

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

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## Research Article

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# Abstract

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

This study examined the teratogenic potential of *Solenstemma argel* extract in pregnant rats. Pregnant rats were treated with *Solenstemma argel* from 7th to 16th day of gestation. The dosage used was 250 mg/kg, intraperitoneal.

*Solenstemma argel* extract treated group showed fetal abnormalities appeared as body hemorrhage, limbs abnormalities and resorption of fetuses. These appears in 25% of the fetuses (P-value = 0.01) which is significantly differed from control group. Furthermore, histopathological findings of liver sections from fetuses of *Solenstemma argel*- treated mothers showed loose liver texture and hepatocytes hemorrhage.

In this study, we conclude that the use *Solenstemma argel* extract during the organogenesis period in pregnant rats has the potential to cause teratogenic effects, as well as abnormalities in liver histopathology.

## 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.

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, kaempferol, 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 vanguardic acid, an unidentified sterol, flavonoids and saponins in different organs, alkaloids and/or nitrogenous bases in the leaves, stems and flowers have been reported also (6).

Also, proteins, sugars, fiber, and vitamins are present with minerals  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Ni}^{+3}$ ,  $\text{Mg}^{+2}$  and  $\text{P}^{+3}$  (7).

*Solenstemma argel* has a wide range of traditional uses with deep cultural believes in developing countries that it is safer to use them even during pregnancy, as well as a lack of access to conventional medication and health care, which promotes their use even more.

*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). *S. 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).

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 5.0 g/kg in albino rats (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.

## Materials And Methods

### *Experimental Animals*

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

the animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals.

### *Plant material*

*Solenstemma argel* leaves were brought from Omdurman market, the plant then taxonomically authenticated at the herbarium of medicinal 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 20 ml 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 evidence of the presence of sterols (green to blue) and or triterpenes (pink to purple) in the sample.

#### *Test for alkaloids*

About 7.5 ml of the *Solenstemma argel* extract were evaporated to dryness on a water bath. 5 ml of 2N HCl was 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 in 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 a 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:

One ml of 1% aluminum chloride solution in methanol was added to 3 ml of the filtrate. Formation of a yellow color indicated the presence of flavonoids, flavones or and chalcone.

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

#### *Tests for Tannins*

Seven milliliters of 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:

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

few drops of ferric chloride test reagent were added to 5 ml of the solution, The formation of blue, black or green was taken as 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 was 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 evidence for the presence of Saponins.

#### *Test for anthraquinone glycosides*

Ten grams of the powdered *Solenstemma argel* leaves were boiled with 10 ml of 0.5 N 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 was shaken with 3 mL 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 coumarins*

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 coumarins.

#### *Induction of pregnancy*

Adult females Wistar rats weighing 150–200 g was allowed to mate with proven male rats in the ratio of 2 females (in estrus cycle) 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.

#### *Experimental design and treatment protocol:*

This study was carried out in accordance with ARRIVE guidelines for the reporting of animal experiments. and approved by the Ethical Research Board (ERB), Al- Neelain University.

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 6 to day 15 of pregnancy [ period of organogenesis in rats(20)].

Fetuses were removed on 21<sup>st</sup> day of pregnancy by caesarian section under light diethyl ether anesthesia. Litter size, alive and dead fetus number of resorptions were recorded. Fetal and placental weights were also recorded. The fetuses were examined for external gross malformation , and histopathological examination of the liver.

#### *Histopathological examination*

Liver samples removed from the fetuses by dissection and 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) (21).

#### *Statistical work:*

The results were analyzed using IBM SPSS Statistics version 24. Results summaries were presented as mean ± Standard Error of the Mean (S.E.M.). Differed statistical tests (t-test, and Chi square test,) were performed. Difference between groups was considered statistically significant at P-value < 0.05. Charts and graphs were drawn by graph pad prism version 9.2.0.

## **Results**

#### *Phytochemical screening*

A preliminary phytochemical analysis conducted on the *S. argel* extract revealed that the plant contains high content of flavonoids, tannins and steroids and moderate contents of Saponins with traces of triterpenes and coumarins, whereas Anthraquinone glycosides are completely absent (Table 1).

#### *Maternal weight:*

Maternal weight from day1 to day 21of gestation, in both control group and *S. argel* treated group, showed a significant increase (P-value = 0.010\* and 0.007\*) respectively, (Fig. 1).

