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Acute and Repeated Oral Toxicity of Antidiabetic Polyherbal Formulation Flax Seed, Fenugreek and Jamun Seeds in Wistar Albino Rat

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Abstract

Polyherbal formulations treating for various diseases bring attention to the human society. The toxicity and its safe dosage should be studied for dose optimization and formulation. The aim of this study was to evaluate acute and repeated oral toxic effect of the hydroalcoholic extract of polyherbal formulation such as flax seed, fenugreek and jamun seed (FFJ) on wistar albino rats. Acute toxicity was tested by single oral stepwise administration of the drug at the dose of 300 mg, 2000 mg and 5000 mg/kg bw. The toxicity and mortality were observed for every 30 minutes for first 24 hours and then day by day for 14 days. At the 29th day, the blood was collected by Sino orbital puncture for analysis of hematological and biochemical parameters. Then the animals were killed by cervical decapitation and organs were separated and weighed. The results showed that no toxic symptoms were observed up to the dose of 2000 mg/kgbw. But slight changes in the stool consistency, tremor, lethargy and sleep were observed at the dose of 5000 mg/kg bw. There is no significant change in the biochemical and liver enzymatic parameters of different treated groups of polyherbal formulations. The control group animals was observed as Blood Glucose (99.7 ± 4.8), Urea (30.6 ± 1.7), Creatinine (1.08 ± 0.07) and Aspartate aminotransferase (AST) (32 ± 2.36), Alanine aminotransferases (ALT) (46 ± 1.80), Alkaline phosphatase (ALP) (164 ± 1.30). Similarly, hematological parameters such as RBC (5.63 ± 0.23), WBC (6.52 ± 0.32), ESR (8 ± 0.53), PCV (44.8 ± 2.314), MCV (79.6 ± 4.18), MCH (26.4 ± 1.2) and MCHC (33.2 ± 2.5) don't show any significant alterations.

Keywords: Antidiabetic formulations; Polyherbal; Acute; Repeated oral toxicity

Introduction

About 80% of the world's population use plant as their primary source of medication [1]. In India, the use of medicinal plants to alleviate specific ailments is in practice from ancient time's onwards [2]. Medicinal plants offer an unlimited opportunity for the discovery of new drugs. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these more than 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and called as botanical garden of the World [3].

In recent times there is an increasing awareness and interest in medicinal plants and their preparations commonly known as herbal medicines [4]. In Indian systems of medicine most practitioners formulate and dispense their own recipes [3], the drugs are primarily dispensed as water decoction or ethanolic extract. The prime impediment to the use of these herbal preparations is the lack of scientific and clinical data in support of better understanding of the effectiveness and safety of the drug [5]. Inappropriate formulation, have led to adverse reactions that are sometimes life threatening or lethal [6].

Studies have indicated that many medicinal plants used in traditional medicine showed adverse effects also [7,8]. In order to increase the confidence in their safety to humans, acute and sub-acute toxicity studies are warranted in suitable experimental subjects. Determination of acute oral toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds [9]. The present study focuses on the acute toxicity studies, which identify a dose causing major adverse effects and an estimation of the minimum dose causing lethality [10].

The medicinal plant *L. usitatissimum* (flax seed), *T. foenumgreacum* (fenugreek) and *S. cumini* (jamun) are widely used for the treatment of many symptoms mainly diabetes mellitus in the traditional system of medicine in India. Hence termed the above mixed polyherbal product as FFJ. Some medicinal plant products were used as common ingredients in food those are called as functional foods [11]. Similarly the above mentioned medicinal plants products were already used in foods for taste, spicy and consistency. But the polyherbal formulations (FFJ) have rich medicinal property such as weight reduction, excess fat burner and as hypoglycemic agent.

The oral study reported on flax seed reduces the blood glucose level by reducing the insulin resistance directly and indirectly by reducing

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the oxidative stress [12]. Numerous studies were already conducted on fenugreek and jamun seed proved that they reduce the blood glucose level by stimulating insulin secretion in the beta cells of the pancreas [13,14].

Despite their traditional use in the treatment of diabetes mellitus in individual drugs, there is no systematic study on exploration of anti-diabetic potential with this herbal formulation. In general, treatment involving herbal drugs spans a long duration of time, further, diabetes mellitus, being a chronic disease, needs a long term treatment and chronic consumption of the herbal formulation may cause toxic effects. However, there is no report on toxicity evaluation with this FFJ herbal formulation deserves this study.

Materials and Methods

Collection and identification of plant

Seeds belonging to the herbal formulation *L. usitatissimum* (flax seed), *T. foenumgreacum* (fenugreek) and *S. cumini* (jamun) (FFJ) were collected from the local market, Pondicherry, India and authenticated by Siddha physician and nodal officer, Siddha unit, Dept of ISM&H, Pondicherry. The seeds were dried under shade and powdered before use.

Extraction of the plant material

Extraction of flax seed: Powdered flax seeds are defatted by petroleum ether (at 60-80°C) in the sox let apparatus. The merc was then hydrolyzed with 1M sodium hydroxide for 1 hr at room temperature by constant rotation followed by extraction with 50% ethanol acidified with 1m Hcl up to the pH: 2-4, further dry the filtrate at 50°C [15].

