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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(6): 298-308

www.thepharmajournal.com
Received: 02-03-2022
Accepted: 06-04-2022

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Oral exposure of *Morinda citrifolia* Linn. fruit extract coated silver nanoparticles: Effects on haematological and biochemical parameters in wistar rats

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Abstract
The present study was carried out to evaluate the effect of *Morinda citrifolia* fruit extract coated SNPs on sub-acute oral toxicity in wistar rats. SNPs synthesized by biological reduction of (AgNO3) with neem 5% extract. SNPs synthesis and coating were confirmed colour pale yellow to dark brown. TEM used for characterization. In this study 50 wistar rats of either sex were divided into 5 groups, each group contain 10 rats. Group I (control), Group II (Low dose), group III (Medium dose), group IV (High dose) and group V (Satellite, highest dose). All treatment groups given orally with 0.17 mg/kg SNPs common offered with given McFE,100 mg/kg McFE (G- II), 200mg/kg McFE (G- III), 400mg/kg McFE (G-IV) and Satellite group (G-V) 800 mg/kg McFE and observed extra 14 day after trial to assess post withdrawal effect of treatment. The haemato-biochemicals observations note within normal limits suggest no adverse effect at all dose.

Keywords: *Morinda citrifolia*, sub-acute oral toxicity, synthesis of silver nanoparticles

1. Introduction
The dwarf is referred to as Nano in Greek. The use of nanotechnology in the diagnosis, monitoring, control, and treatment of diseases is referred to as nanomedicine. Nanotechnology is a combination of sophisticated manufacturing science and engineering in which material synthesis is focused at the nanoscale scale (1-100 nm). Catalysis, energy, the environment, agriculture, optics, sensors, computers, and many more fields rely on it. Nanoparticles are key building blocks of nanotechnology because of their unique physical, chemical, and biological capabilities. The design and synthesis of nanostructures for biomedical applications resulted from developments in nanotechnology in medical sciences.
The potential toxicity of nanoparticles on biological and ecological systems. This is a new topic of research into ultrafine particle toxicity. The chemical reactivity and biological activity of particles with a higher surface area to volume ratio are impacted by scale, shape, purity, crystallinity, electronic characteristics, surface function, solubility, and stability of NPs. Colloidal silver nanoparticles are small particles with diameters ranging from 1 to 100 nanometers that are employed in engineering, manufacturing, and healthcare. Its range and nature of functions have expanded, and it is likely to grow in the future, with significant commercial value.
The plant 'Noni' (*Morinda citrifolia*), which belongs to the Rubiaceae family. It has a wide range of therapeutic uses. Its fruit contains around 200 bioactive chemicals, such as phenolic compounds, anthraquinones, organic acids, beta-carotenoids, terpenoids, alkaloids, volatile and non-volatile components. *M. citrifolia* fruit have been used for treatment of various diseases including diabetes, high blood pressure, inflammation or cancer. *M. citrifolia* traditionally used in bone and wound healing is reported. Noni has been reported to have antibacterial, antiviral, antifungal, antitumour, antihelmintic, analgesic, hypotensive, anti-inflammatory, and immune enhancing properties.
Its aqueous and methanol fruit extracts include a wide range of phytoconstituents, including steroids, cardiac glycosides, phenol, terpenoides, alkaloids, carbohydrates, tannins, flavonoids, reducing sugar, lipids, and fats, as well as acidic chemicals found in the aqueous extract.

2. Materials and Methods
The Present study was carried out to evaluate sub-acute oral toxicity of *Morinda citrifolia* fruit aqueous extract coated silver nano particles in 50 wistar rats as per OECD Guidelines No. 407.

The materials required and methods used during various investigations are presented and at Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra.

3. Experimental Animals

The current study involved 50 wistar rats of either sex, aged 4-6 weeks and weighed 200 to 250g. Rats with a live body weight of less than 20% of their mean body weight at the time of randomization were chosen based on physical and behavioural evaluation. Animals were housed in polypropylene cages, given ad libitum food and water, and kept under conventional laboratory conditions in the Department of Veterinary Pharmacology and Toxicology's laboratory animal home. The Institutional Animal Ethical Committee (IAEC) accepted the experimental procedure in accordance with CPCSEA rules via resolution no. IAEC / 79 / 21, dated October 13, 2021. At least 5 to 7 days prior to the start of the experiment, these animals were kept under continual observation. All precautions and care were taken to ensure that the animals were free of disease.

Acclimatization, grouping and identification of experimental animals

Wistar rats were randomly randomized into five groups, each containing ten rats of either sex, after five days of acclimatisation to experimental laboratory condition. Except for the satalite group (given with the maximum dose), which was kept for an additional 14 days (without treatment) to investigate reversibility, persistence, delayed toxicity, and the post withdrawal effect, all of the animals were kept for the 28-day experimental period. Wistar rats were maintained in separate cages, each with a label, cage number, group details, treatment type, and marking to help researchers identify the animals.

4. Drugs and Chemicals

4.1 Silver nitrate

To produce silver nanoparticles (SNPs) by biological reduction method using *Azadirachta indica* (neem) leaf extract silver nitrate was used.

4.2 *Morinda citrifolia* (Noni) Fruits Extract

Aqueous extract of *Morinda citrifolia* L. fruit was prepared by cold extraction method. Noni fruits were procured from the VNMKV campus, Parbhani (Figure 2) and fruits cut into small pieces to keep for shed dry. For making powder the dried fruit pieces were ground to powder with the help of an grinder (Figure 3). Then, 20% aqueous solution was prepared by dissolving 200 grams of powder in double distilled water and then the final quantity was prepared to 1 liter. It was mixed thoroughly and allowed to soak for 48 hours at 4_0 C in refrigerator with intermittent shaking on flask shaker. Thus, solutions obtained was filtered by using muslin cloth and then by using filter paper. Then it was allowed to dry under shade and thus the aqueous extract of *Morinda citrifolia* fruit was finally done. (Figure 4).

4.3 Synthesis, Coating and Characterization SNPs

Synthesis of Silver nanoparticles was confirmed by color change, which was very well visible with the naked eyes. When 9 ml of 1mM silver nitrate was used solution was reacted with 1ml of 5% *Azadirachta indica (neem)* leaves extract, instantly colour was changed. After 30 minutes of incubation, the solution turned to pale yellow to dark brown and then brownish black. When the tubes were remains for 2 hours for incubation the colour intensified and tuned to brownish-black colour. To confirm this colour change was due to the formation of Silver nanoparticles, one silver nitrate solution chemical was used control (1mM, 10ml) and another extract control (9ml distilled water ± 1ml 5% *Azadirachta indica* leaf extract) was taken to compared (Figure 1).

4.4 Drug Administration

For these present study, given formulation administered by using oral gavage for 28 days of experimental period (Figure 5).

