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Effect of Extracts of Test Plants, Isolated Ingredient and Prepared Analogues on Serum Inorganic Ions (*Sodium, Potassium, Calcium, Magnesium and Phosphorous*) in *Albino Rats*

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Abstract

Medicinal plants are of great value in the field of treatment and cure of diseases. Over the years, scientific research has expanded our knowledge of the chemical effects and composition of the active constituents, which determine the medicinal properties of the plants. It has now been universally accepted fact that the plant drugs and remedies are far safer than that of synthetic medicines for curing the complex diseases like cancer and AIDS. Melghat region is a rich source of medicinal flora. Near about 772 naturalized species found in Melghat region which are used for the cure of various diseases. Butea monosperma, Cassia fistula, Ceasalpinina bonduc, Cassine glauca, and Cassia absus, plants are found in Melghat region. All these plants are frequently used for the eradication of various diseases. In case of serum inorganic ions, the four cations, Na^+, K^+, Mg^{2+} and Ca^{2+}, are widely distributed in all living organisms. These cations play a tremendous variety of roles such as in the transmission of nerves impulses, in muscle contraction, and as enzyme activators and structural factors. In the present study we have undertaken this task to explore the impact of test plants whole extracts; some isolated ingredients and prepared analogues on serum composition of test animals with special reference to inorganic ions.

Keywords: Test plants, isolated ingredient, prepared analogue, serum inorganic ions.

Introduction

Primarily it may be stated that cutaneous lesions are due to influences of source outside the body or which exist or arise within the body. To the former class may be assigned such as depend on temperature and climate, such as are due to traumatism of various kind, such as result from various parasitic invasions, etc. this is in reality but a limited class, on other hand, the etiological factors which arise within the body itself are very numerous. The external causes like excessive heat direct exposure to the Sun may exhibit undue activity of the sudoriferous gland and result in the production of *sudamina* or Sunburn. The excessive cold may result in absolute, congelation of exposed portion of the integument. On the other hand internal causes of skin diseases, however, are far more frequently in operation. In this class we may place those affections of the skin which are due to pre-existing lesions of some part of the nervous system. The nervous system acts as a medium for the transmission of some internal irritation to the surface, thus, the gastric irritation resulting from the ingestion of shell-fish may manifest itself on the skin till another

internal cause of cutaneous lesions is found in ill nutrition or imperfect assimilation. Finally we may have external lesions resulting from the accumulation in the blood of certain infectious material. Sometimes such materials may be generated within the body itself through imperfections in the digestive, assimilative or excretory functions.

In order that nutrition may be healthily carried on in any part there must be a proper state of the blood, a proper condition and behaviour of the tissues to be nourished and a right exercise of the controlling influence exerted by the nerves. These three must work harmoniously together. The theoretical origin, therefore, of diseased changes in the skin may be especially in the blood and here the skin affection is only symptomatic.

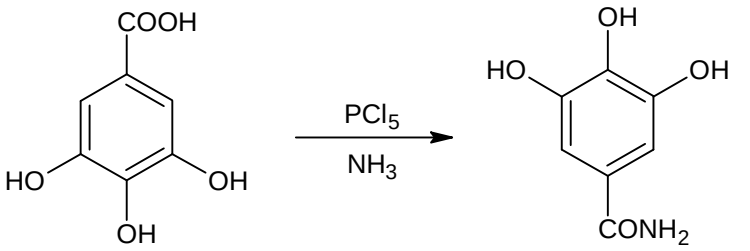
The four cations, Na^+, K^+, Mg^{2+} and Ca^{2+}, are widely distributed in all living organisms These cations play a tremendous variety of roles such as in the transmission of nerves impulses, in muscle contraction, and as enzyme activators and structural factors. The extra- and intracellular concentrations of Na^+, K^+, Mg^{2+} are very different (the values for Ca^{2+} being quite similar). This observation raises the questions about the establishment, maintenance and distribution of dissymmetry[1-4].

In the present study we have undertaken this task to explore the impact of test plants whole extracts, some isolated ingredients and prepared analogues on serum composition of test animals with special reference to inorganic ions.