#### *Pregnancy percentages and litter size:*

In both of control group and *S. argel* treated group 71.43% of the mated rats have life fetuses whereas 28.57% of the mated rats deprived of fetuses (no significance different in the pregnancy percentage between the control group and *S. argel* treated group. P-value =1.00), Fig. 2.

There was no significant effect of *S. argel* extract on maternal litter size percentages. P-value = 8.33 (Fig 3).

### *Fetal and placental weight:*

There was no significant difference in fetal and placental weights between control and *S. argel* group. P-value = 0.722 & 0.917 respectively (Fig. 4).

### *Fetal abnormalities:*

The percentage of abnormal to normal fetuses showed significant difference (P-value = 0.010) between the control group fetuses and *S. argel*/treated group fetuses, However, 25% of the fetuses from the group of *S. argel*/treated mothers for were abnormal. (Fig. 5).

Abnormalities of fetuses appeared in the form of upper or lower limb abnormalities or absent limbs, and body hemorrhage and subcutaneous bleeding. Resorbtion of fetuses was reported also. As presented on Fig. 6 (A,B, C, D, E, F and G),

### *Histopathological findings*

Histopathological examination of the fetal liver sections in the control group showed dense liver texture (compact) with high erythropoietic activity and numerous megakaryocytes. Whereas liver sections from fetuses belong to *S.argel*/treated mothers showed loose liver texture, hepatocyte vaculation, nuclear vesiculations, hepatocyte hemorrhage and erythropoietic activity observed (Fig 7. A, B C, D, E, and F).

**Table 1** phytochemical screening of *Solenstemma argel* extract

<b>Phytochemical component</b>	<b>Result</b>
Alkaloids	+
Anthraquinone glycosides	-
Cumarins	+
Flavonoids	+++
Saponins	++
Tannins	++
Triterpenes	+
Steroids	++

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

## Discussion

Herbal medicines are widely used as traditional remedies that have been consumed more frequently in recent decades (22). 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 (23). Due to these concerns, researchers have been more cautious when studying the effect of herbal extracts during pregnancy (23,24).

This study aimed to explore the teratogenic potential of the crude extract of *S. argel* in pregnant rats. Extract phytochemical screening revealed high flavonoid content and moderate saponin content. The results are consistent with the findings of Kamel Ms. and Shyoub *et al* (5,6). High contents of tannins and steroids, traces of triterpenes and cumarins were detected, where Anthraquinone glycosides are completely absent.

Studies on animal models often cannot distinguish between direct and indirect effects of substances on embryos. Developmental toxicity is frequently related to maternal toxicity in many cases(25).

In our study, administration of *S. argel* extract in dose of 250 mg/kg, intraperitoneally, did not exert any signs of maternal toxicity or maternal dietary restriction. This evident by the significant increase in maternal weight of the *S. argel* treated group during the gestation period from day 1 to day 21. Comparing the increase in maternal weight to the control group, this can be a predictor of normal pregnancy progression and fetal growth. Several studies have linked maternal dietary restriction to adverse newborn outcomes in pregnant animals. It has been reported that a 10% decrease in normal food intake is associated with an increased risk of maternal toxicity, as well as developmental problems(26).

According to pregnancy percentage for mated rats and litter size percentages for the *S. argel* treated group compared to the control group, it could be seen that both groups had the same percentage of pregnant rats carrying live fetuses with no significance difference in litter size. Based on these results, pregnancy index and litter size were not affected by extract administration. These results are in harmony with the findings by Weidner *et al.* (27) who investigated the teratogenic potential of *Zingiber officinale* extract in rats that share the phytochemical components of Saponins and flavonoids with *S.argel* (28).

The fetal and placental weight did not indicate abnormal patterns or significant differences from controls, reflecting normal development of the offspring of the treated group; this corresponds with the follow up of maternal weight during the gestation period mentioned above.

The gestation period between days 6 through to 15 is the period of organogenesis. It is more common for congenital anomalies to occur during this period of exposure to teratogens than spontaneous abortion or resorption of the embryo(29).

Our study found that 25% of the fetuses from the *S. argel* treated group were abnormal, which is significantly different from the control group. The most common abnormalities were hemorrhage in fetal

bodies, external morphological abnormalities, and fetus resorption. 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 (30). Examination of fetal liver sections of *S.argel*/treated mothers in our study, showed loose liver texture, hepatocyte vaculation, nuclear vesiculations and hepatocyte hemorrhage, that is totally in line with histopathological results of the study by Shyoub *et al.* (6) for evaluation of acute toxicity of *S.argel* on Nubian goats.

