Evaluation of pharmacological activity of chadraprabha vati on serum of albino wistar strain rats

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ABSTRACT

The current study is to develop the acute and sub-acute toxicity profile of some ayurvedic Bhasma and understand the side effects due to the presence of heavy metals. Chandraprabha vati pill were weighed, powdered and suspended in water had made into liquid formulation. The animals were classified and treated with the doses of Chandraprabha vati (50 and five hundred mg/kg) in rat. The dose was calculated by extrapolating the equivalent human dose (1 and ten times) and was administered orally between ten and eleven after median daily for 28 days, during all in a very volume not exceeding one ml/100 g rat weight. Blood was collected on 7, 14 and 28 days, later they were sacrificed for histopathological studies.

Keywords: Bhasma; chadraprabha vati; detoxification.

INTRODUCTION

Bhasma are distinctive ayurvedic metallic/minerals preparation, treated with herbal juice or decoction and exposed for Ayurveda, that are best-known in Indian landmass since 7th century A.D. and wide suggested for treatment of a variety of chronic ailments. Animal’s by-product like horns, shells, feathers, metallic, non-metallic and herbs are normally administered as Bhasma. Bhasma that contain iron, Cu, S or different producing method plays a particular role within the final product. Standardization of Bhasma is utmost necessary to substantiate its identity and to see its quality, purity safety, effectiveness and acceptableness of the product. However, the most necessary challenges baby-faced by these formulations are the shortage of complete standardization by physicochemical parameters.

Complications and toxic effects of in toxification

There is a section in Ayurveda referred to as “Rasa shastra” that describes the employment of metals, gems, minerals and poisons for producing special formulations to combat chronic and tough diseases. Rasa Shastra is referred to as “Vedic Chemistry”. As the modern laboratory chemicals were not fictional within the vedic era (3000 years ago), seasoning juices were used instead, largely made of metal ashes those are referred to as rasa product, Saini says. These medicines, additionally called bhasmas, take off of associate ayurvedic tradition practiced for thousands of years in India wherever extremely cytoxic serious metals like lead, arsenic, mercury and atomic number 48 combine with herbs or spices. Serious metal toxicity following the utilization of Ayurvedic remedies is well documented within the literature [11].

Figure 1: Brands of chandraprabha vati

During the standard preparation of bhasmas the metal is “purified-out” through multiple cooling and heating cycles and by addition of specific “mineral
herbs”. In modern formulations, however, the concentration of serious metals could also be excessive as a result of poor quality control permits for contamination, adulteration, or improper purification. 2 recent Canadian province cases highlight the attainable danger of taking Ayurvedic remedies.

A male given to hospital feeling terribly sick with emesis and looseness of the bowels. His bloodwork showed solution abnormalities and anemia with stainability stippling of his red blood cells. For a few years he had been taking one pill daily of associate Ayurvedic medication purchased in India to “increase vigor”. His blood lead level was 5.2µmol/L. Heavy metal analysis showed that every pill contained some 28 mg of lead, 0.70 mg mercury, 0.11 mg arsenic, and negligible amounts of cadmium. He responded well to succimer chelation with no apparent sequelae (11).

**Bhasmas required detoxification**

In preparations of those Bhasmas, the preliminary method that causes detoxification while not harming its medicative properties (gunas) is named as So-dhana (12). The method of sodhana was well accepted by the pioneers of Rasasatra for the purification of herbomineral medicine. These ways were developed to detoxify the staple by chemical transformations and enhance the properties and therapeutic potential. There are issues raised concerning the utilization of metals and minerals in therapeutics. Raising safety issues regarding ancient preparations, the World Health Organization has issued pointers concerning toxicity studies of seasoning medicine (13).

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**Bhasmas required better purification**

There is varied the importance of Bhasma like maintaining optimum alkalinity for optimum health, neutralizing harmful acids that result in illness; as a result of Bhasma don’t get metabolized in order that they do not produce any harmful matter, rather it breakdowns serious metals within the body. ways together with for Bhasma preparation are parpati, rasayoga, sindora etc. Bhasma that contain iron, Cu, S or alternative producing method plays a selected role within the final product (13). Particle size (1-2 µ) reduced considerably, which can facilitate absorption and assimilation of the drug into the body system.

