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Sub-acute Toxicological Effects of Jobelyn® on Pregnant Albino Rats

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Abstract. The aim of the present study was to investigate the sub-acute toxicological effects of Jobelyn® on pregnant albino rats by employing biochemical, haematological and histopathological methods. A total of 32 pregnant female rats were randomly assigned to four different groups of eight rats each. The control group received distilled water and different doses of Jobelyn®; 250, 500, 1000 mg kg^{-1}were administered orally once a day for 2 weeks to the other groups. Biochemical analysis revealed a significant decrease ($p<0.05$) in the levels of alanine aminotransferase, albumin, urea, PCV and Hb in the treatment groups when compared to the control. However, the significant decrease in PCV and Hb was observed solely in the group treated with 1000 mg kg^{-1}body weight, suggesting that this decrease could be dose-dependent. Alkaline phosphatase, total protein, triglycerides, cholesterol, HDL cholesterol, LDL cholesterol, eosinophils, basophils, neutrophils, monocytes, lymphocytes, WBC count, revealed no significant difference ($p<0.05$) when compared to the control. The results show that at an appropriate dosage, the use of Jobelyn® during pregnancy may have no adverse effect on the liver and kidney tissues and may possess hepatoprotective and nephroprotective properties however the histopathological studies revealed that very high levels of Jobelyn may be hepatotoxic.

INTRODUCTION

Jobelyn® is a multifunctional natural ingredient derived from the leaf sheaths of *Sorghum bicolor*, a member of the Poaceae family [1].All parts of the plant, *Sorghum (bicolor)* have been useful in disease treatment, making the plant a phytoceutical. The leaf sheath of *Sorghum bicolor* is commonly used as a remedy against anaemia by traditional medicine healers [2]. The root is used in treating malaria in Southern Rhodesia; the seed has been employed for the treatment of breast disease and diarrhoea while the stem has been used for tubercular swellings treatment [3]. Recently, focus has been on the leaf sheath of *S. bicolor* being used as herbal remedy for anaemia and having a boosting effect on blood concentration; possessing hematinic potentials [2, 4]. This powder extract from *Sorghum bicolor*(Jobelyn®), contains proteins, carbohydrates, dietary fibre as well as bioactive substances known as phytochemicals, which have the potential to significantly affect human health positively [5]. The high concentration of phytochemicals, including proanthocyanidins, anthocyanins, phenolic acids, apigenin, proapigeninidin, apigeninidin, luteolin, naringenins in this wholly, natural, herbal product confers on it, very high antioxidant activity with a total oxygen radical absorbance capacity (ORAC) score of 37,000 µmolTE/g of dry powder[6]. It is capable of inhibiting peroxyl free radicals, hydroxyl free radicals, singlet oxygen and possesses a high capacity for quenching superoxide anions, hence its usefulness in maintaining human health [7]. The inhibition of peroxyl free radicals per gram of jobelyn® is 3,549 µmoleTE/g as compared to 997, 68, 24, 15, 8 and 7 µmoleTE/g of acai berry, cherry tart, blueberry, strawberry, oranges, red grape, respectively [8].This indicates that the antioxidant capacity of Jobelyn® is about 4 times higher than that of acai berry and 50 times than that of tart cherries [8] thussignifying the antiradical properties of Jobelyn® [9]. This herbal formulation is believed to alleviate symptoms of anaemia of diverse origin, among which are malaria, sickle cell disease, leukaemia, stroke, arthritis, pregnancy, enteric fever, helminthiasis, multiple myeloma, and tuberculosis, its use in pregnancy being quite notable [1, 10]. Elevation of packed cell volume (PCV) from 21 percent to 32 per cent has been observed in pregnant women within eight days of ectopic pregnancy operation following the use of Jobelyn® [unpublished]. Despite the wide use of this herbal

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formulation, there is a paucity of studies on its toxicological profile. In relation to its effect on pregnant women, there has been no previous work done to ascertain if it poses health risks on the mother or not. The plethora of previously reported and unreported adverse drug reactions associated with the use of herbal medicines makes it imperative that pre-clinical toxicological studies be carried out on these natural products [11]. Furthermore, the recommendation of the herbal drug as a dietary supplement, establishes the need for toxicological studies on its effects on pregnant women. The primary aim of the present study was to investigate the sub-acute toxicological effect of Jobelyn® by employing the biochemical, haematological, and histological indices in pregnant albino Wistar rats.