5. Experimental Design

Table 1: Experimental design

Group	Name of Group	Treatments	Dose (mg/kg)	Route
I	Healthy control	Deionized water	1 ml	p.o.
II	McFECSNPs (Low Dose)	SNPs	0.17	Oral gavage
		McFE	100	
III	McFECSNPs (Middle Dose)	SNPs	0.17	Oral gavage
		McFE	200	
IV	McFECSNPs (High Dose)	SNPs	0.17	Oral gavage
		McFE	400	
V	Satellite Group (Highest Dose)	SNPs	0.17	Oral gavage
		McFE	800	

6. Collection of Sample

For the experimental trials, Blood samples were collected on the 0thday, 7th day, 14th day & on the last day of experiment (28th day), from retro-orbital plexus with the help of capillary tube for estimations of haematological and biochemical parameters.

7. Parameters Studied

7.1 General Observations

All the animals in all the groups were daily examined for any abnormal behavioral changes, changes in water and feed intake and any mortality if any.

7.2 Haematological and Serum Biochemical Parameter Estimations

In this study the hematological parameters were evaluated to find the out any deviations in Hemoglobin (Hb) by Jain (1996) [7], Total Erythrocyte Count (TEC) by Sastry (1989) [18], Total Leucocyte Count (TLC) by Sastry (1989) [18], Differential Leucocyte Count (DLC) by Weiss and Wardrop (2010) [22], Blood clotting time (BCT) by Benjamin (1978) [1]. The serum biochemical parameters *viz*. AST (by UV kinetic method); ALT (by UV kinetic method), BUN (by Berthelot method), Creatinine (by Alkaline picrate method) and Total Protein (by Biuret method) also been investigated.

7.3 Statistical Analysis

By using Factorial Randomized Block Design (FRBD) and Completely Randomized block Design (CRD) as per requirement and interpreted. The data obtained from various parameters from all the groups was analyzed by as per method suggested by Panse, U.G. and P.V. Sukhatme Statistical methods for Agricultural workers, ICAR, Publication New Delhi.

8. Results and Discussion

8.1 *Morinda citrifolia* fruit extract recovery

The moisture content of fruit powder was 38% (DM basis) was obtained from 8.6 kg wet fruit weight and 3.4 kg dry fruit powder weight after shade drying. Extraction Yield = (Weight of dry powder obtained (W1) x 100) / Total weight of wet fruits taken (W2) = 155 x 100 / 3400 g = 4.56 g%.

8.2 General Observations

Behavioral changes all experimental rats observed for general behaviour during course of experiment and the extended period were alert, active and free from any adverse reaction or toxicity. Feed and water consumption-No alterations in feed and water intake in any of the experimental groups were observed on regular basis (Numerical data are not presented). Mortality-All exposed rats did not showed any mortality in any of the treatment groups and the healthy control, all animals were healthy, alert and active.

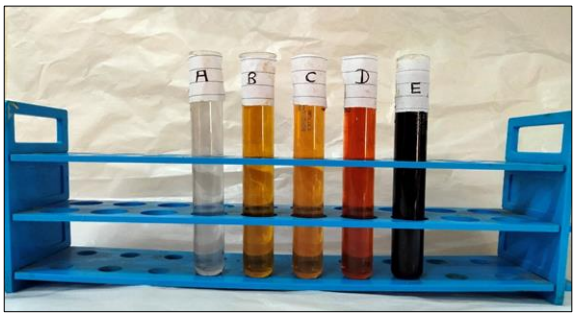

Fig 1: Confirmation of Silver nanoparticles synthesized by observing colour change

Fig 2: *Morinda citrifolia* (Noni) fruits

Fig 3: Powder of *Morinda citri*folia fruits

Fig 4: Aqueous Fruit Extract of *Morinda citrifolia* Linn.

Fig 5: Oral gavaging of McFECSNPs in wistar rats

8.3 Hematological Parameters

The hematological parameters *viz*. hemoglobin, TEC, TLC, DLC and blood clotting time were done on 0, 7th, 14th and 28th day of the experiment. Results obtained are presented and discussed chronologically in light of the available literature in this section.

Table 2: Hb level following subacute oral toxicity of McFECSNPs in different experimental group of wistar rats

Group	Hb (g/dl), Mean values ± SE				Stat	CD
	0 day (Initial)	7th day	14th day	28th Day		
I	13.75 ± 0.23	13.78^{b} ± 0.30	13.35^{c} ± 0.26	13.36^{b} ± 0.35	NS	At 5% (0.686) At 1% (0.892)
II	13.84 ± 0.19	13.65^{b} ± 0.20	13.72^{bc} ± 0.19	13.80^{b} ± 0.18	NS	
III	13.82 ± 0.18	13.82^{b} ± 0.18	13.96 ± 0.09	14.11^{b} ± 0.33	NS	
IV	13.42^{q} ± 0.22	13.52^{bq} ± 0.26	13.66^{bcq} ± 0.20	14.88^{ap} ± 0.16	HS	
V	13.71^{q} ± 0.47	14.75^{ap} ± 0.13	14.82^{ap} ± 0.16	14.98^{ap} ± 0.20	S	
Stat	NS	HS	HS	HS		
CD	At 5% (0.686); AT 1% (0.892)					

There was no toxicity however Hb levels significantly increase in all McFECSNPs treated groups within the normal physiological range.

Superscripts a,b,c,d,e shows significant difference within the column (between different groups on specific day) (p<0.05)

Superscripts p,q,r shows significant difference within the row (between different days in specific groups) (p<0.05)

In the group II (Low Dose) the mean Hb values on 0, 7th, 14th and 28th day were 13.84±0.19, 13.65 ± 0.20, 13.72 ±0.19 and 13.80± 0.18 g/dl respectively. Hb level in this group was not significantly different compared to day 0 value. There was increase in mean Hb values than group I (healthy control), however values were observed within the normal physiological range.

The mean Hb levels in group III on 0, 7, 14th and 28th day were 13.82±0.18, 13.82±0.18, 13.96±0.09 and 14.11±0.33 g/dl respectively. These values not significantly different as compared to day and highly significant on 17th, 14th and 28th day than group I significantly differs from group II when compared to group I (healthy control). There was no significant difference in this group compare to group-II, however these values were found within the normal physiological limit.

The mean Hb values in group IV (high dose-III) on day 0, 7th, 14th and 28th were observed to be 13.42±0.22, 13.52±0.26, 13.66±0.20 and 14.88±0.16 g/dl respectively. Increase in mean Hb value on 28th day as compared to day 0 value and group II and III than group I (healthy control). The rise in Hb level was within the normal physiological limit. differences in mean Hb values were highly significant on 7th, 14th and 28th compared to group I (healthy control) and day 0 value. The mean Hb values in group V (Satellite group) on day 0, 7th, 14th and 28th were 13.71±0.47, 14.75 ±0.13, 14.82 ±0.16 and 14.98 ±0.20 g/dl respectively. These values progressively increase from day 7th, 14th and 28th as compared to 0 day value. There was significant increase in Hb values in this group as compared to healthy control (group I). The increase in Hb levels on 28th day in this group was highly significant in group I but found within the normal physiological ranges.