Standardization of Bhasma is utmost necessary to verify its identity and to work out its quality, purity safety, effectiveness and satisfactoriness of the product; however, the foremost vital challenges visage by these formulations are the shortage of complete standardization by physiochemical parameters (14).

**Chandraprabha vati**

Chandraprabha vati is associate ayurvedic formulation. These classical medicines ought to be exactly analyzed before application to the patient for obtaining a fascinating outcome. There are numbers of multi-drug formulations practiced in ayurveda clinics with success however most of them are anguish from lack of knowledge relating to their details mechanism of action. Such one wide practiced formulation is Chandraprabha vati (16). The drug has been mentioned in Rasaratnasamucchaya within the context of Kustha (skin disorder) and in Bhaishyajyaratnavali within the context of Yakrivikara (liver disorder) (15),

This ancient formulation of ayurveda is victimization for hundreds of years with claimed effectivity and safety in treatment of jaundice and alternative liver and skin disorders (18), it’s used for Hansen’s disease, fever, oedema, obesity, jaundice and alternative hepatic disorders. The drug is additionally smart for lack of appetite, upset stomach and irregular bowls, liver disorders and skin diseases. It acts as an alternate, carminative stomachic (19).

**Table 1: Composition of Chandraprabha vati**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Latin Name</th>
<th>Sanskrit Name</th>
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</thead>
<tbody>
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</tr>
<tr>
<td>2</td>
<td>Commiphora mukul</td>
<td>Guggul</td>
</tr>
<tr>
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</tr>
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<td>Emblica officinalis</td>
<td>Amalaki (Amla)</td>
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</tr>
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<td>Maricha</td>
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<td>Piper longum</td>
<td>Pippali</td>
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<td>Sajikashaar</td>
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<td>16</td>
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<td>Yavikshaar</td>
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<td>Sodium sulphate</td>
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<td>Vida Lavan</td>
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<tr>
<td>22</td>
<td>Cinnamomum tamala</td>
<td>Tejpatta</td>
</tr>
<tr>
<td>23</td>
<td>Elettaria cardamomum</td>
<td>Ela</td>
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MATERIALS AND METHODS

Chandraprabha vati (B.R.) Manufactured by Divya pharmacy, Uttarakhand, India. Chandraprabha vati manufactured from Shree Baidyanath Ayurveda Bhawan (P) LTD, India. Was procured from native medical stores glucose kit, cholesterol kit, Total protein kit, Creatinine kit, hemoglobin kit, alkaline Phosphates kit, amino acid transaminase kit, Aspartate transaminase are procured from biomedical LTD., Solan, India. albumin kit manufactured by Agappe diagnostics LTD, Kerala, India.

Animals: Healthy man and female rats of Wistar strain (180-220 g) were obtained from the animal house facility of Mahati college of Pharmacy, Madanapalle. The animals were kept in a very well-ventilated room and therefore the animals had exposed to 12hours day and night cycle with a temperature between 20±3°C. The animals were housed in massive spacious, sanitary polypropene cages throughout experimental period. The animals were fed with water and rat pellet feed supplied by Sree Venkateshwara agencies, Bengaluru, India. All the experiments were performed after getting previous approval from CPCSEA and IAEC (registration NO: 1952/PO/Re/S/17/CPCSEA/MCP/2018/02-P-COL/DATED 01/05/2018)

Mechanism of toxicity of bhasma preparations

They accumulate and thereby disrupt operate in important organs and glands like the heart, brain, kidneys, bone, liver, etc.

They displace the important nutritional minerals from their original place, thereby, obstructive their biological function. It is, however, not possible to measure in an surroundings freed from significant metals. There ar some ways by that these toxins are often introduced into the body like consumption of foods, beverages, skin exposure, and also the inhaled air.

As per ayurveda, the bioavailability and toxicity of the metals rely on their chemical forms, particularly of mercury, though some authors couldn’t ascertain it by experimentation.

