Shayoub M. the Effect of Solenostemma Argel Leaves Extract on Status of the Effect of Solenostemma Argel Leaves Extract on Status of Induced Lipid. 2015;5(March):3828–30.
17. Plaza A, Perrone A, Balestrieri ML, Felice F, Balestrieri C, Hamed AI, et al. New unusual pregnane glycosides with antiproliferative activity from Solenostemma argel. Steroids. 2005;70(9):594–603.
18. Sukhdev Swami Handa, Suman Preet Singh Khanuja, Gennaro Longo DDR. Extraction technologies for medicinal and aromatic plants (United Nations Industrial Development Organisation and the International Centre for Science and High Technology). Vol. 99, International Centre for Science and High Technology-United Nations Industrial Development Organization, area Science Park Padriciano. 2008. 34012 p.
19. Al-Harbi MM, al-Shabanah OA, al-Gharably NM IM. The effect of maternal administration of enalapril on fetal development in the rat. Res Commun Chem Pathol Pharmacol. 1992;77(3):347–58.
20. El Tahir, K.E.H., Al Tahir, A. Y. and Ageel A. Pharmacological studies on Sesame and Nigella sativa fixed oils: effects on the sensitivities of the adrenoceptors, baroreceptors, platelets and uterus of the rat. Saudi Pharm J,. 1999;7:205–15.
21. Mehrabani G, Aminian S, Mehrabani G, Rabiee M. Dietetic Plans within the Multiple Sclerosis Community: A Review. Int J Nutr Sci. 2019;4(March):14–22.
22. De Santis M, Cesari E, Cavaliere A, Ligato MS, Nobili E, Visconti D, et al. Paternal exposure and counselling: Experience of a Teratology Information Service. Reprod Toxicol. 2008;26(1):42–6.
23. Parivar K, Mohsenikouchesfahani H, MOhammadzadehasl B. Surveying Acyclovir side effects on developing embryos of Balb/C mice strain. Med Sci J Islam Azad Univesity-Tehran Med Branch. 2006;16(4):231–5.
24. Weidner MS, Sigwart K. Investigation of the teratogenic potential of a Zingiber officinale extract in the rat. Reprod Toxicol. 2000;15(1):75–80.
25. Osabor V, Bassey F, Umoh U. Phytochemical Screening and Quantitative Evaluation of Nutritional Values of Zingiber officinale (Ginger). Am Chem Sci J. 2015;8(4):1–6.
26. Sutomo AE, Sitorus TD, Pribadi A. The Teratogenic Effect of The Mindi (Melia azedarach L) Leaves Ethanol Extract on Mice (Mus musculus) Fetus. Althea Med J. 2015;2(2):221–5.