MATERIALS AND METHODS

A total of thirty two healthy cyclic female Wistar albino rats weighing 82-190 g were used in this study. Animals were acclimatized for two weeks prior to commencement of treatment housed under standard environmental conditions and fed with a standard rat diet (obtained from Graceline feeds, Ota, Ogun state) with free access to water. During this period of acclimatization, the animals were exposed to a photoperiodicity of 12h light/12h darkness and were periodically assessed for gross morphological/behavioural changes.

Experimental Design

The animals were divided into 4 groups of 8 rats each and treated daily for 2 weeks; beginning from the 5th to 19th day of gestation during the period of foetal organogenesis. Group I served as the control group and received distilled water while groups II, III and IV received 250, 500 and 1000 mg $kg^{-1}$of Jobelyn®. All animals were weighed and sacrificed on the 20th day of pregnancy. Their uteri were dissected and blood samples were immediately collected. The liverand kidney was collected, trimmed of excess fat and weighed and a portion was separated for histological studies.

Haematological assays

Blood samples were withdrawn from the heart to measure the levels of haemoglobin (Hb), packed cell volume (PCV), white blood cell count (WBC), WBC differentials (neutrophils, monocytes, lymphocytes, basophils and eosinophil) [12]

Biochemical assays

The commercial test kits for liver function test were purchased from Randox Laboratory, United Kingdom. Standard procedures were used to evaluate the protein concentration [13], aspartate aminotransferase (AST) [14], alkaline phosphatase (ALP) [15], alanine aminotransferase (ALT)[16], albumin[17], direct bilirubin [18], urea[19] and creatinine[20].

Histopathological studies

The organtissues were fixed in normal saline for a period of 72 h and cut into thin slices 2.1 mm thick. The tissues were dehumidified using liquor. They were thereafter treated with paraffin wax and cast into blocks; tissue sectionswere then slit into 5µm using microtome and allowed to dry on a slide. The slides were afterwards soiled with haematoxylin-eosin stain, analyzed using a light microscope and photomicrographs recorded [21].

Statistical analysis

Differences between means of biochemical and haematological analysis of all the groups were estimated using a one-way analysis of variance followed by least significant difference test (post hoc) using the SPSS software (SPSS 15 for Windows). The $p<0.05$ were considered statistically significant. Graph pad prism was used in presentation of charts.

RESULTS

TABLE 1. Effect of Jobelyn® on Weight of Selected Organs of Pregnant Albino Rats

Parameters	Control (A)	250 mg kg^{-1} (B)	500 mg kg^{-1} (C)	1000 mg kg^{-1} (D)
LIVER	5.97±0.53	6.03±0.87	6.27±0.45	5.24±0.77
KIDNEY	0.47±0.03	0.47±0.04	0.47±0.02	0.46±0.02
HEART	0.45±0.03	0.47±0.04	0.49±0.02	0.49±0.04
LUNGS	1.13±0.09	1.13±0.13	1.29±0.06	1.15±1.15
BRAIN	1.41±0.06	1.16±0.18	1.32±0.04	1.35±0.05
SPLEEN	0.58±0.06	48.50±5.74	0.76±0.05	1.02±0.18

Values represent mean + SEM of 8 replicates. From the Table 1, there was no significant difference $p<0.05$ in the weight of the kidney, heart, lungs, brain, spleen of treatment groups administered with Jobelyn® when compared with the control group.

TABLE 2. Effect of Jobelyn® on in Liver Homogenate of Pregnant Albino Rats.