Materials And Methods

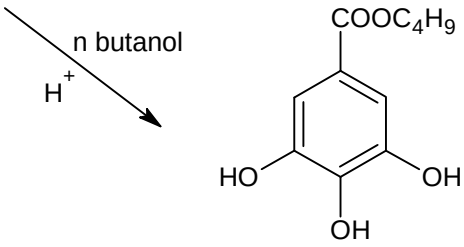
Plant material: The test plant materials of *Butea monosperma, Cassia fistula, Ceasalpinina bonduc, Cassine glauca,* and *Cassia absus,* plants were collected seasonally from the Melghat region of Amravati, District of Maharashtra, India. They were authenticated by the taxonomist Dr. S. P. Rothe with Voucher specimens (ML – 101, ML – 102, ML – 103, ML – 104, ML – 105) and were deposited in the herbarium of Department of Botany, Shri Shivaji College, Akola.

Extraction and Isolation: The test plant materials of *Butea monosperma, Cassia fistula, Ceasalpinina bonduc, Cassine glauca,* and *Cassia absus,* plants were shade dried at room temperature and ground in a manual mill to get coarse powder. The powders were kept in the airtight polythene bags and stored at dry place. These powders were extracted with water as a solvent by using soxhlet apparatus. The extracts were concentrated at 40 ^oC using rotary evaporator. Finally it was dried, crushed and stored in air tight bottle at 4 ^oC for further study. Again by using thin layer and column chromatography techniques, we have isolated the acidic ingredient from Butea monosperma test plant extracts and from the characterization it was found that isolated compound 1 was Gallic acid. The Gallic acid (Compound 1) thus separated from test extracts of Butea monosperma plant was then used for the preparation of its amide analogue (Compound 2) and ester analogue (Compound 3).



3,4,5-trihydroxybenzoic acid
Compound 1

3,4,5-trihydroxybenzamide
Compound 2



butyl 3,4,5-trihydroxybenzoate
Compound 3

Animals: The interdisciplinary part of proposed study was carried out after getting permission from the Institutional Animal Ethical Committee, Pusad (CPCSEA/IAEC/CP_PL/07-2012). The care of laboratory animals was taken according to the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (registration number 729/02/a/ CPCSEA). All the experiments were carried out using adult *albino Wistar male rats* weighing about 130-150 gm. The animals had free access to food and water and they were housed in cages in a natural (12 hrs each) light–dark cycle. The animals were acclimatized to the laboratory conditions for at least 5 days before behavioural experiments which were carried out between 0900 h and 1800 h.

Creation of Burn wound: Dorsal skins of the Wistar rats were shaved at full thickness. The animals were anesthetized by ketamine injection and burn wound of approximate 2 cm in diameter were created (circular area) by the brass probe which was immersed in boiling (100 °C) water until thermal equilibrium was reached. The probe was then placed on the back of the rats for 20 s without applying pressure. They were then housed individually in separate cages after complete recovery from anaesthesia.

Rats were randomly divided into following groups of five animals each:

Control group: Immediately after burning, burn areas were covered with propylene glycol solution once a day for 20 days.

Whole extract of *Butea Monosperma* group: Immediately after burning, burn areas were covered with whole extract of *Butea Monosperma* sample which was prepared in propylene glycol once a day for 20 days.

Whole extract of *Cassia fistula* group: Immediately after burning, burn areas were covered with whole extract of *Cassia fistula* sample which was prepared in propylene glycol once a day for 20 days.

Whole extract of *Ceasalpinina bonduc* group: Immediately after burning, burn areas were covered with whole extract of *Ceasalpinina bonduc* sample which was prepared in propylene glycol once a day for 20 days.

Whole extract of *Cassine glauca* group: Immediately after burning, burn areas were covered with whole extract of *Cassine glauca* sample which was prepared in propylene glycol once a day for 20 days.

Whole extract of *Cassia absus* group: Immediately after burning, burn areas were covered with whole extract of *Cassia absus* sample which was prepared in propylene glycol once a day for 20 days.