Heavy metals could exert their acute and chronic effects on the human skin through stress signals. Findings recommend that significant metals reduced the phosphorylation level of tiny heat shock protein 27(HSP27), which the ratio of p-HSP27 and HSP27 could also be a sensitive marker or further end point for the hazard assessment of potential skin irritation caused by chemicals and their product (18).

Bhasmas and Rasa, might not have understood that the standard formulation contained significant metals requiring special care and oversight. Inhalation of mercury vapour produces acute corrosive respiratory illness and opening inflammation and, if not fatal, could also be related to central system nervous effects like tremor or accrued excitability (18,19).

RESULTS AND DISCUSSION

Chandraprabha vati could be an ancient Ayurvedic formulation used for centuries with claimed efficaciousness and safety in treatment of jaundice and alternative liver and skin disorders, the utilization of those metals in Ayurvedic medicines are done after a rigorous method of purification (Shodhana) and changing the metal into compounds (Marana). However, recently some gold preparations employed in Indian system of drugs are suspected to be harmful, inflicting hepatic, nephritic and neurotoxicity and plenty of alternative side effects. so as to know the potential toxicities of the wide marketed preparations, a sub-acute toxicity had been conducted.

Acute toxicity study (OCED 423)

The acute toxicity study was performed by acute toxic classic methodology. The physiological observational battery of tests shown that the formulation doesn’t show any deadly symptoms up to 500 mg/kg for up to 14 days in albino Wistar rats.

Effect of Chandraprabha vati on body weight

In toxicity study, in all groups at variable test doses, a substantial significant increase in body weight was evident. Body weight was indicative of increase appetite and food intake and effectiveness of a drug might become expressed as increase in body weight. As there was increase in body weight determined, it’s going to be likely that increase in body weight at highest dose level have effect on animals. The results are shown in (Figure 2)

![Figure 2: Effect of Chandraprabha vati on body weight](image)

Effect of Chandraprabha vati on food consumption

Effect of Chandraprabha vati on food consumption over a period of twenty-eight days treatment were recorded on day one, 3, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and twenty-eight within the treatment and control groups and represented in graph.

The treatment groups differ from the control and that they exhibit amendment in food consumption throughout the study period. As there was increase in food intake it doesn’t showed any impact in animals
thus it is considerable. Results are estimated below (Figure 3)

**Food consumption**

![Food consumption graph](image)

**Figure 3: Effect of Chandraprabha vati on consumption of food**

**Effect of Chandraprabha vati on elevated plus maze**

There was important amendment in latencies of Chandraprabha vati treated groups (50 and 500 mg/kg) as compared to normal control on day 1, 14, and 28. However, there was significant (p < 0.001) increase in mean retention transfer latencies on day 28th of Chandraprabha vati treated cluster as compared to normal control cluster that indicate improvement in memory enhancement. The heavy metal (Hg) poisoning happens by deposition in human animal tissue neuron and in a very scattered cluster of neurons within the brain stem and neural structure by generation of free radicals, unleash of intracellular calcium, lysosomal enzyme or cytoskeleton disorganization. The was no amendment determined in Chandraprabha treated cluster the latency time was estimated below (Figure 4Figure 2)

![Elevated plus maze graph](image)

**Figure 4: Effect of Chandraprabha vati on elevated plus maze**

Behavioural and spatial learning deficits in animals due to consumption of bhasmas containing unprocessed heavy metals (Hg) did not showed cognitive impairment in Chandraprabha vati consumed rats and there was increased ability to learning in elevated pulse maze.