Parameters	Control (A)	250 mg kg^{-1} (B)	500 mg kg^{-1} (C)	1000 mg kg^{-1} (D)
ALT (U/I)	94.09±5.01	90.54±1.42	86.57±1.12	88.16±1.84
ALP (U/I)	104.420±23.26	237.65±127.25	77.54±14.93	140.66±66.50
AST (U/I)	118.21±4.10	103.25±17.63	117.02±15.01	136.59±2.57
TP (mg/dl)	2.08±0.41	2.20±0.43	1.79±0.35	1.55±0.28
ALB (mg/dl)	0.61±0.12	0.65±0.89	0.44±0.08	0.57±0.10

Values represent mean ± SEM of 8 replicates $p<0.05$, significantly different from the control group. ALT = Alanine aminotransferase, ALP =Alkaline phosphatase, AST = Aspartate aminotransferase, TP = Total protein, ALB = Albumin, D BIL=Direct Bilirubin. From Table 2, there was no significant difference $p<0.05$ in the activity of ALT, ALP, AST, TP, Albumin and Direct Bilirubin in the liver homogenate of groups administered Jobelyn® when compared with the control group.

TABLE 3. Effect of Jobelyn® in Kidney Homogenate of Pregnant Albino Rats.

Parameters	Control (A)	250 mg kg^{-1} (B)	500 mg kg^{-1} (C)	1000 mg kg^{-1} (D)
UREA (mg/dl)	27.22±1.64	20.98±1.92	10.39±2.63	25.50±9.96
CREA (mg/dl)	1.83±0.32	1.75±0.32	1.27±0.31	1.55±0.48

Values represent mean ± SEM of 8 replicates $p<0.05$, not significantly different from the control group. CREA = Creatinine. From Table 3, there was no significant difference $p<0.05$ in the levels of urea and creatinine in the kidney homogenate of the treatment groups administered with Jobelyn® when compared with the control group.

TABLE 4. Eect of Jobelyn® on Selected Lipid Profile Parameters in Plasma of Pregnant Albino Rats.

Parameters	Control (A)	250 mg kg^{-1} (B)	500 mg kg^{-1} (C)	1000 mg kg^{-1} (D)
TRIGS (mg/dl)	47.02±10.33	41.53±8.05	52.60±8.51	43.48±14.48
T.CHOL (mg/dl)	66.66±8.19	48.16±1.34	59.26±4.46	53.84±5.90

Values represent mean ± SEM of 8 replicates $p<0.05$, not significantly different from the control group. TRIGS = Triglycerides, T. CHOL = Total Cholesterol. From Table 4, there was no significant difference $p<0.05$ in the levels of Triglycerides and Total cholesterol in the plasma of the treatment groups administered with Jobelyn® when compared with the control group.

TABLE 5. Effect of Jobelyn® on Selected Lipid Profile Parameters in Liver homogenate of Pregnant Albino Rats.

Parameters	Control (A)	250 mg kg^{-1} (B)	500 mg kg^{-1} (C)	1000 mg kg^{-1} (D)
TRIGS (mg/dl)	119.52±13.88	102.93±11.85	102.33±9.93	146.34±23.26
T.CHOL (mg/dl)	25.00±5.01	23.41±3.00	36.95±8.20	32.48±9.29
HDL CHOL (mg/dl)	206.89±3.73	201.80±0.83	202.77±0.90	207.11±6.76

Values represent mean ± SEM of 8 replicates $p<0.05$, not significantly different from the control group. TRIGS = Triglycerides, TOTAL CHOL = Total Cholesterol, HDL CHOL = High Density Lipoprotein Cholesterol. From Table 5, there was no significant difference $p>0.05$ in the levels of Triglycerides, Total cholesterol and HDL cholesterol in the liver homogenate of the treatment groups administered with Jobelyn® when compared with the control group.

TABLE 6. Effect of Jobelyn® on Selected Lipid Profile Parameters in Kidney of Pregnant Albino Rats.

Parameters	Control (A)	250 mg kg^{-1} (B)	500 mg kg^{-1} (C)	1000 mg kg^{-1} (D)
TRIGS (mg/dl)	60.24±2.95	60.70±7.55	56.20±3.50	54.52±3.19
T.CHOL (mg/dl)	48.16±3.32	39.94±3.47	52.46±6.95	42.58±6.31
HDL CHOL (mg/dl)	21.19±6.11	15.77±3.60	14.74±1.98	14.62±1.31

Values represent mean ± SEM of 8 replicates $p<0.05$, not significantly different from the control group. TRIGS = Triglycerides, TOT CHOL = Total Cholesterol, HDL CHOL = High Density Lipoprotein Cholesterol. From Table 6, there was no significant difference $p<0.05$ in the levels of Triglycerides, Total cholesterol and HDL cholesterol in the kidney homogenate of the treatment groups administered with Jobelyn® when compared with the control group.