Isolated acid group: Immediately after burning, burn areas were covered with isolated acid sample (Gallic acid) prepared in propylene glycol, once a day for 20 days.

Prepared amide analogue group: Immediately after burning, burn areas were covered with the prepared amide analogue sample prepared in propylene glycol once a day for 20 days.

Prepared ester analogue group: Immediately after burning, burn areas were covered with the prepared ester analogue sample also prepared in propylene glycol once a day for 20 days.

Blood samples were collected from control and treated group test animals and left to clot at room temperature for at least 30 minutes. They were centrifuged at 2000rpm for a minute to remove clot and cell debris. Equal amount of serum from Experimental and control animals were pulled in order to have sufficient material to perform the estimations.

The serum samples were analyzed for their *sodium* and *potassium* contents by flam photometric method, *calcium* and *magnesium* by titration method and *phosphorus* by photo colorimetric method the values were expressed as MEq/lit[5-9].

Observation And Results

Data obtain about composition of serum inorganic ions in *control* and *treated* group test animals are shown in the Tables (Table No. 1.1-1.5) along with their graphical presentations (Fig. No. 1.1-1.5).

During cutaneous infection the concentration of *sodium*, *potassium* and *calcium* get significantly increased. It may be due to histopathological changes in kidneys of test animal. Increased potassium concentration may be due to cellular necrosis which has already been reported in many tissues during cutaneous infections. So also dehydration in such cases has been reported. Increased *calcium* in serum could be ascertained due to its release from bones. The level of *calcium* and *phosphorous* in extra cellular

fluids rise markedly because kidney cannot excrete them rapidly. As a consequence of this excessive phosphorous is reabsorbed by bones.

Table 1.1: Serum *sodium ion* change on exposure to burn and exogenous pharmacological drugs in *Albino rats.*

Day	Control	Induced	Treated							
			1	2	3	4	5	6	7	8
2	1.38 ± 0.17	1.41 ± 0.17	1.37 ± 0.17	1.36 ± 0.17	1.34 ± 0.17	1.35 ± 0.17	1.33 ± 0.17	1.37 ± 0.17	1.35 ± 0.17	1.36 ± 0.17
10	1.35 ± 0.17	1.38 ± 0.17	1.32 ± 0.18	1.34 ± 0.18	1.33 ± 0.18	1.34 ± 0.18	1.31 ± 0.18	1.34 ± 0.18	1.35 ± 0.18	1.35 ± 0.18
15	1.33 ± 0.18	1.47 ± 0.18	1.32 ± 0.18	1.30 ± 0.18	1.32 ± 0.18	1.31 ± 0.18	1.29 ± 0.18	1.32 ± 0.18	1.30 ± 0.18	1.31 ± 0.18
20	1.35 ± 0.18	1.56 ± 0.18	1.35 ± 0.18	1.32 ± 0.18	1.34 ± 0.18	1.33 ± 0.18	1.32 ± 0.18	1.33 ± 0.17	1.34 ± 0.17	1.34 ± 0.17

1= *Butea monosperma*, 2= *Cassia fistula*, 3= *Caesalpinia bonduc*, 4= *Cassine glauca*, 5= *Cassia absus*, 6= Gallic acid, 7= Amide and 8= Ester analogues of gallic acid.