Extraction of fenugreek and jamun seeds: Dried powdered seeds were extracted with 50% ethanol using sox let apparatus for 20-24 hrs. The extract was concentrated under vacuum and dried at 50°C. Finally the powdered extract of flax, fenugreek and jamun seeds were formulated 1:1:1 ratio for further study.

Experimental animal: Wister strain albino rats weighing 180-220 gm were used for the study. Before the commencement of the experiments proper IACE permission was obtained (IAEC NO: JKKMMRF/IAEC/2013/014). Rats were housed under standard laboratory conditions with food and water provided *ad libitum*.

Procedure for acute oral toxicity

The acute oral toxicity effect of (hydroalcoholic extracts) herbal formulation FFJ (1:1:1) was evaluated in wistar albino rats by the procedures, described by Organization for Economic Co-operation and Development (OECD) guidelines 423 [16]. Animals were divided into 4 groups with six animals each group. Group1 is control, which receives only 0.9% saline. Group 2, 3, 4 are treated groups which receives herbal formulation FFJ (1:1:1) dissolved in 0.9% saline. The animals were kept fasting for overnight providing only water after which single oral administration of the hydroalcoholic extraction (herbal formulation) FFJ in a stepwise procedure at a fixed dose of 300 mg, 2000 mg and 5000 mg at the rate of 2 ml /100 mg bw. No food or water was given up to 4hrs after drug administration.

Animal were observed for toxicity and mortality every 30 minutes for first 24 hours and then daily for 14 days. If mortality was observed in animals, then the dose administered was assigned as toxic dose. If mortality was observed in single animal, then the same dose was repeated again to confirm the toxic dose. If no mortality observed in single animal it is considered as non toxic and animal treated with next higher dose. The general observations like body weight, skin, fur, lacrimation, ptosis, tremor, salivation, stool consistency, lethargy, sleep and coma are also observed continuously for every 30 minutes of the administration for first 24 hours and then daily for 14 days.

Procedure of repeated oral toxicity test

Repeated dose oral toxicity study was carried out according to OECD Guideline 407 [17]. The animals were divided into 6 groups with 10 animals in each group (5 males and 5 females). The hydroalcoholic herbal formulation FFJ (1:1:1) was dissolved in 0.9% saline and administered orally at different dosage (200 mg, 300 mg, 400 mg / kg bw) on daily basis for 28 days. An additional group was devised as the satellite group in order to observe the reverse sign of any toxicity for another 14 days after the regimen.

Group1: Control received 0.9% saline.

Group 2: Control received 0.9% saline (satellite group)

Group 3: Received herbal formulation FFJ at the dose of 200 mg / kg bw

Group 4: Received herbal formulation FFJ at the dose of 300 mg / kg bw

Group 5: Received herbal formulation FFJ at the dose of 400 mg / kg bw

Group 6: Received herbal formulation FFJ at the dose of 400 mg / kg bw (satellite group)

Mortality, body weight, food and water consumption as well as observation for general toxicity signs of the animals were evaluated daily for 28 days. At the 29th day blood was taken by sinus puncture and animals were killed by cervical dislocation. Organs were separated, weighed and stored in 10% of the formalin solution for histopathological analysis.

Determination of hematological parameters

Red blood cell (RBC) was estimated by haemocytometer method [18]. the white blood cell count was estimated by method of John [19]. The haemoglobin (Hb) content was determined according to Jain, using the cyanomethaemoglobin method [20]. The packed cell volume (PCV) was determined by the microhaematocrit method according to Dacie and Lewis [21]. Erythrocyte sedimentation rate (ESR) was estimated by the method of wintrobe [21]. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) were calculated according to Jain [20].

Determination of plasma biochemical parameters

The blood glucose level were measured by using GOD-PAP kit (agappe, kerala). plasma Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) Alkaline phosphatase was assayed by using kit purchased from agappe diagnostic ltd, kerala [22]. The blood urea was measured by using the method of Natelson et al. [23]. The level of creatinine and urea were measured by using the method of Slot [24].

Statistical analysis

All the data were represented as (±SD) were analyzed statistically by using one way analysis of variance (ANOVA) followed by turkey's

multiple comparison. The Values P<0.05 is considered statistically significant.

Result

Acute oral toxicity

The acute toxicity study of hydro alcoholic extract of herbal formulation FFJ in wistar albino rats are given in Table 1. The Table 1 demonstrates that no toxic symptoms and mortality were observed up to the dose of 2000 mg/kg bw during the 14 days of the observation. There is no visible change in the food intake and body weight when compared to control. The herbal formulation FFJ treated group showed slight changes in the general behavior such as decreased stool consistency, tremor, lethargy and sleep at the dose of 5000 mg/kg bw.

Repeated oral toxicity

In the repeated oral toxicity study, there is no mortality and intoxication were observed at the treated dose of 200 mg, 300 mg, 400 mg/kg bw throughout the dosing period of 28 days. The mean body weight of the animal during the dosing period, given in Table 2. The percentage increase (6-7%) in body weight of the control and herbal formulation FFJ treated animals were found to be same throughout the dosing period of 28 days in all male and female rats.

The repeated oral toxic effect of herbal formulation FFJ on hematological parameters in male and female rats given in Table 3**.** There **is** no changes in the RBC count, WBC count, Packed cell volume, Hemoglobin content, ESR and the values of MCV, MCH, MCHC at the of end 28 days and the results are almost similar to control animal.