TABLE 7. Effect of Jobelyn® on Haematological Parameters in Plasma of Pregnant Rats.

Parameters	Control	250 mg kg^{-1}	500 mg kg^{-1}	1000 mg kg^{-1}
EOSINO (x 10^{12}/L)	41.00±3.49	34.80±4.76	38.11±1.88	40.29±4.14
NEUTRO (x10^{12}/L)	46.50±1.55	56.80±5.32	52.67±1.70	54.50±2.81
BASO (x 10^{12}/L)	4.0±1.73	3.4±1.03	2.25±0.37	1.86±0.55*
LYMPH (%)	4.25±0.63	5.00±1.30	5.22±0.97	3.83±1.08
MONO (%)	1.50±0.29	1.0±0.0	1.57±0.30	1.40±0.24
PCV (x 10^{12}/L)	55.5±6.51	48.50±5.74	51.38±1.46	41.50±1.74*
Hb (g/L)	17.90±2.10	15.65±1.85	16.57±0.47	13.39±0.56*

Values represent mean ± SEM of 8 replicates.*$p<0.05$: significantly different from the control group. WBC COUNT = White Blood Cell Count, NEUTRO = Neutrophils, EOSINO: =Eosinophils, BASO = Basophils, LYMPH = Lymphocytes, MONO = Monocytes, PCV: =Packed Cell Volume, Hb = Haemoglobin. From Table 7, there was no significant difference $p<0.05$ in the levels of neutrophils, eosinophils, basophils, lymphocytes, monocytes, white blood cell count, whereas, there was a significant difference $p<0.05$ in levels of haemoglobin and packed cell volume in the plasma of the treatment groups administered with Jobelyn® when compared with the control group.

Histopathological examination

In the histopathological examination, hepatoytes in control group revealed normal nuclei membrane, rough endoplasmic reticulum and mitochondria (Fig. 3A). But in the 250mg kg^{-1} group, mild to moderate portal inflammation was observed (Fig. 3B). The 500 mg kg^{-1} group (Figure 3C) revealed moderate inflammation, while moderate to severe portal inflammation was observed in the 1000 mg kg^{-1} (Fig. 3D).

DISCUSSION

Toxicological assessment of drugs, herbs and extracts are necessary to establish the safety limit of these substances in animals. These are then commonly used to assess the possible health risk in humans [22]. The liver is a metabolically active organ responsible for many vital life functions, some of such primary functions include; bile production and excretion; excretion of bilirubin, cholesterol, hormones, and drugs; metabolism of fats, proteins, and carbohydrates; storage of glycogen, vitamins, and minerals; synthesis of plasma proteins, such as albumin, and clotting factors, as well as blood detoxification and purification [23]. Due to these important activities, the liver is one of the body's organs most subject to injury. It is the central organ in the metabolism and detoxification of drugs and toxins. Consequently, drugs affect the liver more frequently than any other organ and place the liver at great risk for toxic damage [24]. The cells in the liver contain proteins called enzymes that drive these chemical reactions. When liver cells are damaged or destroyed, the enzymes in the cells leak out into the blood. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) are specific markers of