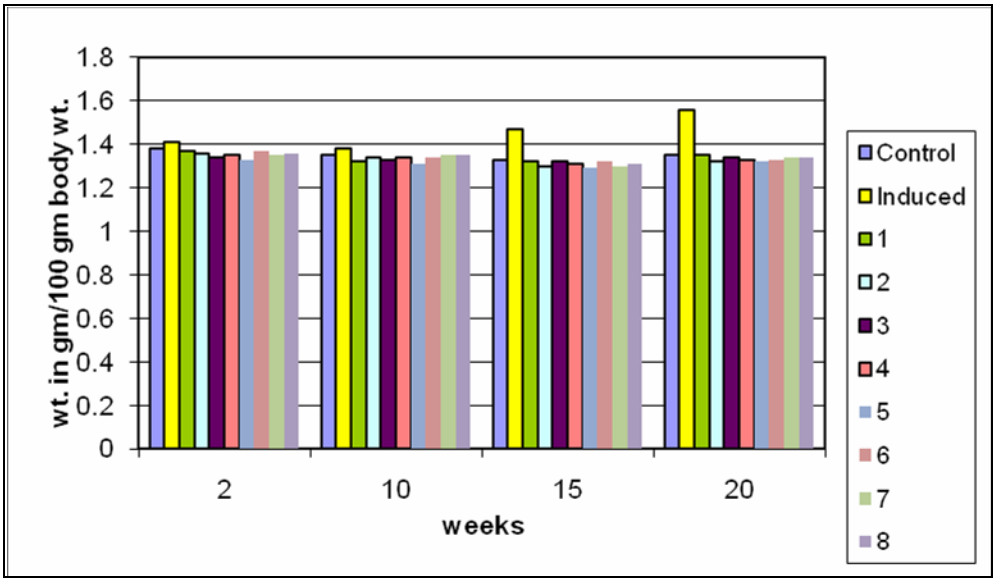


Fig. 1.1: Serum *sodium ion* change: *Wistar Albino rat*

Table 1.2: Serum *potassium ion* change on exposure to burn and exogenous pharmacological drugs in *Albino rats*.

Day	Control	Induced	Treated							
			1	2	3	4	5	6	7	8
2	6.08 ± 0.20	6.07 ± 0.20	6.01 ± 0.20	6.05 ± 0.20	6.03 ± 0.20	6.01 ± 0.20	6.02 ± 0.20	6.05 ± 0.20	6.07 ± 0.20	6.06 ± 0.20
10	5.96 ± 0.17	7.39 ± 0.22	5.88 ± 0.18	5.86 ± 0.17	5.88 ± 0.17	5.89 ± 0.17	5.87 ± 0.17	5.90 ± 0.18	5.92 ± 0.18	5.93 ± 0.18
15	6.18 ± 0.17	8.29 ± 0.23	6.12 ± 0.18	6.09 ± 0.17	6.13 ± 0.17	6.11 ± 0.17	6.14 ± 0.17	6.13 ± 0.18	6.17 ± 0.18	6.17 ± 0.18
20	6.08 ± 0.18	9.39 ± 0.22	6.03 ± 0.18	6.02 ± 0.18	6.04 ± 0.18	6.01 ± 0.18	6.02 ± 0.18	6.08 ± 0.17	6.06 ± 0.17	6.05 ± 0.17

1= *Butea monosperma*, 2= *Cassia fistula*, 3= *Caesalpinia bonduc*, 4= *Cassine glauca*, 5= *Cassia absus*, 6= Gallic acid, 7= Amide and 8= Ester analogues of gallic acid.