Figures

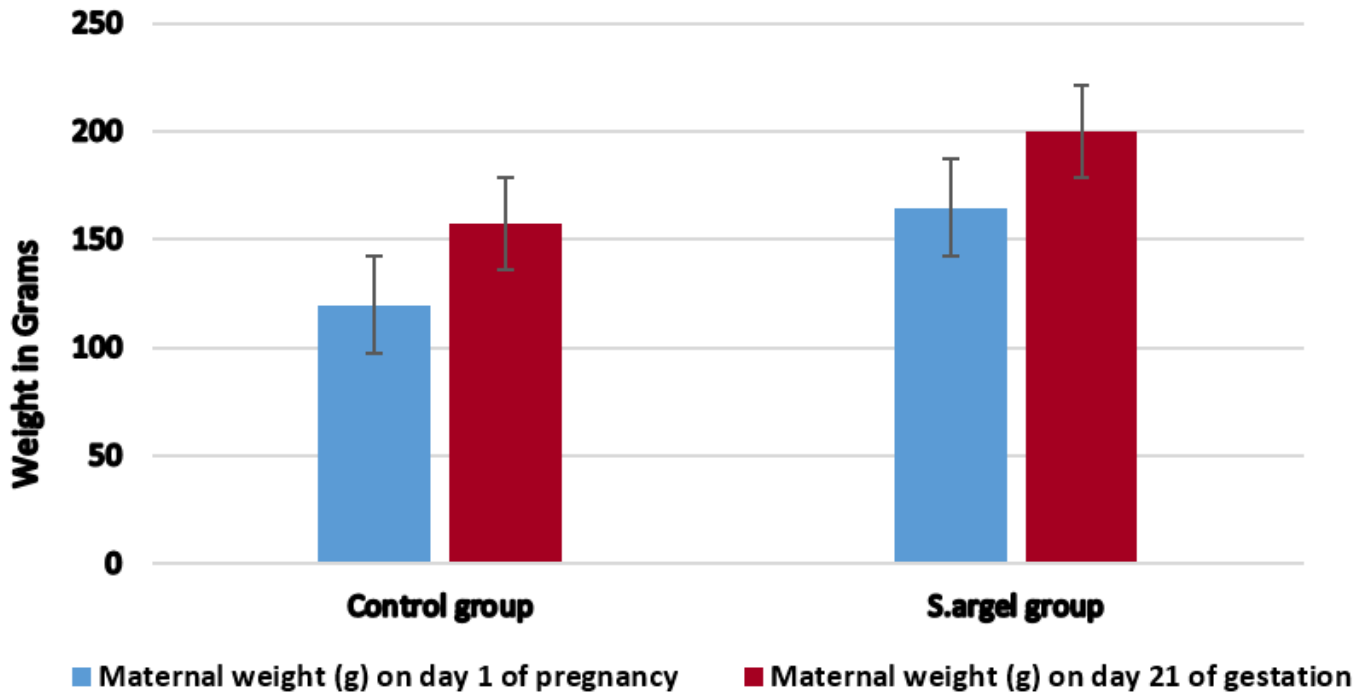

Figure 1

Effect of Solenstemma argel extract on maternal weight between day 1 & day 21 of gestation. Data was expressed as mean ± SEM. SEM = Standard error of the mean. * p = 0.010 between day 1& day 21 of gestation in control group. * *p = 0.007 between day 1& day 21 of gestation in S. argel treated group.