In summary administration of *S. argel* extract to female pregnant rats results in fetal hemorrhage, limbs abnormalities (missed limbs), resorbtion and loose liver texture with hepatocyte hemorrhage.

## Conclusion

In conclusion, the use of *S. argel* extract during the organogenesis period of pregnancy in rats induced teratogenic effects, and liver histopathological abnormalities. We recommend, to conduct further studies to test which of the phytochemical components of the plant is responsible for the teratogenic effect.

## Abbreviations

S. argel: Solenstemma argel

H&E: hematoxylin and eosin.

ERB: Ethical Review Board

ARRIVE: Animal Research: Reporting of In Vivo Experiments

## Declarations

### Authors' contributions

Nazik Mohamed Elamin Suliman Mustafa carried out the experimental part and wrote the manuscript editing tables and figures.

Tarig Mohamed Hashim Elhadayah conception design of the experiment revised the manuscript.

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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### **Declarations**

### **Ethics approval and consent to participate**

The experimental procedures and methods were in accordance with the international guidelines of the care and use of laboratory animals in scientific investigations and Ethical approval for this study was obtained from the Ethical Review Board (ERB)- Al-Neelain University- Sudan.

All methods are reported in accordance with ARRIVE guidelines for the reporting of animal experiments.

### **Consent for publication**

All authors declare no conflict of interest.

### **Competing interests**

The authors declare that they have no competing interests

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## Figures

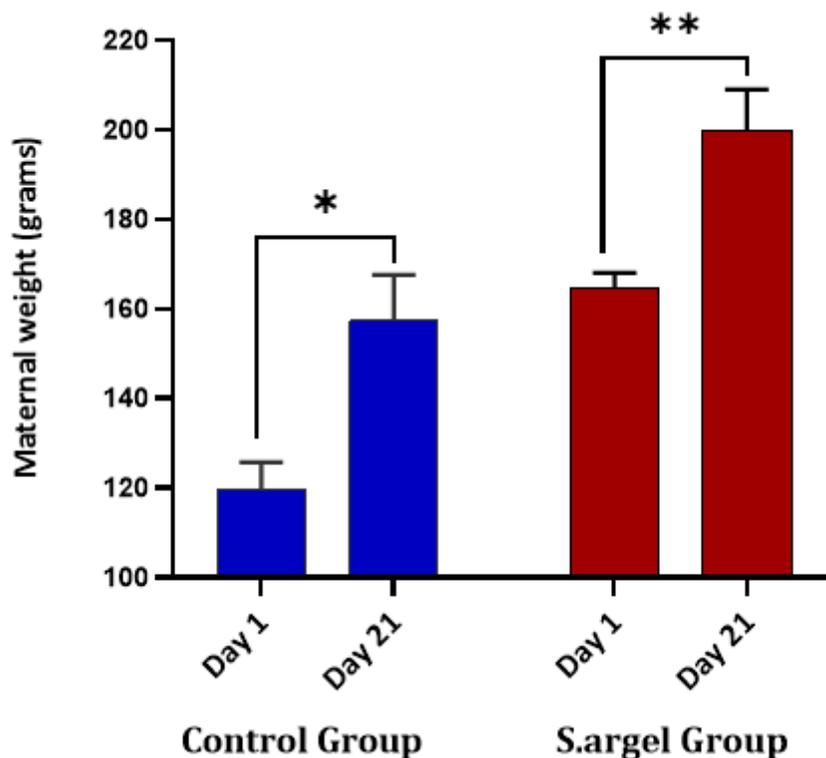
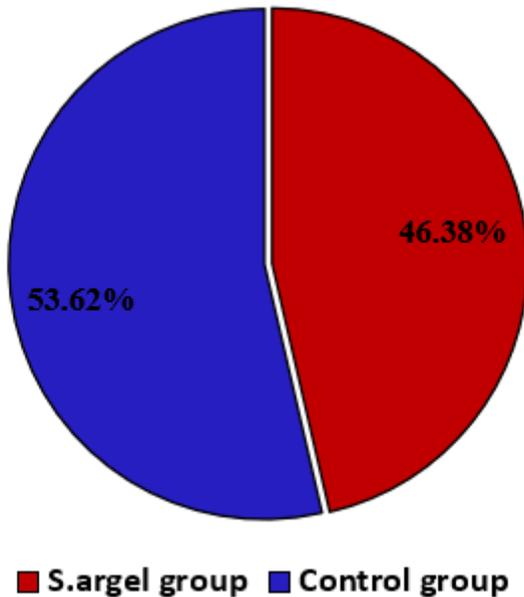