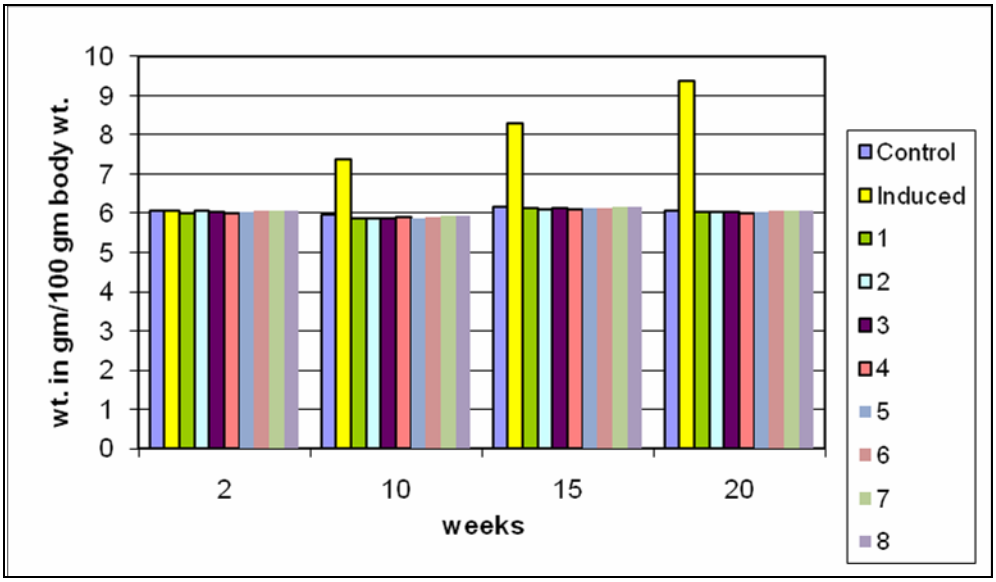


Fig. 1.2: Serum *potassium ion* change: *Wistar Albino rat*

Table 1.3: Serum *calcium ion* change on exposure to burn and exogenous pharmacological drugs in *Albino rats*.

Day	Control	Induced	Treated							
			1	2	3	4	5	6	7	8
2	6.5 ± 0.21	8.9 ± 0.18	6.0 ± 0.21	5.9 ± 0.21	6.3 ± 0.21	6.1 ± 0.21	6.3 ± 0.21	6.4 ± 0.21	6.3 ± 0.21	6.3 ± 0.21
10	6.0 ± 0.21	8.36 ± 0.20	5.8 ± 0.34	5.8 ± 0.34	5.9 ± 0.34	5.7 ± 0.34	5.8 ± 0.34	5.7 ± 0.34	5.9 ± 0.34	5.9 ± 0.34
15	6.4 ± 0.39	9.20 ± 0.37	6.0 ± 0.38	6.2 ± 0.38	6.0 ± 0.38	6.1 ± 0.38	6.1 ± 0.38	6.3 ± 0.34	6.2 ± 0.38	6.2 ± 0.38
20	6.1 ± 0.20	9.42 ± 0.29	5.8 ± 0.34	5.9 ± 0.34	5.8 ± 0.34	6.0 ± 0.34	5.8 ± 0.34	5.9 ± 0.34	6.0 ± 0.34	6.0 ± 0.34

1= *Butea monosperma*, 2= *Cassia fistula*, 3= *Caesalpinia bonduc*, 4= *Cassine glauca*, 5= *Cassia absus*, 6= Gallic acid, 7= Amide and 8= Ester analogues of gallic acid.

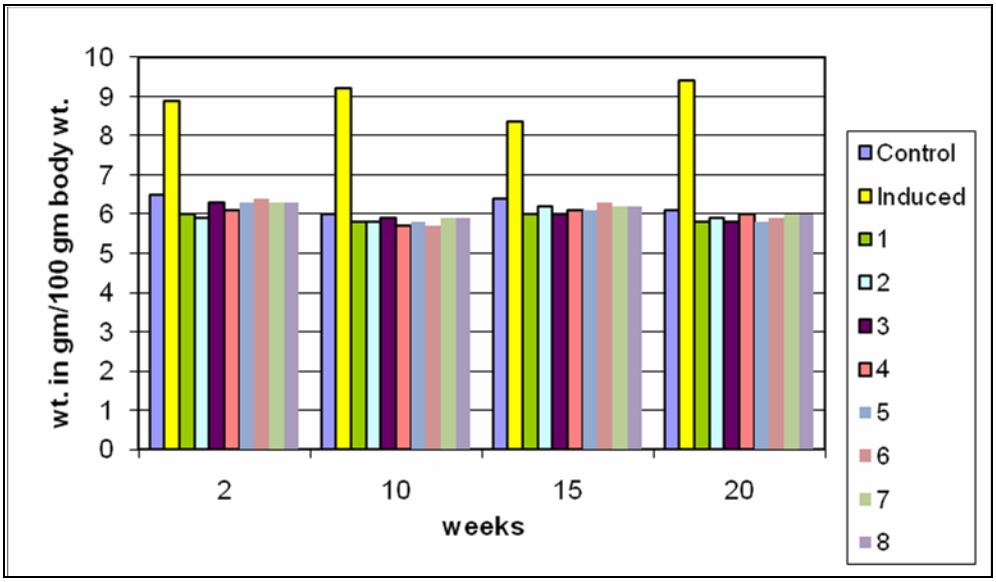


Fig. 1.3: Serum *calcium ion* change: *Wistar Albino rat*

Table 1.4: Serum *magnesium ion* change on exposure to burn and exogenous pharmacological drugs in *Albino rats*.