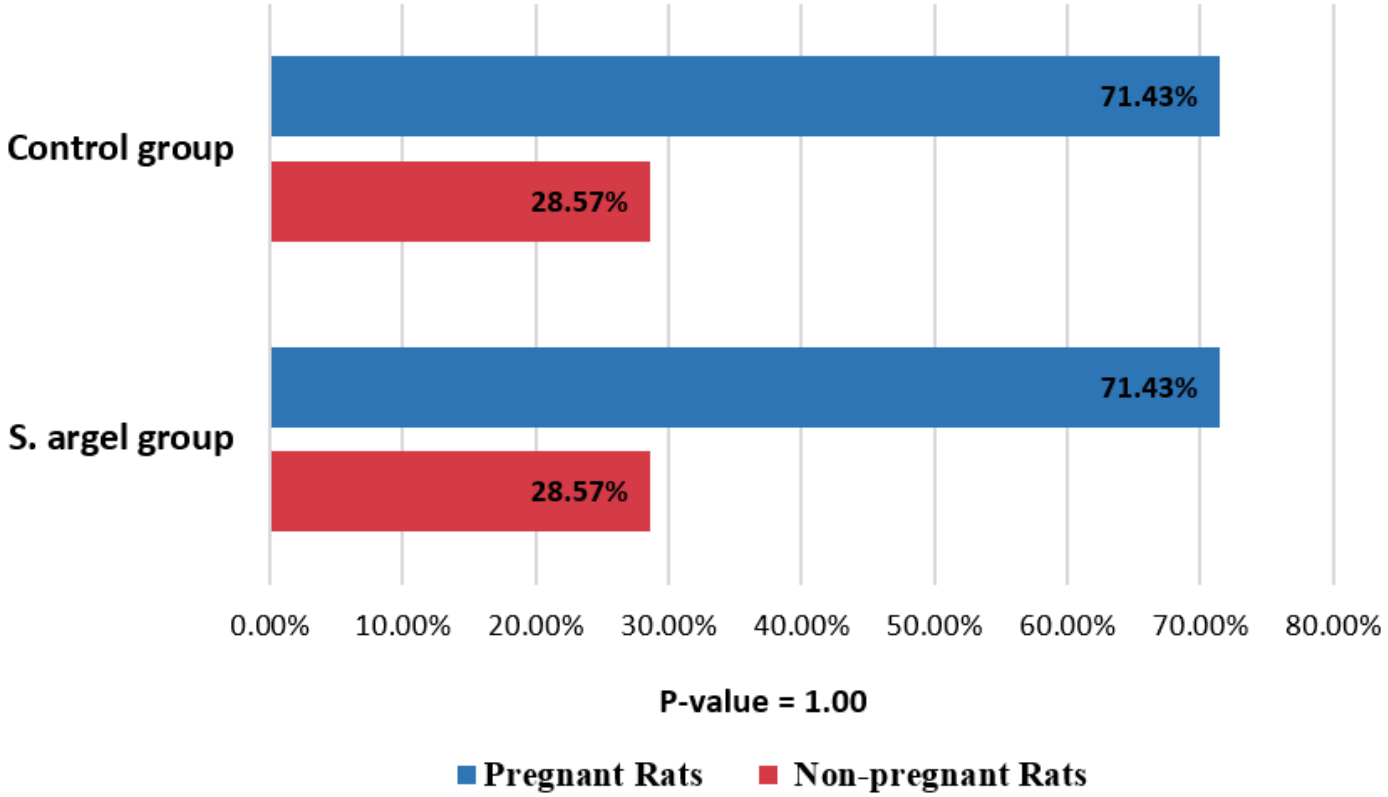

Figure 2

Effect of Solenstemma argel extract on pregnancy percentage. Data was expressed as percentages of pregnant to non-pregnant rats in control and S. argel treated group. P>0.05 no significance different in between the two groups P-value = 1.00.

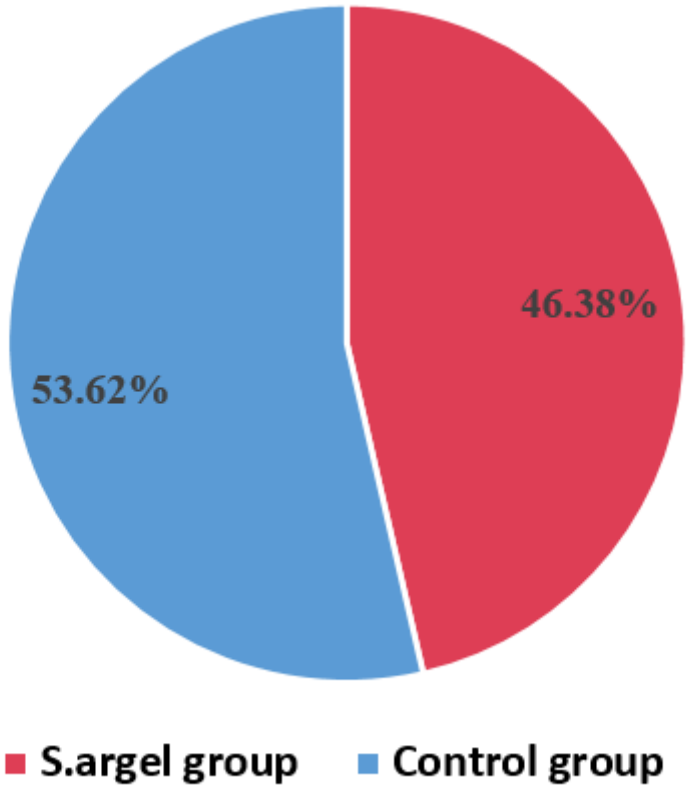

Figure 3

Effect of Solenstemma argel extract on litter size. Data was expressed as percentages of litter size in control and S. argel treated group. P>0.05 no significance difference between the two groups P-value = 0.833.