The repeated oral toxic effect of herbal formulation FFJ on biochemical parameters was given in Figures 1 and 2. There is no significant alteration in the control group parameters such as Blood Glucose (99.7 ± 4.8), Urea (30.6 ± 1.7), Creatinine (1.08 ± 0.07) and Aspartate aminotransferase (AST) (32 ± 2.36), Alanine

Parameter	Control (group1)	Herbal formulation FFJ (gm/kgbw)		
		300 mg (group2)	2000 mg (group3)	5000 mg (group4)
Skin changes	-	-	-	-
Fur changes	-	-	-	-
Lacrimation	-	-	-	-
Ptosis	-	-	-	-
Tremor	-	-	-	+
Salivation	-	-	-	-
↓Stool consistency	-	-	-	+
Lethargy	-	-	-	+
Sleep	-	-	-	+
Coma	-	-	-	-

Table 1: Acute Toxic Effect of Herbal Formulation FFJ at the dose of 2000 mg, 3000 mg, 5000 mg/kg bw: (-) represents the absence of Parameters, (+)(-) represents the Presence of Parameters.

	Days	Group 1 (control)	Group 2 (satellite control)	Group 3 (200 mg/ kgbw)	Group 4 (300 mg/ kgbw)	Group 5 (400 mg/ kgbw)	Group 6 (satellite group)
Male	1st day	209.3 ± 10.7	208.2 ± 10.0	210.1 ± 10.8 A^{NS}	206.5 ± 10.8A^{NS}	207.6 ± 12.2 A^{NS}	207.6 ± 10.3 A^{NS}
	7th day	215.8 ± 9.51	214.8 ± 9.3	216.8 ± 11.4 A^{NS}	212.6 ± 11.4A^{NS}	213.3 ± 10.3 A^{NS}	214.8 ± 11.7 A^{NS}
	21st day	221.1 ± 10.3	220.2 ± 10.3	222 ± 10.8 A^{NS}	219 ± 10.3 A^{NS}	220.5 ± 10.15 A^{NS}	220.4 ± 10.1A^{NS}
	28th day	227.6 ± 10.3	226.8 ± 10.3	229.3 ± 11.4 A^{NS}	225.5 ± 10.2A^{NS}	227.5 ± 10.01 A^{NS}	226.8 ± 10.9 A^{NS}
Female	1st day	208.8 ± 9.7	206.2 ± 9.6	207.5 ± 9.3 B^{NS}	205.1 ± 8.3 B^{NS}	206.5 ± 11.4 B^{NS}	209 ± 9.6 B^{NS}
	7th day	213.3 ± 9.5	212.6 ± 11.6	214.8 ± 9.8 B^{NS}	211.3 ± 9.09B^{NS}	212.1 ± 10.3 B^{NS}	215.4 ± 10.9 B^{NS}
	21st day	219.8 ± 10.3	218 ± 11.3	220.8 ± 9.2 B^{NS}	218.3 ± 10.2B^{NS}	218.8 ± 8.9 B^{NS}	221.2 ± 10.3 B^{NS}
	28th day	225.3 ± 10.2	224 ± 12.2	227.5 ± 9.6 B^{NS}	224.6 ± 11.09B^{NS}	225.3 ± 10.01 B^{NS}	227.4 ± 10.8 B^{NS}

Values are expressed as mean ± SD of six animals, NS-no significance, A-compared with control male, B-compared with control female

Table 2: Repeated Oral Toxic Effect of Herbal Formulation FFJ on Body Weight (In gms/Animal) In wistar albino Rats.