Day	Control	Induced	Treated							
			1	2	3	4	5	6	7	8
2	3.4 ± 0.18	3.6 ± 0.19	3.0 ± 0.18	3.2 ± 0.18	3.0 ± 0.18	3.1 ± 0.18	3.3 ± 0.18	3.3 ± 0.18	3.2 ± 0.18	3.2 ± 0.18
10	3.4 ± 0.18	4.8 ± 0.18	3.1 ± 0.17	3.1 ± 0.17	3.3 ± 0.17	3.2 ± 0.17	3.0 ± 0.17	3.1 ± 0.17	3.2 ± 0.18	3.3 ± 0.18
15	3.2 ± 0.18	3.7 ± 0.18	3.1 ± 0.18	3.0 ± 0.18	3.0 ± 0.18	2.9 ± 0.18	3.1 ± 0.18	3.0 ± 0.17	3.1 ± 0.17	3.1 ± 0.17
20	3.2 ± 0.18	3.8 ± 0.18	3.0 ± 0.17	3.1 ± 0.17	2.9 ± 0.17	3.2 ± 0.17	3.0 ± 0.17	3.0 ± 0.18	3.1 ± 0.18	3.2 ± 0.18

1= *Butea monosperma*, 2= *Cassia fistula*, 3= *Caesalpinia bonduc*, 4= *Cassine glauca*, 5= *Cassia absus*, 6= Gallic acid, 7= Amide and 8= Ester analogues of gallic acid.

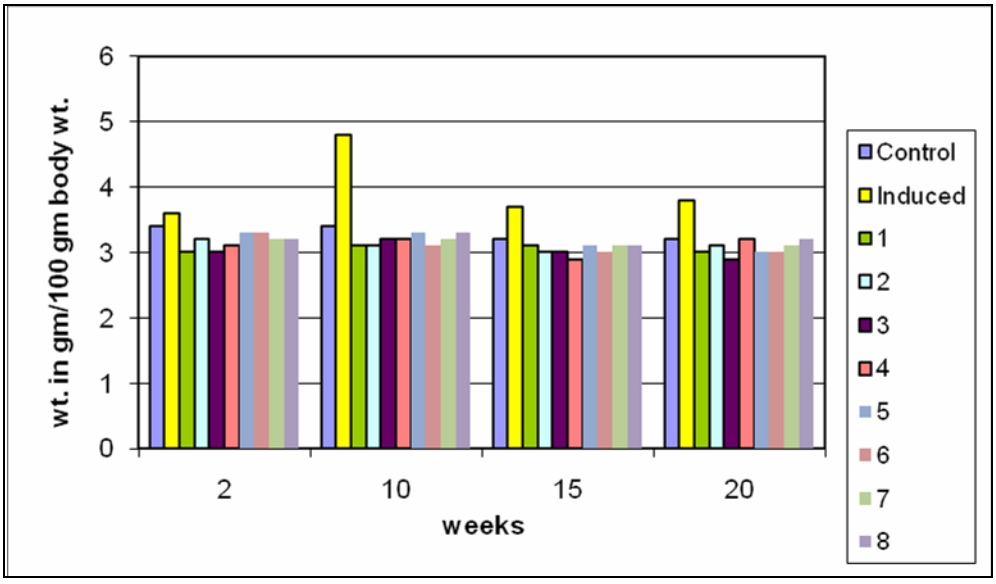


Fig. 1.4: Serum *magnesium ion* change: *Wistar Albino rat*

Table 1.5: Serum *phosphate ion* change on exposure to burn and exogenous pharmacological drugs in *Albino rats*.