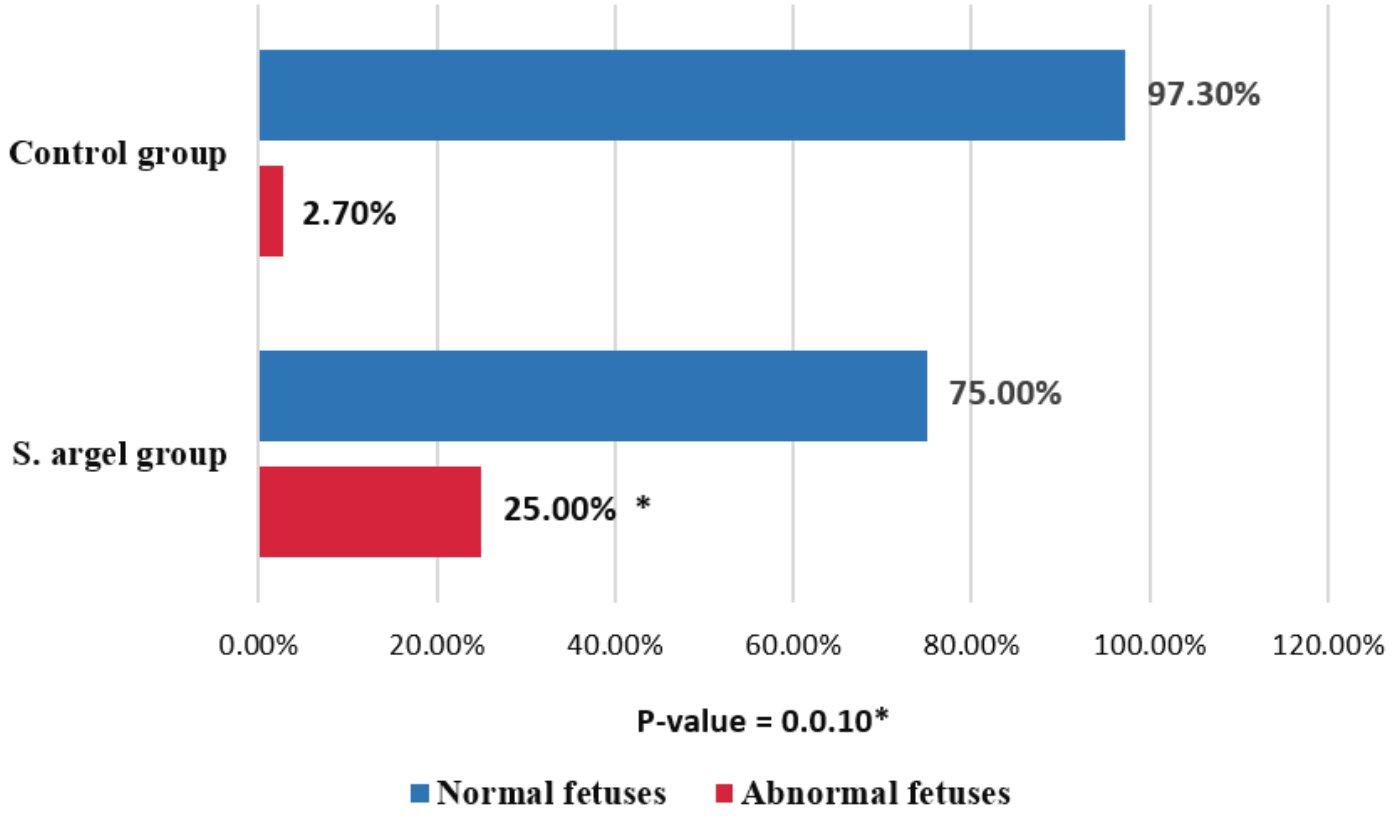

Figure 4

Effect of S. Solenstemma argel extract on fetal abnormalities. Data was expressed as percentages of normal to abnormal fetuses in control and S. argel treated group. 25% of fetuses in S.argel treated group were abnormal. * P-value= 0.010 shows significance different from the control group.