	parameter	Group 1 (control)	Group 2(satellite control)	Group 3 (200 mg/ kgbw)	Group 4 (300 mg/ kgbw)	Group 5 (400 mg/ kgbw)	Group 6 (satellite group)
Male	RBC(x10^6/cumm)	5.63 ± 0.23	5.65 ± 0.26	5.58 ± 0.19A^{NS}	5.64 ± 0.21 A^{NS}	5.73 ± 0.18 A^{NS}	5.69 ± 0.21 A^{NS}
	WBC(x10^3/cumm)	6.52 ± 0.32	6.45 ± 0.31	6.69 ± 0.34 A^{NS}	6.73 ± 0.29 A^{NS}	6.33 ± 0.19 A^{NS}	6.35 ± 0.21 A^{NS}
	PCV (%)	44.8 ± 2.3	44 ± 1.7	46 ± 2.0 A^{NS}	46 ± 1.2 A^{NS}	46 ± 2.0 A^{NS}	45.6 ± 1.9 A^{NS}
	Hb(gm%)	14.8 ± 0.53	15 ± 0.46	14.9 ± 0.70 A^{NS}	15.1 ± 0.70 A^{NS}	14.9 ± 0.54 A^{NS}	14.8 ± 0.54 A^{NS}
	ESR(mm/1hr)	3.55 ± 0.19	3.62 ± 0.1	3.48 ± 0.19 A^{NS}	3.58 ± 0.19 A^{NS}	3.5 ± 0.18 A^{NS}	3.48 ± 0.19 A^{NS}
	MCV(μm^3)	79.6 ± 4.18	77.9 ± 3.35	82.3 ± 4.3 A^{NS}	81.5 ± 3.6 A^{NS}	80.3 ± 4.8 A^{NS}	80.1 ± 3.48 A^{NS}
	MCH(Pg)	26.4 ± 1.2	26.5 ± 1.3	26.6 ± 1.2 A^{NS}	26.7 ± 1.1 A^{NS}	26 ± 1.4 A^{NS}	26 ± 1.0 A^{NS}
	MCHC (%)	33.2 ± 2.5	34.1 ± 2.0	32.4 ± 2.6 A^{NS}	32.8 ± 1.9 A^{NS}	32.47 ± 2.3 A^{NS}	32.5 ± 2.1 A^{NS}
Female	RBC(x10^6/cumm)	4.98 ± 0.2	5 ± 0.22	4.78 ± 0.15B^{NS}	4.69 ± 0.17 B^{NS}	4.86 ± 0.22 B^{NS}	4.6 ± 0.18 B^{NS}
	WBC(x10^3/cumm)	6.14 ± 0.28	6.25 ± 0.1	6.12 ± 0.24 B^{NS}	6.17 ± 0.28 B^{NS}	6.22 ± 0.16 B^{NS}	6.18 ± 0.13 B^{NS}
	PCV (%)	42.1 ± 2.0	42.2 ± 2.28	42.1 ± 1.7 B^{NS}	42 ± 1.2 B^{NS}	42 ± 1.2 B^{NS}	41.6 ± 1.14 B^{NS}
	Hb(gm%)	12.8 ± 0.47	12.8 ± 0.53	12.8 ± 0.53 B^{NS}	12.7 ± 0.53 B^{NS}	12.9 ± 0.54 B^{NS}	12.9 ± 0.6 B^{NS}
	ESR(mm/1hr)	5.3 ± 0.28	5.2 ± 0.15	5.2 ± 0.18 B^{NS}	5.4 ± 0.24 B^{NS}	5.6 ± 0.27 B^{NS}	5.54 ± 0.26 B^{NS}
	MCV(μm^3)	84.7 ± 4.2	84.4 ± 4.6	88.2 ± 3.2 B^{NS}	89.5 ± 3.2 B^{NS}	86.5 ± 4.8 B^{NS}	89.0 ± 3.4 B^{NS}
	MCH(Pg)	25.8 ± 1.5	25.6 ± 1.7	26.8 ± 0.9 B^{NS}	27.0 ± 0.67 B^{NS}	26.7 ± 1.3 B^{NS}	27.7 ± 1.4 B^{NS}
	MCHC (%)	30.4 ± 1.2	30.4 ± 1.3	30.4 ± 0.7 B^{NS}	30.2 ± 1.2 B^{NS}	30.9 ± 1.3 B^{NS}	31.1 ± 1.8 B^{NS}

Values are expressed as mean ± SD of Six animals, NS-no significance, A-compared with control male, B-compared with control female

Table 3: Repeated Oral Toxic Effect of Herbal Formulation FFJ on Hematological Parameters in wistar Albino Rats.

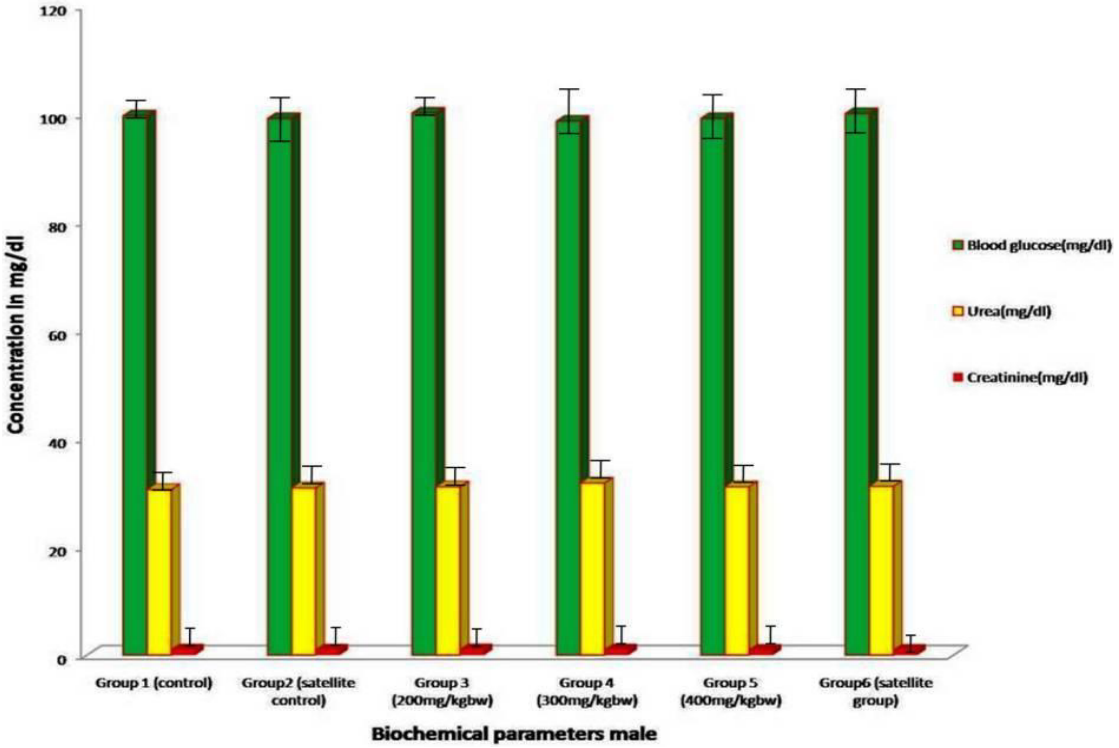

Figure 1: Effect of (various concentration) FFJ on Biochemical parameters in male wistar rats.