Day	Control	Induced	Treated							
			1	2	3	4	5	6	7	8
2	6.0 ± 0.20	4.2 ± 0.18	5.9 ± 0.17	5.7 ± 0.17	5.9 ± 0.17	5.6 ± 0.17	5.6 ± 0.17	5.9 ± 0.18	5.8 ± 0.18	5.8 ± 0.18
10	6.3 ± 0.20	4.8 ± 0.18	6.0 ± 0.18	5.9 ± 0.18	5.8 ± 0.18	6.21 ± 0.18	6.1 ± 0.18	6.2 ± 0.18	6.0 ± 0.18	6.1 ± 0.18
15	6.20 ± 0.20	7.20 ± 0.20	6.15 ± 0.18	6.17 ± 0.18	6.16 ± 0.18	6.18 ± 0.18	6.17 ± 0.18	6.19 ± 0.17	6.17 ± 0.18	6.18 ± 0.18
20	6.5 ± 0.20	8.3 ± 0.20	6.3 ± 0.20	6.0 ± 0.20	6.2 ± 0.20	6.2 ± 0.20	6.3 ± 0.20	6.4 ± 0.20	6.2 ± 0.20	6.3 ± 0.20
1= *Butea monosperma*, 2= *Cassia fistula*, 3= *Caesalpinia bonduc*, 4= *Cassine glauca*, 5= *Cassia absus*, 6= Gallic acid, 7= Amide and 8= Ester analogues of gallic acid.										

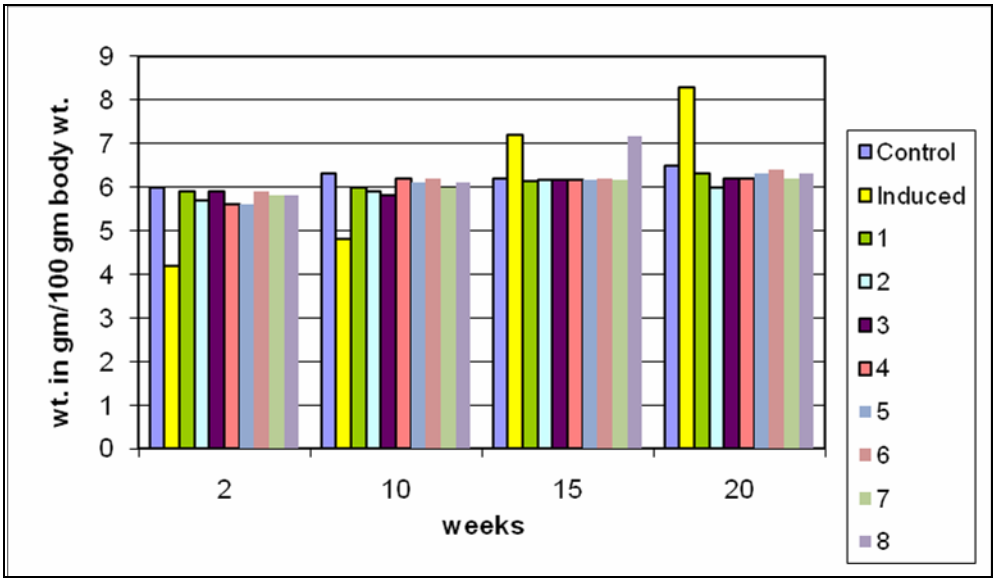


Fig. 1.5: Serum *phosphate ion* change: *Wistar Albino rat*

Conclusion:

Cutaneous infections adversely influence the ion exchange permeability of the skin. This leads to creating barriers in the supply of vital materials (ingredients) to the infected sites resulting into the deterioration of the natural composition and also the texture of the skin. Since the infected part of the skin becomes more susceptible to bacterial infections, the proportion of metal ions present in serum gets abnormally altered. From the above data it was very obvious that the concentration of the *sodium*, *potassium*, *calcium*, *magnesium* and *phosphate* ions for treated animals restored almost to the state of normalcy. It has also been revealed that isolated ingredients of the plant extracts and their chemical analogues are more effective in restoring the normalcy of the skin as compared whole plant extracts. However the author would like to mention that further extensive and systematic study in this field can lead to revealing many more life saving properties and potentials of these herbal preparations.

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