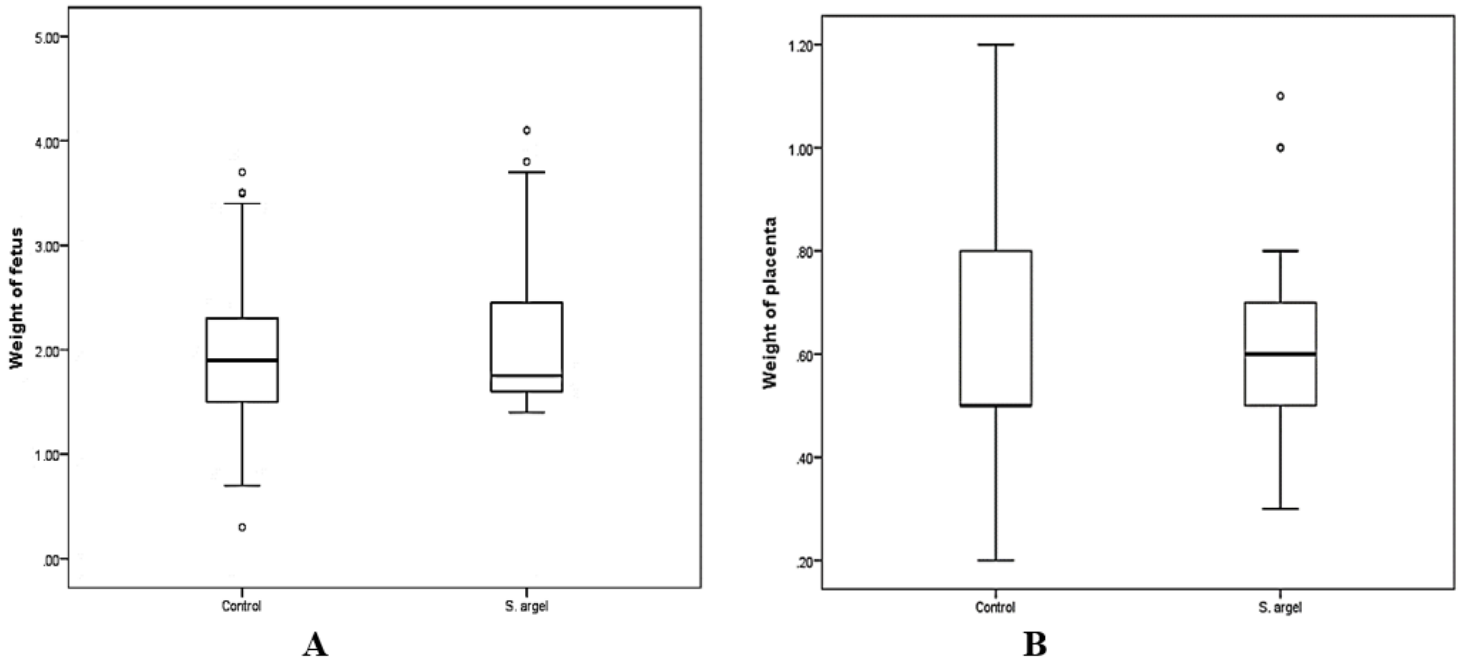

Figure 5

Effect of S. Solenstemma argel extract on fetal weight A and placental weight B. Data was expressed as mean ± SEM. SEM = Standard error of the mean. P>0.05 no significance difference in fetal weight and placental weight between the control and S. argel treated group. P-value = 0.722 & 0.917 respectively.