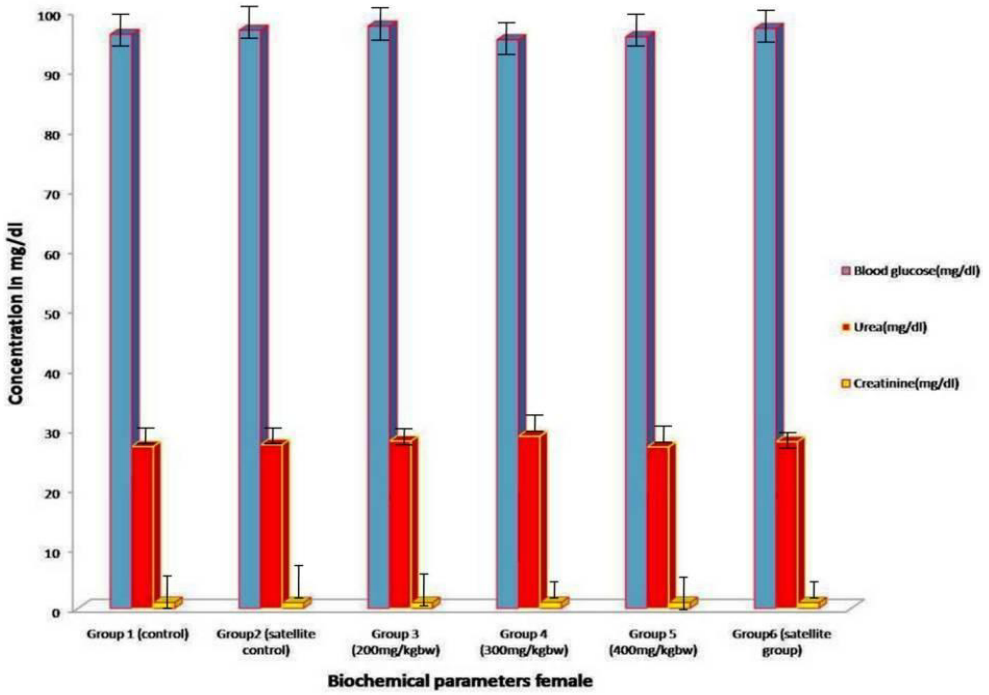

Figure 2: Effect of (different concentration) FFJ on Biochemical parameters in Female wistar rats.

aminotransferases (ALT) (46 ± 1.80), Alkaline phosphatase (ALP) (164 ± 1.30). Similarly, hematological parameters such as RBC (5.63 ± 0.23), WBC (6.52 ± 0.32), ESR (.8 ± 0.53), PCV (44.8 ± 2.314), MCV (79.6 ± 4.18), MCH (26.4 ±1.2) and MCHC (33.2 ± 2.5), when compared to treated groups shown in Figures 3 and 4 in both sex.