hepatic injury and hepatocellular necrosis [25]. A Significant decrease ($P<0.05$) in the activity of alanine aminotransferase (ALT) was observed in groups treated with 500 and 1000 mg/kg body weight of Jobelyn® when compared with the control (Fig 1). Hence, suggesting that Jobelyn® may possess hepatoprotective properties. ALT is localized in the cytosol of the hepatocytes and is a more sensitive marker of hepatocellular damage as opposed to AST which can also be produced from other tissues like heart, kidney and pancreas other than the liver [26]. However, AST and ALP levels were not significantly different ($P<0.05$) in the treated groups when compared with the control groups. A significant decrease ($P<0.05$) in albumin levels in the group administered 500 mg/kg body weight of Jobelyn® was observed (ig 1). A similar study conducted by Salawu*et al*. [27] and Mancini-Filho[28] revealed similar results. The possible hepatoprotective effects of Jobelyn® could be attributed to the presence of significant levels of flavonoids such as tannins and anthocyanidins which help to clear up free radicals. It has been established that free radicals play a significant role in various pathogenesis, inflammatory diseases and can result in necrosis of the liver [29, 30]. Flavonoids are known for their ability to exhibit antioxidant potential, and their effects on human health and nutrition are considerable. The mechanisms of action of flavonoids are through chelating or scavenging processes [31]. Chemically, the remarkable antioxidant properties of flavonoids is due to the hydrogen donating substituents (hydroxyl groups), which are attached to the aromatic ring structures of flavonoids, enabling the flavonoids to undergo a redox reaction and thus helping them to rapidly scavenge free radicals easily. Flavonoids also mediate their antioxidant effect by a stable delocalization system, which consist of heterocyclic and aromatic rings as well as multiple unsaturated bonds, and helps to delocalize the resulting free radicals [32]. Finally, the presence of certain structural moieties which are capable of forming transition metal chelating complexes that can regulate the production of reactive oxygen species such as O_2- and OH is also a mechanism through which flavonoids mediate their antioxidant effect [33]. *Sorghum bicolour*, the plant extract of Jobelyn® is known to possess high concentration of flavonoids which include tannins and anthocyanins, significantly possessing high concentrations of dimeric 3-deoxy-anthocyanidins [34, 35]. Phenolic compounds, which have also been found to exist in high concentrations in *Sorghum bicolor* [36] are considered to play an important role as dietary antioxidants for theprevention of oxidative damage in living system, further establishing the antiradical properties of Jobelyn®[36].Epidemiological studies have suggested association between the comsumption of phenolic acid - rich foods or beverages and the prevention of many diseases [37]. The potential hepatoprotective properties of this wholly herbal drug is further supported by the significant decrease ($P<0.05$) in albumin levels in the group administered 500 mg/kg body weight of Jobelyn®. This could have been pregnancy-induced, as albumin is often decreased in normal pregnancy as a consequence of haem dilution. It is noteworthy though, that a low albumin level is often temporary, and is not a reliable way to diagnose liver disease. In contrast, the concentrations of the transaminases (alanine and aspartate) and γ-glutamyltransferase normally increase during pregnancy. Thus, the decreased levels of ALT observed in pregnant rats treated with Jobelyn® is a further proof of the possible hepatoprotective effect of Jobelyn® The histological findings revealed mild to moderate portal inflammation in the liver of animals treated with 250 and 500 mg/kg bodyweight of Jobelyn®, while the liver of rat treated with 1000 mg/kg bodyweight of Jobelyn® showed moderate to severe portal inflammation (Fig 3). This is possibly as a result of the dose of Jobelyn® administered to the animals as the severity of liver abnormality increased with increasing dosage. Bilirubin levels were not significantly different ($p<0.05$) when compared with the control. Bilirubin is a waste product from the breakdown of red blood cells. The liver processes bilirubin so it can be excreted in stool. Bilirubin flows through the liver's bile ducts, dissolved in bile. Bilirubin blood levels may be elevated in people with impaired bile flow. This can occur in severe liver disease, gallbladder disease, or other bile system conditions. Very high bilirubin levels cause jaundice, in which the skin and whites of the eyes turn yellow [38]. Bilirubin can be a useful liver function test in people with a known bile flow problem. Higher than normal levels of direct or indirect bilirubin may indicate different types of liver problems. Occasionally, higher bilirubin levels may indicate an increased rate of destruction of red blood cells. Hence, the stable level of bilirubin in animals treated with Jobelyn® further supports the safety and possible hepatoprotective role of Jobelyn®. A significant decrease ($p<0.05$) in Urea concentration was observed, suggesting that Jobelyn® may have protective effects on renal function. Salawu*et al.* [27] also observed similar effects when *Sorghum bicolor* aqueous extract was administered to rats fed with low and high iron diet. This implies that the administration of Jobelyn® has no toxic effect on the kidney and may thereby preserve the integrity of the kidney. Urea is the major end product of protein metabolism in humans and other mammals. Factors which tend to increase urea excretion include increases in glomerular filtration rate, increased dietary protein intake, protein catabolic conditions, and water diuretic states. Factors which reduce urea excretion include low protein intake and conditions which result in low urine output such as dehydration), although low urea levels are seen during normal pregnancy. Biochemical analysis revealed significant decrease ($p<0.05$) in the levels of Hb and PCV in only the group administered 1000mg/kg which could be due to the high-dose (Table 7). The PCV (packed cell volume) determines the percentage of red blood cells in the plasma. During Pregnancy, however, decreased hematocrit levels are observed**,** especially in the last trimester as plasma volume increases. Haemodilution occurs physiologically in pregnancy [39]. This may result in lower haemoglobin concentrations than in the non-pregnant state. Sorghum contains such hard-to-find nutrients as iron, calcium and potassium. In the past, doctors prescribed sorghum as a daily supplement for those low in these nutrients. No significant difference was observed in