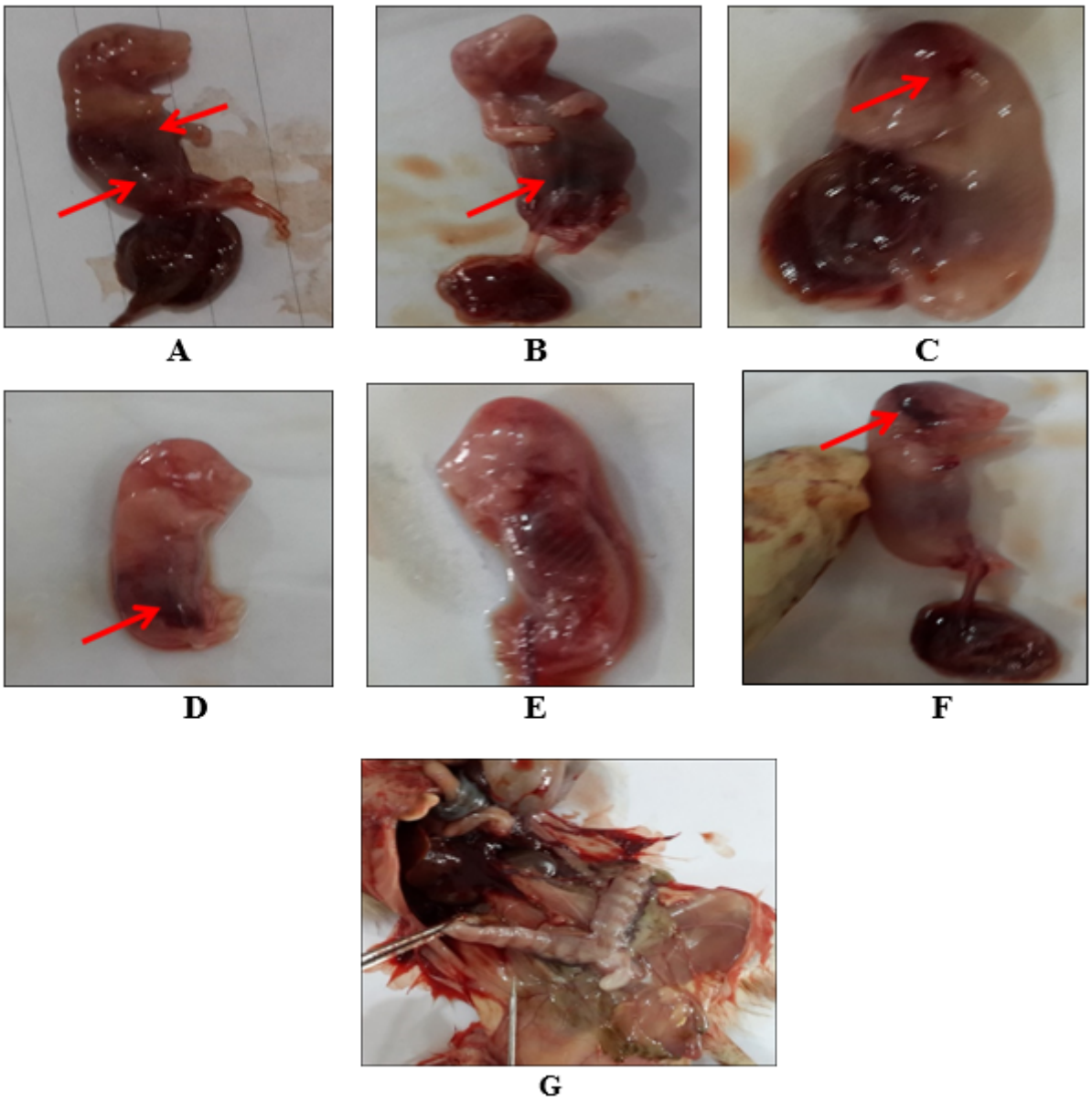

Figure 6

Different abnormalities of fetuses from rats treated with Solenstemma argel. Missed limb& body clot (A), Body clots (B), Clot on the head (C), Clots in the lower part (D), Missed limbs & abnormal body(E), Body& neck clots (F) and resorbtion of all fetuses in the uterus (G).