Table 4 shows no changes was observed in weight of the liver, kidney

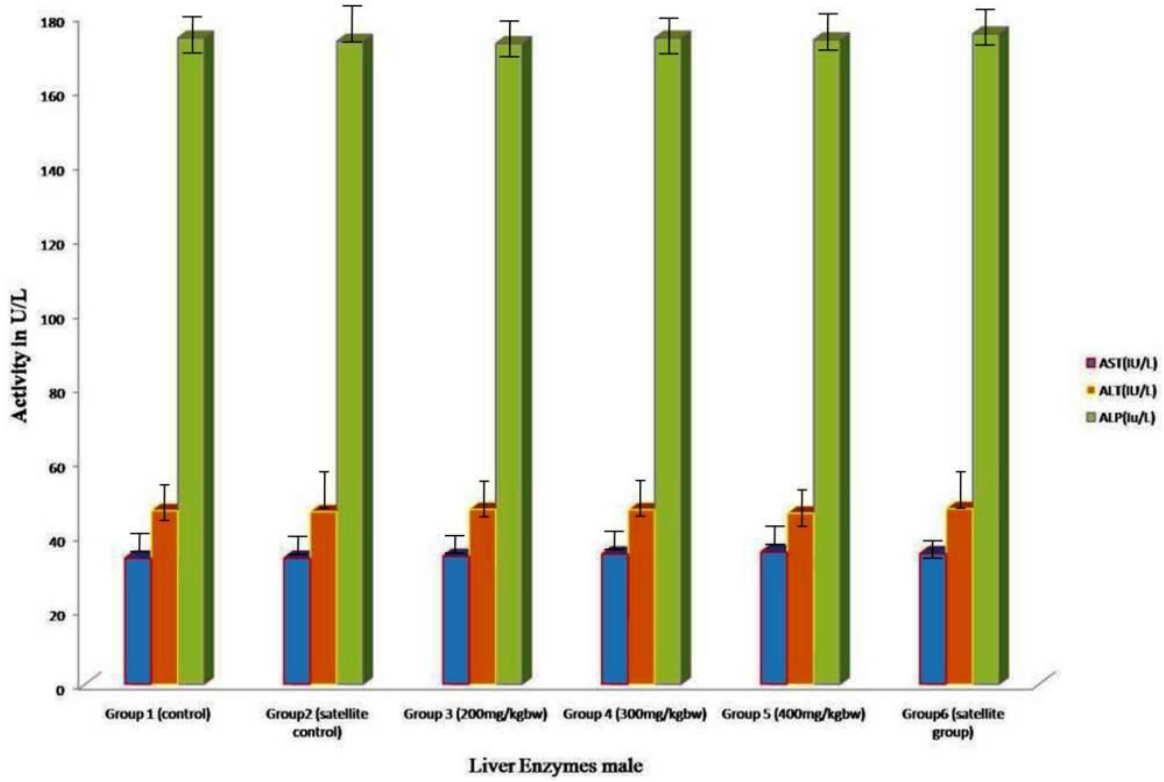

Figure 3: Effect of FFJ (with various concentration-treated groups) on liver enzymes in male wistar rats.

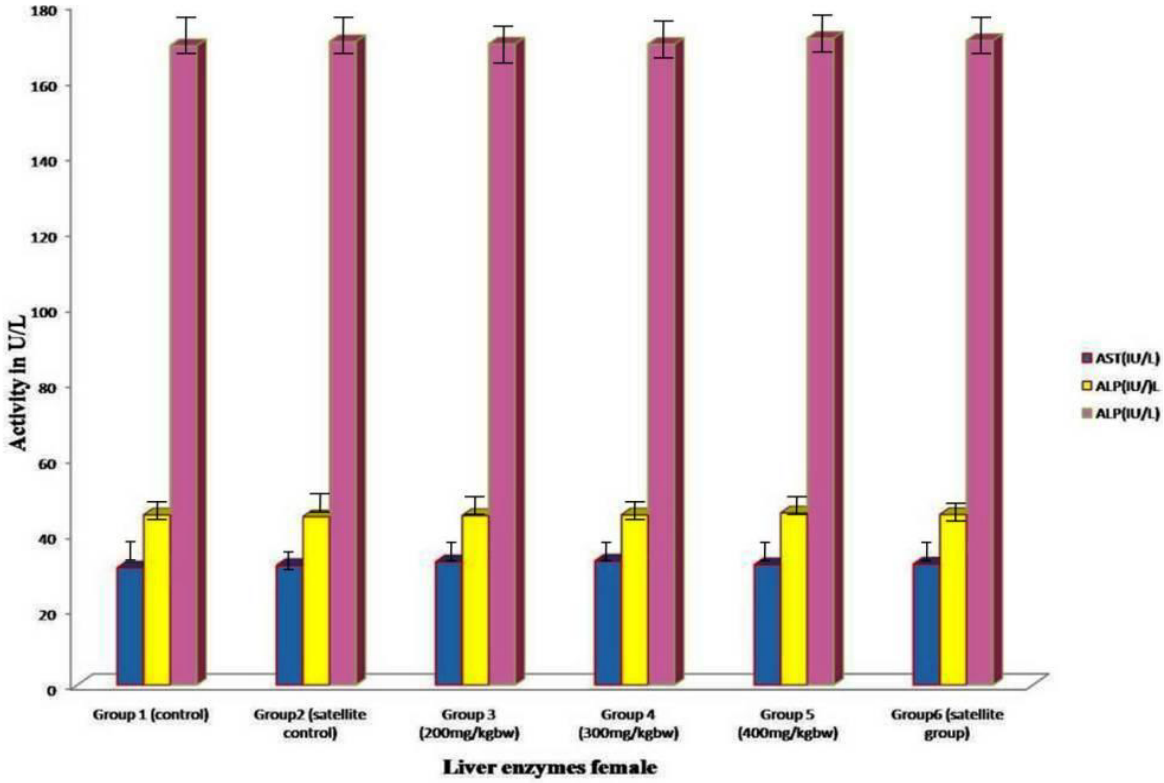

Figure 4: Effect of FFJ (with various concentration-treated groups) on liver enzymes Female wistar rats.

	Organs (gms)	Group 1 (control)	Group 2 (satellite control)	Group 3 (200 mg/ kgbw)	Group 4 (300 mg/ kgbw)	Group 5 (400 mg/ kgbw)	Group 6 (satellite group)
Male	Liver	4.69 ± 0.23	4.77 ± 0.16	4.73 ± 0.20 A^{NS}	4.66 ± 0.13 A^{NS}	4.72 ± 0.19 A^{NS}	4.77 ± 0.17 A^{NS}
	kidney	0.486 ± 0.01	0.480 ± 0.017	0.471 ± 0.01 A^{NS}	0.496 ± 0.01 A^{NS}	0.479 ± 0.02 A^{NS}	0.492 ± 0.018 A^{NS}
	pancreas	0.288 ± 0.008	0.286 ± 0.006	0.286 ± 0.007 A^{NS}	0.289 ± 0.008 A^{NS}	0.281 ± 0.007 A^{NS}	0.291 ± 0.008 A^{NS}
Female	Liver	4.23 ± 0.21	4.37 ± 0.07	4.28 ± 0.15B^{NS}	4.24 ± 0.21 B^{NS}	4.32 ± 0.15 B^{NS}	4.26 ± 0.23 B^{NS}
	kidney	0.410 ± 0.01	0.405 ± 0.013	0.428 ± 0.01 B^{NS}	0.417 ± 0.01 B^{NS}	0.418 ± 0.01 B^{NS}	0.416 ± 0.012 B^{NS}
	pancreas	0.271 ± 0.008	0.271 ± 0.009	0.275 ± 0.006 B^{NS}	0.277 ± 0.008 B^{NS}	0.281 ± 0.007 B^{NS}	0.281 ± 0.008 B^{NS}