Figure 1

Effect of *Solenstemma argel* extract on maternal weight between day 1 & day 21 of gestation. Data was expressed as mean  $\pm$  SEM. SEM = Standard error of the mean. \*  $p = 0.010$  between day 1 & day 21 of gestation in control group (t-test). \*\*  $p = 0.007$  between day 1 & day 21 of gestation in *S. argel* treated group (t-test).

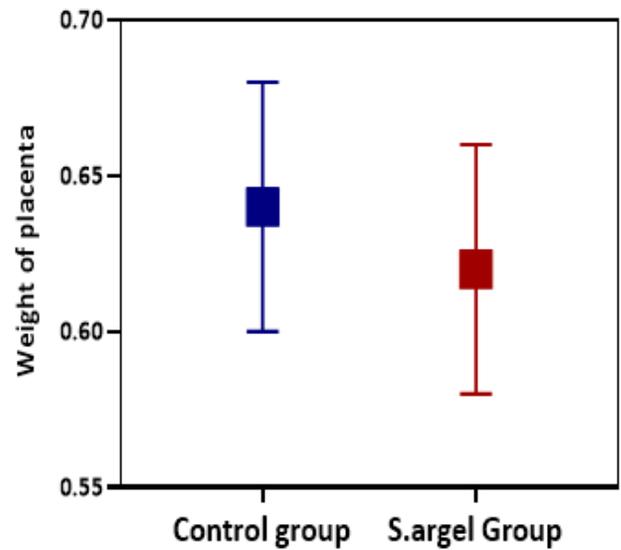
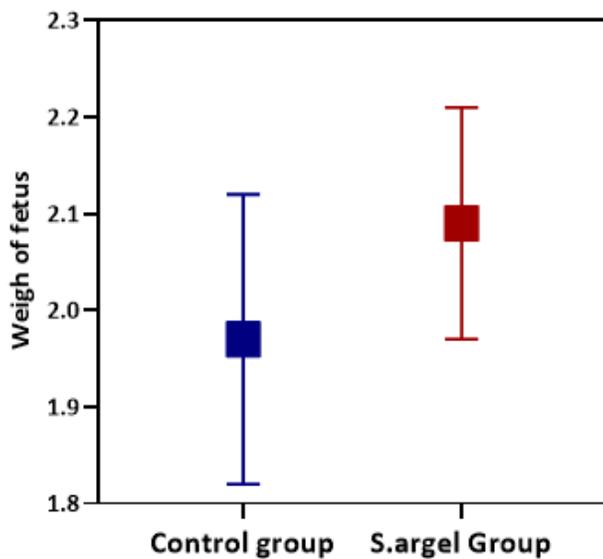
**Figure 2**

Effect of *Solenstemma argel* extract on pregnancy percentage. Data was expressed as percentages of rats with live fetuses to mated rats without fetuses in control and *S. argel* treated group.  $P > 0.05$  no significance difference in between the two groups  $P\text{-value} = 1.00$  (chi square test).



**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  $P\text{-value} = 0.833$  (chi square test).



**A**

**B**

**Figure 4**

Effect of *S. Solenstemma argel* extract on fetal weight A and placental weight B. Data were expressed as mean  $\pm$  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 (t-test).

**Figure 5**

Effect of *S. Solenstemma argel* extract on fetal abnormalities. Data were 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 significant difference from the control group (chi square test).

**Figure 6**

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

**Figure 7**

Histopathological investigation on fetus liver (control group A&B), fetus liver of *Solenstemma argel* extracts treated mothers (C, D, E&F). Frequent Megakaryocytes (H&E × 400) (A), Compact liver texture (H&E × 100) (B). Cytoplasmic vesiculations (C), Nuclear vaculation(D), Hepatocyte hemorrhage (E), Loose liver texture with dilated sinusoids (F). All images C, D, E, and F are (H&E × 400).