Table 3: Total Erythrocytes Count (TEC) following subacute oral toxicity of McFECSNPs in different groups of wistar rats

Group	TEC (x10^6 / µl), Mean values ± SE				Stat	CD
	0 day	7th day	14th day	28th day		
I	$6.67^{p} \pm 0.33$	$6.70^{p} \pm 0.19$	$6.65^{q} \pm 0.25$	$6.82^{b} \pm 0.24$	NS	At 5% (0.664) At 1% (0.871)
II	$6.55^{q} \pm 0.18$	$6.05^{q} \pm 0.22$	$6.33^{q} \pm 0.15$	$7.36^{bp} \pm 0.29$	HS	
III	6.66 ± 0.15	6.64 ± 0.27	6.55 ± 0.15	$7.15^{b} \pm 0.26$	NS	
IV	$6.86^{q} \pm 0.07$	$6.55^{q} \pm 0.15$	$6.22^{q} \pm 0.20$	$8.62^{ap} \pm 0.27$	HS	
V	6.57 ± 0.15	6.69 ± 0.15	6.36 ± 0.15	6.78 ± 0.21	NS	
Stat	NS	NS	NS	HS		
CD	At 5% (0.664); At 1% (0.871)					

There was no significant elevation in TEC on 28th day in all treated groups, however changes observed were within normal physiological limits.

The mean TEC values in group I (healthy control) rats on 0, 7th, 14th and 28th day were 6.67±0.33, 6.70±0.19, 6.65±0.25 and 6.82±0.24 million/µl respectively. There was no nonsignificant difference in TEC values observed on 0, 7th, 14th and 28 day of trial. However these values were observed within the normal physiological limits. There were nonsignificant differences in mean TEC values on 28th day as compared to 0 day, these values also observed within normal physiological limits.

On the day 0, 7th, 14th and 28th of the trial TEC values in group II rats were 6.55 ± 0.18, 6.05 ± 0.22, 6.33 ± 0.15 and 7.36 ± 0.29 million/µl respectively. There was significantly arise in TEC values on 28th day was noted as compared to 0day value. However these values found within the normal physiological limits. There was significant altered observed in TEC value in Group II as compared to healthy control throughout the experiment, but the mean values of TEC was relatively reduced in this group on 0 day when compared to Group-1(healthy control).

Superscripts a,b,c,d,e shows significant difference within the column (between different groups on specific day) (p>0.05)

Superscripts p,q,r shows significant difference within the row (between different days in specific groups) (p = 0.05)

On day 0, 7th, 14th and 28th the TEC values in group III were observed to be 6.66 ± 0.15, 6.64 ± 0.27, 6.55 ± .0.15 and 7.15 ± 0.26 million/µl respectively. There was non-significant increase in TEC values on 28th day as compared to 0 day of group II. However these values were within the normal physiological limits. There was significantly increase in TEC values on 28th day in this group as compared to healthy control (Group-I). Also when compared to the low dose of McFECSNPs (group-II). There were non significant changes noticed throughout the experiment.

The TEC values in group IV rats on day 0, 7th, 14th and 28th were 6.86 ± 0.07, 6.55 ± 0.15, 6.22 ± 0.20 and 8.62 ± 0.27 million/µl respectively. In this group TEC values on 28th day of the experiment was significantly increased as compared to health control (Group I). These values were found within the normal physiological limits. The TEC values were significantly increased on 28th day as compared to 0, 7th and 14th day of the experiment. Similarly noted in the healthy control group (group I) on 28th day.

TEC values in Group V (Satellite Group) on 0, 7th, 14th and 28th day were 6.57 ± 0.15, 6.69 ± 0.15, 6.36 ± 0.15 and 6.78 ± 0.21 million/µl respectively. On 28th day TEC values as against 0 day elevated non significantly. On 28th day TEC values were significantly altered as compared to other groups. However these values found within the normal physiological limits.

Table 4: Effect of McFECSNPs on TLC (Mean values x10^6 / µl ± SE,) following oral toxicity in different experimental groups of rats

Group	TLC (x10^6 / µl), Mean values ± SE				Stat	CD
	0 day	7th day	14th day	28th day		
I	8.05 ± .09	8.90 ± 0.34	8.90 ± 0.27	8.78 ± 0.29	NS	At 5% (0.852) At 1% (1.114)
II	$8.20^{p} \pm 0.52$	$8.76^{p} \pm 0.21$	$9.02^{q} \pm 0.49$	$9.12^{q} \pm 0.48$	S	
III	8.47 ±0.57	8.50 ± 0.30	8.50 ± 0.38	9.01 ± 0.50	NS	
IV	8.50 ± 0.30	8.67 ± 0.16	8.71 ± 0.19	9.12 ± 0.48	NS	
V	8.66 ± 0.16	8.70 ± 0.19	8.80 ± 0.28	9.08 ± 0.51	NS	
Stat	NS	NS	NS	NS		
CD	At 5% (0.852); At 1% (1.114)					

Subacute oral toxicity of McFECSNPs did not altered TLC values except in group II, these values were found within the normal physiological limits.

Superscripts a,b,c,d,e shows significant difference within the column (between different groups on specific day) (p>0.05)

Superscripts p, q, r shows significant difference within the row (between different days in specific groups) (p>0.05)

In group I (healthy control), the TLC values on day 0, 7th, 14th and 28th were found to be 8.05±0.09, 8.90±0.34, 8.90±0.27 and 8.78± 0.29 thousand/µl respectively. TLC values in this group were not significantly different within the group and between interval days when compared to their respective control, however these values were found within the normal physiological limit.

In group II (Low Dose) rats the TLC values on 0, 7th, 14th and 28th day were 8.20±0.52, 8.76±0.21, 9.02±0.49 and 9.12±0.48 thousand/ µl respectively. There was significant difference in TLC values observed in this group when compared with group I (healthy control) and group III, IV and V. significantly elevated TLC value was observed in this group on 28th day compared to 0 day, however these values were observed within the normal physiological limits.

TLC values in group III, IV and V were not significantly different within the group and between interval days when compared to their respective control groups and the values found within the normal physiological limits. This indicates that the higher dose of McFECSNPs had not altered TLC values in wistar rats.