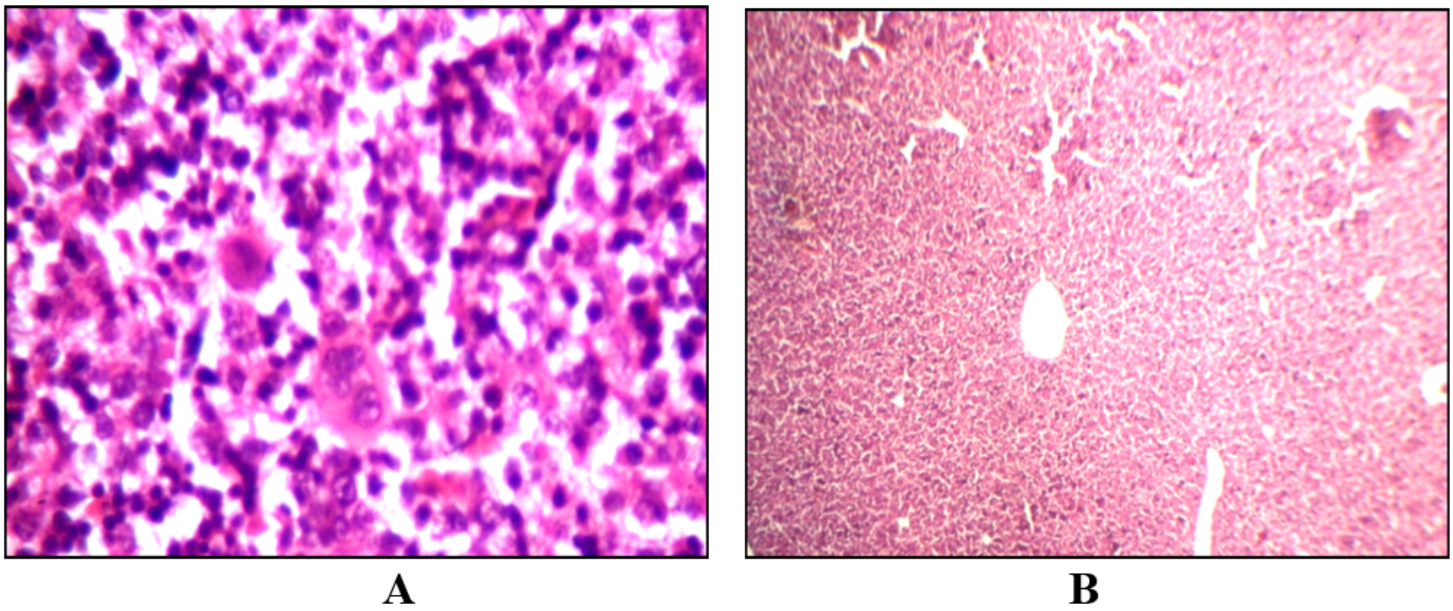

Figure 7

Histopathological investigation on fetuses liver (control group). Frequent Megakaryocytes (H&E × 400) (A), Compact liver texture (H&E × 100) (B). liver texture of control rats appeared dense (compact) with high erythropoietic activity. Megakaryocytes were frequently observed.