Values are expressed as mean ± SD of six animals, NS-no significance, A-compared with control male, B-compared with control female

Table 4: Repeated Oral Toxic Effect of Poly Herbal Formulation FFJ on Organ Weight in Male Albino Rat.

and pancreas when compared to control in the 28 days treatment of the drug at different doses. The values are almost similar to control. No reversible symptoms were observed in the satellite group on another 14 days observation when compared to satellite control.

Histopathology

The histopathology of the liver, kidney and pancreas shows no morphological changes after the 28 days treatment with herbal formulation FFJ at the doses of 200 mg, 300 mg, 400 mg/kg bw when compared to control.

Discussion

In the present study, the acute toxicity effect of antidiabetic herbal formulation FFJ had reported that no toxicity or mortality were observed up to the dose of 2000 mg/kg bw but higher concentration of drug such as 3000 mg,5000 mg/kg bw caused slight changes in the general behavior like tremor, decreased stool consistency, lethargy and sleep. This might be due to the certain Phytochemicals present in the individual drugs in the herbal formulation FFJ. Phytochemicals are constitutive metabolites that enable plants to overcome temporary or continuous threats integral to their environment, but the inherent biological activity of such constituents often causes dramatic adverse consequences in other organisms that may be exposed to them. Similar study conducted by the Abdel-Barry and Al-Hakiem [25] proved that use of fenugreek cause transient diarrhea, flatulence, mild hepatitis and dizziness. Phytochemicals are constitutive metabolites that enable plants to overcome temporary or continuous threats integral to their environment, but the inherent biological activity of such constituents often causes dramatic adverse consequences in other organisms that may be exposed to them. Fenugreek seeds contain oils, alkaloids, amino acids (lysine, argenine, tryptophan, threonine, valine and methionine), mucilages (galactomannan), vitamin A, C, D, B1 and minerals such as calcium, iron and zinc [26]. High level of alkaloids exerts toxicity and adverse effects to humans, especially in physiological and neurological activities. Some Alkaloids may produce nausea, vomiting, diarrhea and in large quantity can cause acute necrosis of liver and death [27].

The flax seed contain cyanogenic glycosides which are toxic to animals and humans [28]. Cyanogenic glycosides undergo enzymatic degradation to produce hydrogen cyanide, resulting in acute cyanide poisoning. Clinical symptoms of acute cyanide poisoning include rapid respiration, drop in blood pressure, rapid pulse, headache, dizziness, vomiting, diarrhea, mental confusion, stupor, blue discoloration of the skin due to lack of oxygen (cyanosis), twitching and convulsions [29-35]. The cyanogenic glycoside might be responsible for acute toxic symptoms of herbal formulation FFJ at the dose of 5000 mg/kgbw. The repeated dose toxicity revealed that herbal formulation FFJ will not interfere in the body weight and normal functions of the animal. This is supported by the study conducted in mice, after oral administration of various doses of jamun (100, 200, 500, 1000 and 2000 mg/kg) shown no behavioral changes or mortality up to 72 h after treatment and Sub acute toxicity studies with jamun at the dose of 1 g/kgbw in rats indicated no significant change in body weight, food and water intake, organ weight, hematological parameters (hemoglobin and WBC count), liver function (total bilirubin, SGOT, SGPT, alkaline phosphatase, total protein and albumin) and renal function (blood urea and creatinine) tests [36,37].

Conclusion

The present study concludes that the toxicity results of polyherbal formulation FFJ and their individual components emphasized to consider it as an alternative medicine to diabetic treatment. This study also validated the traditional use of natural remedies as indigenous plants origin for the treatment of diabetes mellitus. This study finally emphasis that the herbal formulation of FFJ was very safe up to the dose of 2000 mg/kgbw in animal model further the clinical trial in human volunteers developed a promising antidiabetic drug to the diabetic society.

References

1. Abubakar AA, Salka MN (2011) Pelagia Research Library. Asian Journal of Plant Science and Research 1: 95-101.
2. Jain N (1986) Schalms Veterinary haematology (4thedn) Lea and Febiger, Philadelphia.
3. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TP (2007) Indian herbs and herbal drugs used for the treatment of diabetes. J Clin Biochem Nutr 40: 163-173.
4. Ogbonnia SO, Nkemehule FE (2009) Evaluation of acute and subchronic toxicity of Stachytarpheta angustifolia (Mill) Vahl (Fam. Verbanaceae) extract in animals. African Journal of Biotechnology.
5. Talalay P, Talalay P (2001) The importance of using scientific principles in the development of medicinal agents from plants. Academic Medicine 76: 238-247.
6. Elvin-Lewis M (2001) Should we be concerned about herbal remedies. J Ethnopharmacol 75: 141-164.
7. Ernst E (1998) Harmless herbs? A review of the recent literature. Am J Med 104: 170-178.
8. De Smet PA (2002) Drug therapy: Herbal remedies. The New England journal of medicine
9. Akhila JS, Alwar M (2007) Acute toxicity studies and determination of median lethal dose. Curr Sci 93: 917-920.
10. Robinson S, Ockert D (2007) Challenging the regulatory requirement for conventional acute toxicity studies in pharmaceutical drug development. Toxicology 231: 96.
11. Scicchitano P, Cameli (2014) Nutraceuticals and dyslipidaemia: Beyond the common therapeutics. Journal of Functional Foods 6: 11-32.
12. Elshal, MF, Kumosani TA (2012) Influence of defatted flaxseed diet on insulin sensitivity, vascular permeability and lipid profile in a rat model of type 2 diabetes mellitus. Journal of Medicinal Plants Research 6: 2188-2193.