Table 5: Lymphocytes count (%) following subacute oral toxicity of McFECSNPs in different experimental groups of wistar rats

Group	Lymphocytes Count (%), Mean ± SE				Stat	CD
	0 day	7th day	14th day	28th day		
I	64.40 ± 0.68	64.90^{b} ± 0.46	65.57^{ab} ± 0.58	64.60^{bc} ± 0.72	NS	At 5% (2.515) At 1% (3.304)
II	64.24^{q} ± 0.87	63.35^{bqr} ± 1.22	61.67^{cr} ± 0.37	67.26^{ap} ± 0.45	HS	
III	64.14^{q} ± 0.62	63.57^{bq} ± 0.43	62.75^{bcq} ± 0.83	66.47^{abp} ± 0.82	HS	
IV	65.62 ± 1.64	67.82^{a} ± 1.04	67.61^{a} ± 0.96	66.01^{ab} ± 0.64	NS	
V	65.52 ± 0.92	63.23^{b} ± 0.61	61.25^{c} ± 1.91	63.80^{c} ± 0.54	NS	
Stat	NS	HS	HS	HS		
CD	At 5% (2.515) ; At 1% (3.304)					

Superscripts a,b,c,d,e shows significant difference within the column (between different groups on specific day) (p<0.05)

Superscripts p,q,r shows significant difference within the row (between different days in specific groups) (p<0.05)

There was significant increase in lymphocytes count and the difference was highly significant on 28th day than 7th and 14th in group- II and III when compared to healthy control (Group I).

In group I (healthy control) rats the lymphocyte counts on the day 0,7th, 14th and 28th were observed to be 64.40±0.68, 64.90±0.46, 65.57±0.58 and 64.60±0.72% respectively. No significant difference in lymphocytes count was observed within the time interval days and the values observed within the normal physiological range.

In group II the lymphocytes count in rats on day 0, 7th, 14th and 28th were observed 64.24±0.87, 63.35 ±1.22, 61.67 ± 0.37 and 67.26±1.45% respectively. In this group there was decrease in lymphocyte counts on day 7th and 14th and increased in lymphocytes count on day 28th were observed as compared to day 0 value (Group I). However lymphocytes cunt observed within the normal physiological ranges. On 28th day there was significant increase in lymphocytes count than the healthy control (group I) and the difference was statistically highly significant in this group when compared to healthy control (Group I).

On day 0, 7th, 14th and 28th the lymphocyte counts in group III (McFECSNPs Dose-II) rats were 64.14±0.62, 63.57±0.43, 62.75±0.83 and 66.47 ± 0.82% respectively. There was significant decrease in lymphocytes count on 7th and 14th day and increased on 28th day were observed when compared to 0 day value (Group I), however the values were observed within the normal physiological range. Also there was difference in lymphocytes count observed on 14th day as compared to healthy control (Group I). In this group there was increased in lymphocytes count on 28th day was highly significant when compared to healthy control (Group I). But there was slight difference in lymphocytes counts in Group III and Group II, but significant difference was observed throughout the experiment in both groups when compared to healthy control (Group I).

In group IV (High dose) the lymphocytes count in rats on day 0, 7th, 14th and 28th were 65.62±1.64, 67.82±1.04, 67.61±0.96 and 66.01± 0.64,% respectively. These values within the groups was highly significant compared to group I (healthy control), however in this group there was significantly higher lymphocytes count than group II, III and V when compared to group I (healthy control), however these values found within the normal physiological limits. In this group there was significant increase in lymphocytes count at 14th day as compared to group II and III.

The lymphocytes count on day 0, 7th, 14th and 28th in group V (satellite group) were observed to be 65.52±0.92, 63.23±0.61, 61.25 ±1.91 and 63.80±0.54, % respectively. In this group there was decrease in lymphocytes count on 7th, 14th and 28th day and the difference was non-significant when compared to day 0 values and the values were found within the normal physiological limits.

Table 6: Monocytes count following subacute oral toxicity of McFECSNPs in different experimental groups of wistar rats

Group	Monocytes Count (%), Mean ± SE				Stat	CD
	0 day	7th day	14th day	28th day		
I	3.69 ± .35	3.43 ± .25	3.57 ± 0.39	3.86± 0.36	NS	At 5% (0.970) At 1% (1.287)
II	3.26 ± 0.24	4.18 ±0.52	3.76 ±0.55	3.71 ± 0.16	NS	
III	3.42 ± 0.28	4.04 ± 0.51	4.25 ± 0.35	3.13 ± 0.31	NS	
IV	3.88 ± 0.33	4.05 ± 0.52	4.10 ± 0.23	3.22 ± 0.25	NS	
V	3.88 ± 0.33	3.22 ± .33	3.55 ± 0.26	3.81 ± 0.28	NS	
Stat	NS	NS	NS	NS		
CD	At 5% (0.970); At 1% (1.287					

Superscripts a,b,c,d,e shows significant difference within the column (between different groups on specific day) (p<0.05)
Superscripts p,q,r shows significant difference within the row (between different days in specific groups) (p<0.05)
In group I (healthy control) rats the monocyte counts on day 0, 7th, 14th and 28th were 3.69±0.35, 3.43±0.25,3.57± 0.39 and 3.86±0.36% respectively. There was decrease in monocytes count, the difference in monocytes observed on 14th day was non-significant when compared to day 0 value and values observed within the normal physiological limits.
The monocytes count was not significantly different within the groups and between interval days when compared to their respective control groups, however, these values were found within normal physiological limits.
From the above results it is revealed that, there was increase in monocytes count in all treatment groups except in group V, where the treatment of McFECSNPs at all dose levels had no significant effect on monocytes count as compared to healthy control (Group I) and day 0 values of respective treatment groups. However the monocytes values were found within the normal physiological limits.

Table 7: Neutrophils Count (%) following subacute oral toxicity of McFECSNPs in different experimental group of wistar rats

Group	Neutrophils Count (%), Mean ± SE				Stat	CD
	0 day	7th day	14th day	28th day		
I	27.21 ± 1.82	27.25 ± 1.87	27.31 ± 1.19	27.37 ± 1.08	NS	At 5% (4.278) At 1% (5.614)
II	27.22 ± 2.00	27.23 ±2.48	27.50 ± 1.37	27.98 ± 1.36	NS	
III	27.65 ± 1.61	27.87 ± 1.91	28.45 ± 1.61	28.22 ± 1.33	NS	
IV	27.17 ± 1.62	27.12 ± 1.17	27.22 ± 1.45	28.03 ± 1.30	NS	
V	27.21 ± 1.28	28.62 ± 1.51	28.81 ± 1.51	28.85 ± 1.75	NS	
Stat	NS	NS	NS	NS		
CD	At 5% (4.278); At 1% (5.614)					

Superscripts a, b, c, d, e shows significant difference within the column (between different groups on specific day) (p<0.05)
Superscripts p, q, r shows significant difference within the row (between different days in specific groups) (p<0.05)
The neutrophils count was numerically increased but had no significant difference within the groups and between interval days when compared to their respective controls, however, these values were found within the normal physiological limits.