the levels of triglyceride, total cholesterol and high density cholesterol. Implying that Jobelyn® poses no threat on the body as relates to diseases caused by increased cholesterol such as atherosclerosis. In addition to the high content of anti-inflammatory phenolic compounds, sorghum contains several groups of bioactive compounds with the capacity to induce pro-inflammatory immune responses. Water-soluble beta-glucans are found in sorghum that showed biologically active beta-glucans capable of initiating macrophage activation.

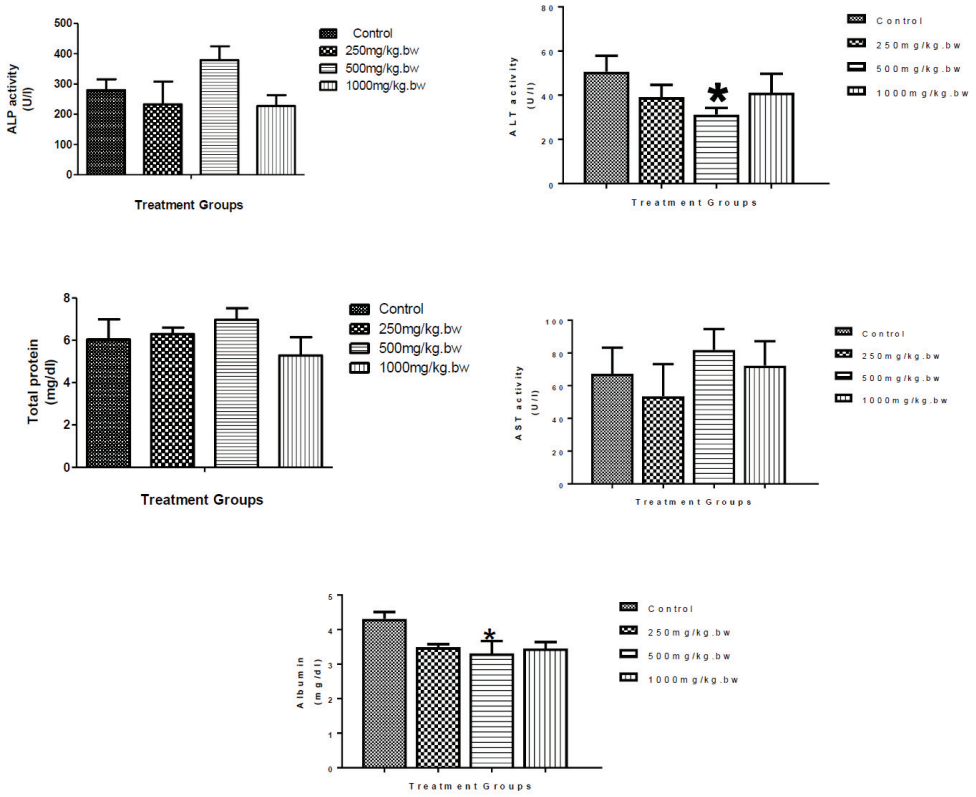

FIGURE 1. Effects of Jobelyn on plasma of pregnant albino rats. ALT = Alanine aminotransferase, ALP = Alkaline phosphatise, AST = Aspartate transaminase, TP = Total protein, ALB = Albumin. Values are presented as mean ± standard error of mean (SEM) of 8 replicates; *significant at $p < 0.05$ as compared with control