13. Broca C, Manteghetti M, Gross R, Baissac Y, Jacob M, et al. (2000) 4-Hydroxyisoleucine: effects of synthetic and natural analogues on insulin secretion. Eur J Pharmacol 390: 339-345.

14. Sharma BG, Viswanath (2008) Effects of flavonoid-rich extract from seeds of Eugenia jambolana (L.) on carbohydrate and lipid metabolism in diabetic mice. Food chemistry 110: 697-705.

15. Ghule AE, Jadhav SS (2012) Effect of ethanolic extract of seeds of Linum usitatissimum (Linn.) in hyperglycaemia associated ROS production in PBMNCs and pancreatic tissue of alloxan induced diabetic rats. Asian Pacific Journal of Tropical Disease 2: 405-410.

16. No OT (2001) 423: Acute oral toxicity-acute toxic class method. OECD Guidelines for the Testing of Chemicals pp: 1-14.

17. Lilienblum W, Dekant W, Foth H, Gebel T, Hengstler JG, et al. (2008) Alternative methods to safety studies in experimental animals: role in the risk assessment of chemicals under the new European Chemicals Legislation (REACH). Arch Toxicol 82: 211-236.

18. Ghai C (2012) A textbook of practical physiology. JP Medical Ltd.

19. John M (1972) Laboratory Medicine Hematology. CV Mosby Co, St. Louis.

20. Dacie J, Lewis S (1991) Practical Textbook of Haematology. (7thedn) Edinburgh. Church Livingstone.

21. Wintrobe MM, Landsberg JW (1935) A standardized technique for the blood sedimentation test. The American Journal of the Medical Sciences 189: 102-114.

22. Raji R, Latha M (2014) Curative effect of elephantopus scaber linn. on n'-nitrosodiethylamine induced hepatocellular carcinoma in experimental rats. International Journal of Pharmaceutical Sciences and Research 5: 3942.

23. Natelson S, Scott ML, Beffa C (1951) A rapid method for the estimation of urea in biologic fluids. Am J Clin Pathol 21: 275-281.

24. Slot C (1965) Plasma creatinine determination a new and specific Jaffe reaction method. Scandinavian journal of clinical and laboratory investigation 17: 381-387.

25. Abdel-Barry JA, Al-Hakiem MH (2000) Acute intraperitoneal and oral toxicity of the leaf glycosidic extract of Trigonella foenum-graecum in mice. J Ethnopharmacol 70: 65-68.

26. Rathore S, Saxena S (2013) Potential health benefits of major seed spices. Int J Seed Spices 3: 1-12.

27. Chatterjea M, Shinde R (2011) Textbook of medical biochemistry (8thedn) Jaypee Brothers Medical Publishers Limited.

28. McMahon JM, White WL (1995) Review Article Cyanogenesis in cassava (Manihot esculenta Crantz). Journal of Experimental Botany 46: 731-741.

29. Baskin, S, Brewer T (2004) Cyanide poisoning. Textbook of military medicine: medical aspects of chemical and biological warfare. The Virtual Naval Hospital.

30. Baskin SI, Rockwood GA (2002) Neurotoxicological and behavioral effects of cyanide and its potential therapies. Military Psychology 14: 159.

31. Dart R, Bogdan G (2004) Acute cyanide poisoning: causes, consequences, recognition and management. Frontline First Responder 2: 19-22.

32. Keyes DC (2005) Medical response to terrorism: Preparedness and clinical practice, Lippincott Williams & Wilkins.

33. Gracia R, Shepherd G (2004) Cyanide poisoning and its treatment. Pharmacotherapy 24: 1358-1365.

34. Mushett CW, Kelley KL, Boxer GE, Richards JC (1952) Antidotal efficacy of vitamin B12a (hydroxo-cobalamin) in experimental cyanide poisoning. Proc Soc Exp Biol Med 81: 234-237.

35. Borron SW (2006) Recognition and treatment of acute cyanide poisoning. J Emerg Nurs 32: S12-18.

36. Chaturvedi A, Kumar MM, Bhawani G, Chaturvedi H, Kumar M, et al. (2007) Effect of ethanolic extract of Eugenia jambolana seeds on gastric ulceration and secretion in rats. Indian J Physiol Pharmacol 51: 131-140.

37. Vennila S, Bupesh G (2015) Efficacy of Aqueous Extract of Helicteres isora on Glucose Level in Type-2 Diabetic Patients Practicing Yoga. A Cohort Study. Journal of Diabetes & Metabolism.