Table 8: Eosinophils count following subacute oral toxicity of McFECSNPs in different groups of wistar rats

Group	Eosinophils Count (%), Mean ± SE				Stat	CD
	0 day	7th day	14th day	28th day		
I	4.50 ± 0.27	4.43 ± 0.26	4.40 ± 0.22	4.02^{c} ± 0.24	NS	At 5% (0.664) At 1% (0.875)
II	4.32 ± 0.30	4.32 ± 0.29	4.33 ± 0.35	4.11^{bc} ± 0.15	NS	
III	4.60 ± 0.18	4.46 ± 0.30	4.35 ± 0.29	4.61^{ab} ± 0.22	NS	
IV	4.80^{p} ± 0.21	4.01^{q} ± 0.34	4.02^{q} ± 0.17	5.09^{ap} ± 0.13	HS	
V	4.40 ± 0.25	5.05 ± 0.31	5.10 ± 0.29	4.70^{a} ± 0.15	NS	
Stat	NS	NS	NS	HS		
CD	At 5% (0.664); At 1% (0.875)					

Superscripts a, b, c, d, e shows significant difference within the column (between different groups on specific day) (p<0.05)
Superscripts p, q, r shows significant difference within the row (between different days in specific groups) (p<0.05)
On the day 0, 7th, 14th and 28th, the eosinophils count in group I (healthy control) rats were observed to be 4.50±0.27, 4.43±0.26, 4.40±0.22 and 4.02±0.24, % respectively. There was significant decrease in eosinophils count observed on day 7th, 14th and 28th as compared to 0 day, however the eosinophils count is observed within the normal physiological range.
The eosinophil counts in group II rats on 0, 7th, 14th and 28th day were 4.32±0.30, 4.32±0.29, 4.33±0.35 and 4.11±0.15, % respectively. These values were not significantly different when compared to day 0 value, However there was significant difference in eosinophils count in this group compare with group III IV and V when compared to group I (healthy control). However these values were observed within the normal physiological limits.
On the day 0, 7th, 14th and 28th, the eosinophils count in group III rats were 4.60±0.18, 4.46±0.30, 4.35±0.29 and 4.61±0.22, % respectively. There was no significant difference in eosinophils count on 7th 14th and 28th day compared to 0 day counts, however difference in eosinophil counts of this group was highly significant as compare to group II IV and V than the healthy control (Group I). However these values were within the normal physiological limits. On 7th and 14th day eosinophils count was not significantly different compared to 0 day, also there was non significant increases in eosinophils count on 28th day than healthy control group.
In Group IV the eosinophils count on day 0, 7th, 14th and 28th were 4.80±0.21, 4.01±0.34, 4.02±0.17 and 5.09±0.13, % respectively. Eosinophils count of this group and on 28th day significantly increased than the healthy control (Group I). Statistically there was highly significant difference in eosinophils count on 28th day as compared to 0 day value and also the healthy control (Group I). However these values were found within the normal physiological limits.
On the day 0, 7th, 14th and 28th, the eosinophils count in group V (satellite group) rats were 4.40±0.25, 5.05±0.31, 5.10 ±0.29 and 4.70±0.15, % respectively. There was increase in eosinophils count of this group on 28th day than 7th and 14th day as compared to day 0 value, however had no significant difference, these values observed within the normal physiological limits.
The McFECSNPs at higher doses increased the eosinophils count, however the difference was highly significant on 28th day as compared to healthy control (group I).

Table 9: Basophils count following subacute oral toxicity of McFECSNPs in different experimental group of wistar rats

Group	Basophils Count (%), Mean ± SE				Stat	CD
	0 day	7th day	14th day	28th day		
I	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.02	0.13 ± 0.02	NS	At 5% (0.162) At 1% (0.218)
II	0.12 ± 0.01	0.15 ± 0.12	0.18 ± 0.11	0.18 ± 0.11	NS	
III	0.12 ± 0.01	0.15 ± 0.12	0.18 ± 0.11	0.18 ± 0.11	NS	
IV	0.13 ± 0.01	0.14 ± 0.01	0.15 ± 0.02	0.15 ± 0.02	NS	
V	0.13 ± 0.01	0.14 ± 0.02	0.18 ± 0.02	0.18 ± 0.02	NS	
Stat	NS	NS	NS	NS		
CD	At 5% (0.162); At 1% (0.218)					

Superscripts a, b, c, d, e shows significant difference within the column (between different groups on specific day (p<0.05)

Superscripts p, q, r shows significant difference within the row (between different days in specific groups (p<0.05)

On the day 0, 7th, 14th and 28th, the basophils count in group I rats (healthy control) were observed to be 0.12±0.01, 0.12±0.01, 0.13±0.02 and 0.13±0.02,% respectively. There was no significant difference in basophils count of this group at different time intervals when compared to day 0 values, however these values observed within the normal physiological rages.

On the day 0, 7th, 14th and 28th the basophil counts in group II rats were 0.12±0.01, 0.15±0.12, 0.18±0.11 and 0.18±0.11,% respectively. These values were not significantly different than 0 day values, however, observed values were within the normal physiological ranges. The numerically increase in basophils count was not statistically different within the group and between interval days when compared to respective day 0 and the healthy control values (group I). On the day 0, 7th, 14th and 28th, the blood clotting time in rats of Group III were 52.35±0.21, 52.43±0.21, 52.51±0.16 and 53.50±0.14, seconds respectively. When compared these values between interval day on 7th, 14th, 28th than 0 day found highly significant and on 28th day were found to be significant as slightly increased in this group. The clotting time on 28th day in this group was significantly increased than healthy control and day 0 value, however there was non significant difference observed on on 7th and 14th day in this group when compared to healthy control (group I).

On the day 0, 7th, 14th and 28th, the blood clotting time in group IV rats were observed to be 52.25±0.13, 52.30±0.15, 52.50±0.62 and 52.60±0.66, seconds respectively. These values when compared to group I and day 0 value were found to be not significant, except on day 28th where the blood clotting time was numerically reduced but not differs than group I.

On the day 0, 7th, 14th and 28th, the blood clotting time in group V (satellite group) rats were 52.28±0.14, 52.33±0.16, 52.40±0.21 and 52.50±0.22, seconds respectively. These values progressively increased as compared to 0 day value and not significatly differs from group I, however there was no significant difference on 28th day as compared to 0 day value.

Oral toxicity assessment of McFECSNPs in rats reveled significant increase in blood clotting time at all dose levels, however significant increase in blood clotting time was observed on 28th day in group III.