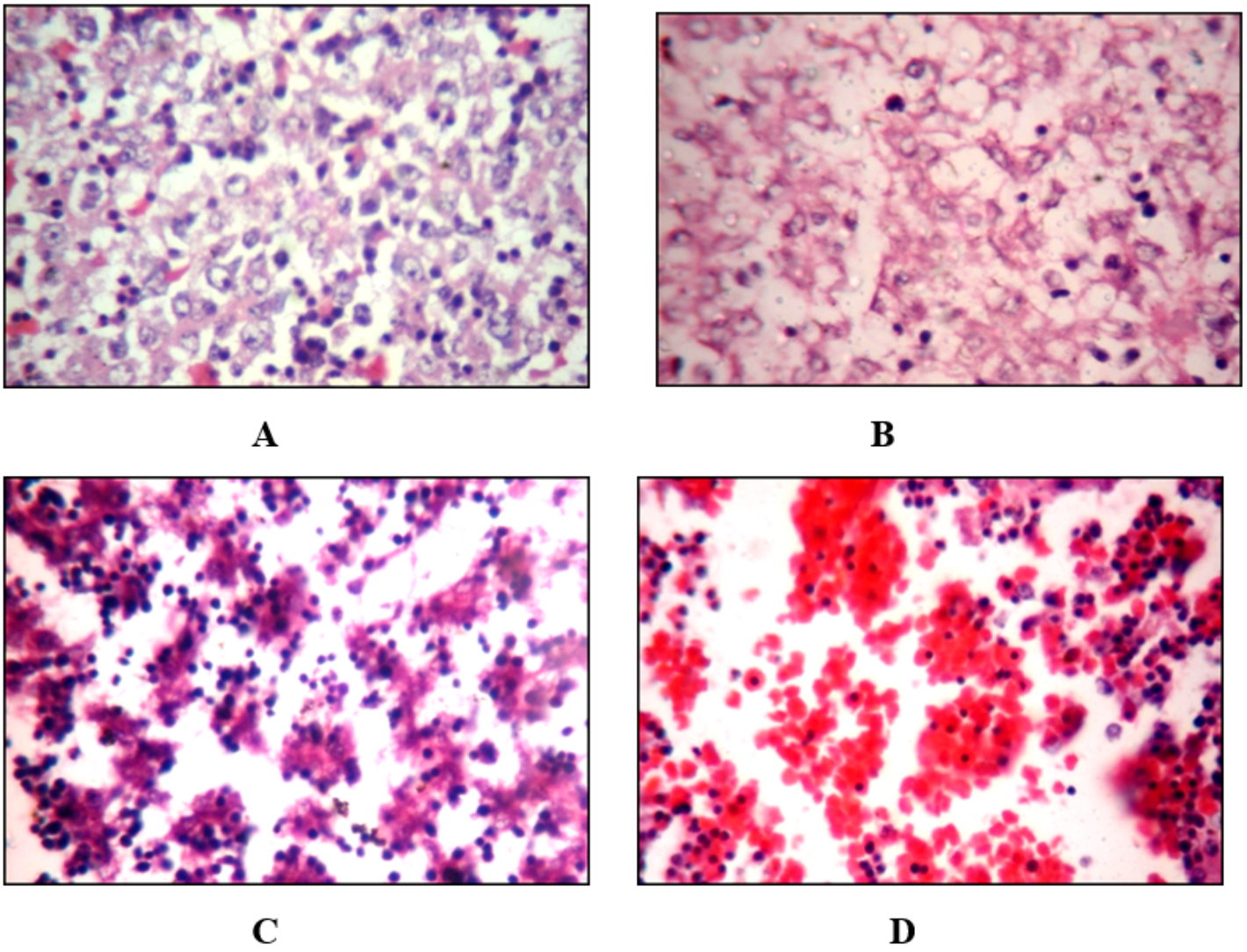

Figure 8

Histopathological investigation on fetuses fetus liver of Solenstemma argel extract treated mothers. cytoplasmic vesiculations (A), nuclear vaculation(B), Hepatocyte hemorrhage (C), loose liver texture with dilated sinusoids (D). All images H&E × 400. liver sections showed hepatocyte vaculation, nuclear vesiculations and hemorrhage. One case showed dissociated hepatic cells. Erythropoietic activity observed but megakaryocytes were not easily recognized. Liver texture (density) appeared loose with dilated sinusoids. Central veins were obvious.