**Effect of Chandraprabha vati on rota rod**

Chandraprabha vati (50 and 500 mg/kg) treated groups show significant amendment within the time spent on the spindle of the rota rod as compared to normal control cluster on day 1, 14, and 28. However, there was significant decrease in time spent on spindle of Chandraprabha vati treated cluster on day 28th compared to regulate cluster that showed motor in coordination (p > 0.001). (Figure 5)

![Rota rod graph](image)

**Figure 5: Effect of Chandraprabha vati on rota rod**

The α-2-adrenergic receptor agonists produce variable levels of sedation, analgesia, muscle relaxation, and anxiolysis. The rota rod check assesses motor coordination and balance. Intact (healthy) animals are going to be able to stay on the rotating rod for extended periods due to preserved limb coordination and balance. In contrast, animals with neurological alterations or receiving neurotoxin compounds or sedatives usually show shorter latencies to fall. In our study, rats that treated with the heavy metal canting bhasmas displayed significant difference in latency to fall compared to intact rats.

Motor coordination deficits in animals due to consumption of bhasmas containing heavy metals (Hg) did not Showed in coordination of mussels in Chandraprabha vati consumed rats and there was no change in ability to stay on rota rod.

**Effect of Chandraprabha vati on hemoglobin**

In toxicity study, in all groups at varied test doses of Chandraprabha vati treated groups shows a substantial significant decrease in hemoglobin levels as compared to normal control group. As there was decrease in hemoglobin levels determined, it should be likely that decrease in hemoglobin levels at highest dose level have no result on animals. Results was demonstrated below (Figure 6)

![Haemoglobin graph](image)

**Figure 6: Effect of Chandraprabha vati on haemoglobin**
There was significant change in Chandraprabha vati treated groups (50 and 500 mg/kg) as compared to normal control on day 1, 14 and 28. However, there was significant \( p < 0.001 \) decrease in hemoglobin on day 28 of Chandraprabha vati treated group as compared to normal control group which didn't show effect on animals.

**Effect of Chandraprabha vati on hematocrit**

The hematocrit of blood determines the percentage of red blood cells (RBCs) in the blood. Blood is composed mainly of red blood cells and white blood cells suspended in an almost clear fluid called serum. The hematocrit indicates the percentage of blood by volume that is composed of red blood cells. The condition called "anaemia" results from having too few red blood cells. Results were showed below (Figure 7)

![Figure 7: Effect of Chandraprabha vati on Hematocrit](image)

**Effect of Chandraprabha vati on platelet count**

A platelet count was to measure how many platelets are present in blood. Platelets are parts of the blood that helps the blood clot. They are smaller than red or white blood cells. Platelets are produced in the bone marrow and released into the blood where they play an important role in coagulation (blood clotting), helping to stop bleeding when blood vessels are injured. The results were demonstrated below (Figure 8)

![Figure 8: Effect of Chandraprabha vati on platelet count](image)

**Effect of Chandraprabha vati on sodium levels**

Among the observations of test group of rats was finding that, the results of their ability to conserve water, was normal in all the groups. There was no dehydration proven by an Chandraprabha vati treated animals.

Plasma sodium concentration were compared with normal control group rats and results was showed below (Figure 9)

![Figure 9: Effect of Chandraprabha vati on sodium levels](image)

The observed significant reduction in sodium levels by Chandraprabha vati treated group that is 1, 14 and 28 days. The sodium levels significant \( p < 0.001 \) decrease caused by Chandraprabha vati may not be efectible to animals. So, the doses of the Chandraprabha vati treated groups are considerable.

**Effect of Chandraprabha vati on potassium levels**

Rats which develop low blood pressures as results of low potassium diet are shown to possess down serum metal levels. Once the blood pressure is restored to normal by injection and 24 hours feeding of metal, serum metal levels conjointly return to traditional. There was no amendment within the potassium levels in Chandraprabha vati treated animals. Results was discussed below (Figure 10)

Figure 10: Effect of Chandraprabha vati on potassium levels

The observed significant reduction in potassium levels by Chandraprabha vati treated group that is 1, 14 and 28 days. The potassium levels significant \((p < 0.001)\) decrease caused by Chandraprabha vati may not be effectible to animals. So the doses of the Chandraprabha vati treated groups are considerable.