Table 10: Presents the mean blood clotting time (seconds) following subacute oral toxicity of McFESNPs in wistar rats

Group	Blood Clotting Time (Seconds)				Stat	CD
	0 day	7th day	14th day	28th day		
I	52.43 ± 0.21	52.55 ± 0.64	52.55 ± 0.64	52.65^{a} ± 0.24	NS	At 5% (0.947) At 1% (1.242)
II	52.49 ± 0.22	52.50 ± 0.62	52.55 ± 0.64	52.55^{a} ± 0.16	NS	
III	52.35^{q} ± 0.21	52.43^{q} ± 0.21	52.51^{q} ± 0.16	53.50^{bp} ± 0.14	HS	
IV	52.25 ± 0.13	52.30 ± 0.15	52.50 ± 0.62	52.60^{a} ± 0.66	NS	
V	52.28 ± 0.14	52.33 ± 0.16	52.40 ± 0.21	52.50^{a} ± 0.22	NS	
Stat	NS	NS	NS	S		
CD	At 5% (0.947); At 1% (1.242)					

Superscripts a, b, c, d, e shows significant difference within the column (between different groups on specific day) (p<0.05).

Superscripts p, q, r shows significant difference within the row (between different days in specific groups) (p<0.05)

On day 0, 7th, 14th and 28th the mean blood clotting time in group I (healthy control) rats were observed to be 52.43±0.21, 52.55±0.64, 52.55±0.64 and 52.65 ±0.24, seconds respectively. These values on the day 0, 7th, 14, 28th were not significantly different and increased the clotting time within the normal range when compared to healthy control (group I).

On the day 0, 7th, 14th and 28th rats in Group II were observed to be 52.49±0.22, 52.50±0.62, 52.55±0.64 and 52.55±0.16 seconds, respectively. These values were not significantly different when compared to 0 day values and group I (healthy control), except the values observed on day 28th which was found to be similar when compared with group I.

On the day 0, 7th, 14th and 28th, the blood clotting time in rats of Group III were 52.35±0.21, 52.43±0.21, 52.51±0.16 and 53.50±0.14, seconds respectively. When compared these values between interval day on 7th, 14th, 28th than 0 day found highly significant and on 28th day were found to be significant as slightly increased in this group. The clotting time on 28th day in this group was significantly increased than healthy control and day 0 value, however there was non significant difference observed on on 7th and 14th day in this group when compared to healthy control (group I).

On the day 0, 7th, 14th and 28th, the blood clotting time in group IV rats were observed to be 52.25±0.13, 52.30±0.15, 52.50±0.62 and 52.60±0.66, seconds respectively. These values when compared to group I and day 0 value were found to be not significant, except on day 28th where the blood clotting time was numerically reduced but not differs than group I.

On the day 0, 7th, 14th and 28th, the blood clotting time in group V (satellite group) rats were 52.28±0.14, 52.33±0.16, 52.40±0.21 and 52.50±0.22, seconds respectively. These values progressively increased as compared to 0 day value and not significantly differs from group I, however there was no significant difference on 28th day as compared to 0 day value.

Oral toxicity assessment of McFECSNPs in rats reveled significant increase in blood clotting time at all dose levels, however significant increase in blood clotting time was observed on 28th day in group III.

8.4 Serum Biochemical Parameters

The biochemical parameters *viz*. AST, ALT, serum total protein, BUN, creatinine in serum were estimated on 0, 7th, 14th and 28th day of experiment. Thus the results obtained are presented and chronologically discussed with the available literature in this section.

Table 11: Serum AST / SGOT activity following sub-acute oral toxicity of McFECSNPs in different experimental groups of wistar

Group	Serum AST / SGOT (g/dl); Mean ± SE				Stat	CD
	0 day (Initial)	7th day	14th day	28th day		
I	131.51 ± 2.13	129.17 ± 7.30	133.08 ± 6.39	133.32 ± 6.49	NS	At 5% (12.951) At 1% (17.025)
II	130.70 ± 5.03	129.34 ± 2.06	132.65 ± 1.14	131.96 ± 6.30	NS	
III	130.78 ± 5.06	131.75 ± 6.28	132.16 ±3.41	134.78 ± 3.06	NS	
IV	130.35 ± 1.91	130.41 ± 1.86	132.73 ± 2.99	133.01 ± 6.38	NS	
V	132.31 ± 1.10	130.85 ± 7.17	132.86 ± 1.09	134.91 ± 6.24	NS	
Stat	NS	NS	NS	NS		
CD	At 5% (12.951); At 1% (17.025)					

Superscripts a, b, c, d, e shows significant difference within the column (between different groups on specific day) (p>0.05)

Superscripts p, q, r shows significant difference within the row (between different days in specific groups) (p>0.05)

In group I (healthy control) rats the AST activity on day 0, 7th, 14th and 28th were observed to be 131.51±2.13, 129.17±7.30, 133.08 ±6.49 and 133.32±6.49, IU/L respectively.

AST activity within the groups and between interval days were not significantly different as compared to their respective controls (0 day) (initial value) and healthy control (group I). However, these values were observed within the normal physiological ranges. AST activity on day 7th and 14th was slightly increased and on 14th day was reduced as compared to healthy control (group I). However, AST activity not significantly altered compared to their respective controls.

McFECSNPs treatment at all dose levels did not significantly alter AST activity, however values observed within the normal physiological ranges.

Table 12: Serum ALT/SGPT level (g/dl) following oral toxicity of McFECSNPs in different experimental group of wistar rats

Group	ALT/SGPT (g/dl), Mean ± SE				Stat	CD
	0 day	7th day	14th day	28th day		
I	64.57 ± 1.12	65.55^{c} ± 1.15	66.05 ±1.88	66.29 ± 3.77	NS	At 5% (7.348) At 1% (9.652)
II	64.52 ± 1.92	69.77^{bc} ± 73.33	70.02 ±3.42	69.88 ± 3.25	NS	
III	64.81^{p} ± 1.40	73.33^{abq} ± 3.01	73.45^{qr} ± 4.70	73.47^{r} ± 4.90	S	
IV	64.55^{q} ± 1.71	75.12^{abp} ± 2.08	75.77^{p} ± 1.78	75.41^{p} ± 3.46	HS	
V	65.05^{q} ± 1.65	77.05^{ap} ± 2.59	77.10^{p} ± 2.29	77.62^{p} ± 2.49	HS	
Stat	NS	S	NS	NS		
CD	At 5% (7.348); At 1% (9.652)					

Superscripts a, b, c, d, e shows significant difference within the column (between different groups on specific day) (p>0.05)

Superscripts p, q, r shows significant difference within the row (between different days in specific groups) (p>0.05)

In group II rats, the ALT values on day 0, 7th, 14th and 28th were 64.52±1.92, 69.77±2.31, 70.02±3.42 and 69.88±3.25, IU/L. The activity at 7th, 14th and 28th days were not significant, on day 7th and 14th day there was increase in ALT activity but the difference was non significant. On 7th day there was significant increase in AST activity as compared to 0 day and healthy control (Group I).