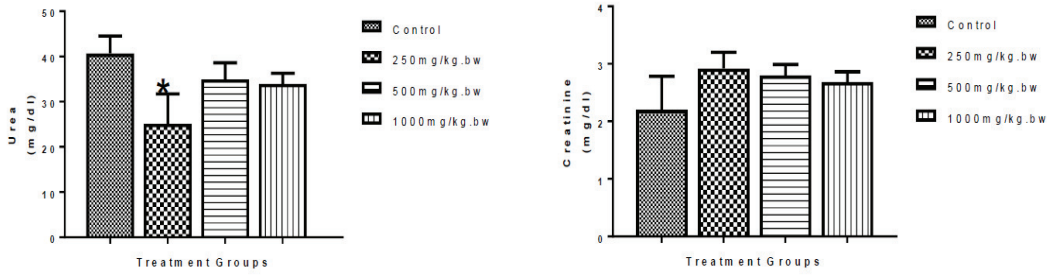

FIGURE 2. Effects of Jobelyn on Kidney functions of pregnant albino rats. Values are presented as mean ± standard error of mean (SEM) of 8 replicates; *significant at $p < 0.05$ as compared with control.

RECOMMENDATION

Jobelyn® should be subjected to further analysis to clearly ascertain the mechanisms involved in its suggested haematocrit boosting property, as well as its proposed hepatoprotective and renal protective property, with emphasis on pregnant women. This would help further confirm the safety of the drug for consumption by pregnant women.

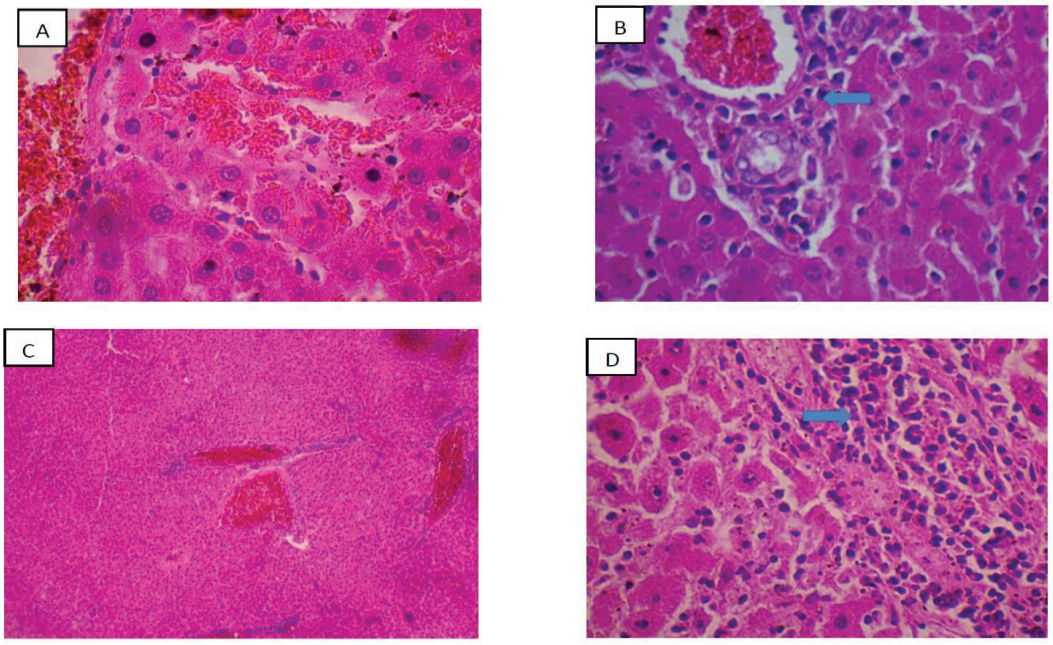

FIGURE 3. Photomicrograph of liver tissues of rat (H&E x 400)(A) with normal features in the control (B) treated with 250mg/kg bodyweight of Jobelyn® Showing mild to moderate portal inflammation, H&E x 400 (C) treated 500 mg/kg bodyweight of Jobelyn®(D)treated with 1000 mg/kg bodyweight of Jobelyn®.

CONCLUSION

In conclusion, Jobelyn may enhance liver and kidney functions in pregnant women when taken at an appropriate dose. It may also boost haematocrit level, and thus could be ideal for intake by pregnant women.

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