In group III rats (Moderate Dose), the ALT activity on 0, 7th, 14th and 28th day were 64.81±1.40, 73.33±3.01, 73.45 ±4.70 and 73.47 ±4.90, IU/L respectively. There was significant increase in ALT activity on 7th, 14th and 28th days when compared to day 0 activity. In this group increased ALT activity on 28th day not significantly different from the day 7th and 14th compared to low dose (group II) and healthy control (group I).

In the group IV in rats (High Dose group), the ALT activity on 0, 7th, 14th and 28th day were 64.55±1.71, 75.12 ±2.08, 75.77±1.78 and 75.41±3.46, IU/L respectively. Progressive increase in ALT activity in this group was highly significant compared to day 0 activity. Similarly there was increased ALT activity on 7th day was higher than low (group II) and medium dose (group III) groups as compared to healthy control (group I).

In the group V rats (highest dose), the ALT activity on day 0, 7th, 14th and 28th were 65.05±1.65, 77.05±2.59, 77.10±2.29 and 77.62±2.49, IU/L respectively. In this group ALT activity difference within interval days was highly significant as compared to 0 day activity. On 7th day ALT activity were differed significantly than activity in group II, III, and IV as compared to healthy control (group I).

McFECSNPs increase ALT activity in dose and duration of treatment dependent manner. The highest dose on 14th and 28th day had significantly altered ALT activity in serum but not significantly different than healthy control (Group I).

Table 13: Serum Total Protein levels following subacute oral toxicity of McFECSNPs in wistar rats

Group	Serum TP (g/dl), Mean ± SE				Stat	CD
	0 day	7th day	14th day	28th day		
I	7.40 ± 0.47	7.45 ± 0.31	7.57^{b} ± 0.16	7.25 ± 0.26	NS	At 5% (0.700) At 1% (0.923)
II	7.38^{q} ± 0.17	7.95^{q} ± 0.31	8.72^{ap} ± 0.16	7.60^{q} ± 0.22	HS	
III	7.56^{p} ± 0.32	7.93^{p} ± 0.25	8.70^{ap} ± 0.26	7.76^{q} ± 0.19	S	
IV	7.32 ± 0.26	7.58 ± 0.28	7.85^{b} ± 0.15	7.86 ± 0.18	NS	
V	7.48^{q} ± 0.20	7.94^{q} ± 0.38	8.4^{ap} ± 0.15	7.55^{q} ± 0.16	HS	
Stat	NS	NS	HS	NS		
CD	At 5% (0.700); At 1% (0.923)					

Superscripts a, b, c, d, e shows significant difference within the column (between different groups on specific day) (p>0.05)

Superscripts p, q, r shows significant difference within the row (between different days in specific groups) (p>0.05)

On the day 0, 7th, 14th and 28th, the TP values in group I (healthy control) rats were 7.40±0.47, 7.45±0.31, 7.57±0.16 and 7.25±0.26 g/dl, respectively. There was no significant difference in TP values observed between interval days when compared to day 0 values and these values observed within the normal physiological limits.

On the day 0, 7th, 14th and 28th, the TP values in group II rats were 7.38±0.17, 7.95±0.31, 8.72±0.16 and 7.60±0.22, g/dl respectively. In this group there was significant increase in TP value on 7th, 14th and 28th day as compared to 0 day and healthy control (group I) and difference was highly significantly when compared to healthy control (group I).

On day 0, 7th, 14th and 28th, the TP values in group III rats were 7.56±0.32, 7.93±0.25, 8.70±0.26 and 7.76±0.19, g/dl respectively. There was significant increase in TP values on 14th day than 7th and 28th day as compared to 0 day value. There was increase in TP value in group II (low dose) and V (highest dose) than group III (moderate dose) as compared to healthy control (group I) and difference was highly significant. Increasing TP values suggest ensuing renal damage in rats.

In group IV rats the TP values on 0, 7th, 14th and 28th day were 7.32±0.26, 7.58±0.28, 7.85± 0.15 and 7.86±0.18, g/dl respectively. These values differ non-significantly as compared to 0 day value. There was significant decrease in TP values in this group on 14th day than group II, IV and V as compared to group I (healthy control). This indicates that the highest dose on 14th day of McFESNPs treatment may have mild renal damage in treated wistar rats.

On the day 0, 7th, 14th and 28th, the TP values in group V (highest dose group) were 7.48±0.20, 7.94±0.38, 8.44±0.15 and 7.55±0.16. g/dl respectively. There was significant increase in TP values observed on 14th day than 7th and 28th as compared to healthy control (group I).

Subacute oral toxicity of McFECSNPs increase TP in serum was dose and duration of treatment dependent manner had no toxicological relevance since the treated rats were survived healthy and alert.

Table 14: Effect of McFECSNPs on BUN level (g/dl) following oral toxicity in different experimental groups of rats

Group	BUN (g/dl), Mean values ± SE,				Stat	CD
	0 day	7th day	14th day	28th day		
I	22.81 ± 0.83	22.83^{c} ± 0.51	22.85^{d} ±1.20	22.38 ± 1.30	NS	At 5% (2.772) At 1% (3.645)
II	21.66^{q} ± 0.84	23.72^{bcpq} ±1.52	26.08^{cp} ±1.22	24.36^{pq} ± 0.68	S	
III	22.92^{r} ± 0.98	26.56^{abq} ± 1.0	31.86^{abp} ± 0.74	25.60^{q} ± 0.55	HS	
IV	22.68^{q} ± 0.78	25.90^{abcq} ± 1.57	31.23^{bp} ± 1.19	25.31^{q} ± 0.79	HS	
V	22.28^{r} ± 0.85	27.75^{aq} ± 0.67	34.30^{ap} ± 1.09	25.88q ± 1.26	HS	
Stat	NS	S	HS	NS		
CD	At 5% (2.772); At 1% (3.645)					

Superscripts a, b, c, d, e shows significant difference within the column (between different groups on specific day) (p>0.05)

Superscripts p, q, r shows significant difference within the row (between different days in specific groups) (p>0.05)

On day 0, 7th, 14th and 28th, the BUN values in the group I rats (healthy control) were 22.81±0.83, 22.83±0.51, 22.85 ±1.20 and 22.38±1.30, mg/L respectively. These values were not significantly differs when compared to day 0 value, however found within the normal physiological limit.

On day 0, 7th, 14th and 28th, the BUN values in group II rats were 21.66±0.84, 23.72±1.52, 26.08±1.22 and 24.36±=0.68, mg/L respectively. There was significant increase in BUN level in this group on 14th day than 7th and 28th day when compared to day 0 values and healthy control (group I).

On day 0, 7th, 14th and 28th the BUN values in group III rats were 22.92±0.98, 26.56±1.0, 31.86±0.74 and 25.60±0.55, mg/L respectively. There was significant increase in BUN level on 14th day and the difference was highly difference in this group than 7th and 28th day compared to day 0 value.

On day 0, 7th, 14th and 28th, the BUN levels in group IV (rats were observed to be 22.68±0.78, 25.90±1.57, 31.23±1.19 and 23.31±0.79, mg/L respectively. The increase in BUN value was highly significant on 14th day than day 0 and healthy control (group I), however observed BUN values on 14th day was within the normal physiological level was increased as compared to 0 day and healthy control (group I). This indicates that the high dose of McFENPs treatment increase BUN on 14th day, however increase was within the normal physiological limit.

On day 0, 7th, 14th and 28th, the BUN level in rats of group V (satellite group) were 22.28±0.85, 27.75±0.67, 34.30±1.09 and 25.88±1.26, mg/L respectively. On day 14th there was increase in BUN level was highly significant within the group and between interval days as compared to their respective control. This indicates that the higher doses of McFESNPs have increased BUN levels was within the normal physiological range with minimal toxicity in rats. From the

results, it is concluded that The high doses of McFESNPs increase BUN levels however within the normal range in rats.

Table 15: Effect of McFECSNPs on creatinine level (g/dl) following oral toxicity in different experimental groups of wistar rats

Group	Serum creatinine (mg/dl), Mean ± SE				Stat	CD
	0 day	7th day	14th day	28thday		
I	0.30 ± 0.026	0.29 ± 0.029	0.27 ± 0.031	0.34^{c} ± 0.057	NS	At 5% (0.265) At 1% (0.35)
II	0.32^{q} ± 0.025	0.33^{q} ± 0.044	0.35^{q} ± 0.062	0.77^{bcp} ± 0.15	HS	
III	0.32^{q} ± 0.016	0.33^{q} ± .059	0.32^{q} ± 0.058	0.92^{bp} ± 0.18	HS	
IV	0.33^{q} ± 0.032	0.31^{q} ± .022	0.27^{q} ± 0.041	1.90^{ap} ± 0.29	HS	
V	0.33^{q} ± 0.037	0.31^{q} ± 0.034	0.26^{q} ± 0.032	0.65^{bcp} ± 0.11s	HS	
Stat	NS	NS	NS	HS		
CD	At 5% (0.265); At 1% (0.35)					

Superscripts a, b, c, d, e shows significant difference within the column (between different groups on specific day) (p<0.05)

Superscripts p, q, r shows significant difference within the row (between different days in specific groups) (p<0.05)

On day 0, 7th, 14th and 28th, the CREA values in group I (healthy control) rate were 0.30±0.026, 0.29 ±0.029, 0.27±0.031 and 0.34±0.057, mg/dl respectively. These values were not significantly different when compared to day 0 value and found within the normal physiological rages.

On day 0, 7th, 14th and 28th, the CREA values in group II rats were 0.32±0.025, 0.33±0.044, 0.35±0.0062 and 0.77±0.15, mg/L respectively. These values were significantly increase on 28th day and not significantly different from day 7th and 14th as compared to day 0 value. However, difference was highly significant than day 0 and on 28th day values of this group as compared to healthy control (group I).

On the day 0, 7th, 14th and 28th, the CREA values in group III rats were 0.32±0.016, 0.33±0.059, 0,32±0.058 and 0.92±0.18, mg/L respectively. There was significant increase in creatinine on 28th day in this group which was highly significant when compared to group I (healthy control).

On the day 0, 7th, 14th and 28th, the CREA values in group IV were observed to be 0.35±0.05, 0.33±0.032, 0.31±0.022, 0.27± 0.041 and 1.90±0.29, mg/L respectively. There was significant increase in creatinine levels between interval days was highest on 28th day observed in this group as compared to 0 day and healthy control (group I). There was significant increase in CREA value on 28th day than 7th and 14th day.

On day 0, 7th, 14th and 28th, the CREA values in group V (satellite group) rats were observed to be 0.33±0.037, 0.31±0.034, 0.26±0.032 and 0.65±0.11, mg/dl respectively. There was significant increase in serum CREA value in this group on 28th day than on the day 7th and 14th compared to day 0 value.

Serum CREA was significantly increase on 28th day treatment of McFECSNPs suggests renal damage, however no significant changes were noted on day 7th and 14th as compared to 0 day and healthy control (group I).

There was increase in serum CREA on 28th day in high dose groups (III, IV and V) were observed within the normal physiological limits.

For Aqueous extract process, by using cold extraction method the Aqueous extract of *Morinda citrifolia* fruit was made and final extractability was found to be 4.56%. By biological reduction (green synthesis) of silver nitrate(AgNo3) with *Azadirachta indica* (neem) leaf extract, Silver nanoparticles were synthesized, after coating the characterization of SNPs by colour results deep yellow to ruby red was confirmed. For treatment of experimental wistar rats these nanoparticles were coated with *Morinda citrifolia* fruit extract (McFE) which was used throughout whole trial period.

Among haematological parameters, the hematological analysis revealed increase in Hb, TEC, TLC on 28th day by McFECSNPs at all dose levels. There was increase in these parameters and difference was highly significant as compared to respective control. TEC significantly increase on 28th day in dose and duration of treatment dependent manner. On 28th day these parameters were increase at low dose group and in satellite group (highest dose) as compared to healthy control (group I), but these values were within the normal physiological ranges that indicate mild toxicity of McFECSNPs at all dose levels. The significantly reduced blood clotting time on 28th day at the medium dose level and the difference was highly significant as compared to healthy control.

There were no significant alterations in differential leucocytes of monocytes, neutrophils and basophils, however their values observed within normal physiological ranges. The increase in lymphocytes and eosinophils on 28th day in all treated groups differs significantly than 7th and 14th day as compared to 0 day and healthy control (group I). Similarly the lymphocytes were increased within the normal range in group II and III as compared to group I.

The serum biochemical estimations The AST values were significantly altered on 7th day in all treatment groups however within normal limits. There was significant increase in AST levels as compared to healthy control which suggest the mild liver and kidney damages.

TP significantly incarese on 14th day in group II, III and group IV (High dose) statistically similar to group I. The BUN significantly increased on 7th and 14th day compared to 0 day whigh indicates slightly changes in kidney function. On 28th day CREA levels in all treatment groups was significantly increase and highest increase noted on 28th day in high dose group IV than the healthy control which suggests relatively slightly more kidney damage, however values observed within the normal physiological limits.

According to the present findings, it can be concluded that *Azadirachta indica* leaf extract silver nanoparticles have no any adverse effects and so, it can be can be used for further therapeutic purposes.

9. Acknowledgement

All the authors are thankful to the Dean, COVAS, Parbhani and MAFSU, Nagpur for providing the necessary facility and financial assistance to conduct this study.

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