Effect of Chandraprabha vati on glucose levels

Glucose is key to keeping the mechanisms of the body in top working order. When glucose levels are optimal, it often goes unnoticed. Results showed below (Figure 11)

![Figure 11: Effect of Chandraprabha vati on glucose levels](image)

Figure 12: Effect of Chandraprabha vati on total cholesterol

The observed significant change in Cholesterol levels by Chandraprabha vati treated group that was in 1, 14 and 28 days. The significant \((p < 0.001)\) decrease in Cholesterol levels caused by Chandraprabha vati cause low density lipoproteins \((LDL-C)\) may not be effectible to animals. So, the doses of the Chandraprabha vati treated groups are considerable.

Effect of Chandraprabha vati on total creatinine

Creatinine was a waste product in blood that comes from muscle activity. It was normally removed from blood by kidneys, but when kidney function slows down, the creatinine level rises. Results were discussed below (Figure 13)

![Figure 13: Effect of Chandraprabha vati on total creatinine](image)

Effect of Chandraprabha vati on total protein

Proteins are important building blocks of all cells and important for body growth, development, and health. a total protein test is habitually accustomed facilitate value a person’s overall health standing and will even be accustomed evaluate conditions like disease, nephrosis, absorption, cancers or infections. Results was discussed below (Figure 14)
In rat's total super molecule values are normal within the primary week of neonatal life. The humour protein levels of Chandraprabha vati wear slightly minimized from 1, 14 and 28 of more days compared with the control group. As there's modification in protein levels this are not effectible to the rats. The statistical significant (p < 0.001) decrease difference is considerable.

**Effect of Chandraprabha vati on albumin**

Albumin, synthesized in the liver, is the protein of the highest concentration in plasma. albumen transports several little molecules within the blood (for example, bilirubin, calcium, progesterone, and drugs), and is of prime importance in maintaining the osmotic pressure of the vascular system. Results was discussed below (Figure 15)

The mean levels of albumin of Chandraprabha vati and control groups were statistically differing from day 1, 14 and 28. However, the values of albumin were lower compared to the values presented by the animals in control group, but they are in the considerable range. Thus, there was no statistically significant difference.

**Effect of Chandraprabha vati on alanine amino transferase**

Proteins referred to as enzymes facilitate the liver break down different proteins so your body will absorb them a lot of easily. ALT is one in all these enzymes. It plays an important role in metabolism, the process that turns food into energy. ALT is often found within liver cells. However, once liver is dam-

aged or inflamed, ALT may be free into your blood. This causes humour ALT levels to rise. Results was discussed below (Figure 16)

The Chandraprabha vati value have distinction between pre-treatment and post treatment the of days one, 14 and 28.ALT levels were decreased as compared to control group therefore it can be concluded significant (p < 0.001) decrease that there has not significant impact for ALT Chandraprabha vati treated group.

**Effect of Chandraprabha vati on aspartate amino transferase**

Amino transferase (AST) is a catalyst that’s present in varied components of your body. A catalyst may be a protein that helps trigger chemical reactions of body must function. AST is found within the highest concentrations in muscles, heart, red blood cells, and liver. A tiny low quantity of AST was generally in blood. Results was discussed below (Figure 17)

Serum AST level of normal control and Chandraprabha vati within the days of one, 14 and 28 respectively in normal vary as compared with normal control group, there was no important distinction within the liquid body substance AST levels of treated Chandraprabha vati group.

**Effect of Chandraprabha vati on alkaline phosphatase**
The alkaline phosphatase test (ALP) is employed to assist observe liver disease or bone disorders. In conditions affecting the liver, damaged liver cells unleash accumulated amounts of ALP into the blood. If one or a lot of them are obstructed, as an example by a tumour, then blood levels of mountain can typically be high. Results was discussed below (Figure 18)

Figure 18: Effect of Chandraprabha vati on alkaline phosphatase

Serum mountain level of normal control and Chandraprabha vati within the days of 1, 14 and 28 severally diminished. As compared the normal control group, there was no significant difference within the body fluid ALP levels of treated Chandraprabha vati group.

Histopathology in 28 days’ toxicity study

The higher dose level and low dose levels (50mg/kg and500mg/kg) of Chandraprabha vati treated group did not show any significant histopathological changes within the various organs when subjected to histopathological studies on termination of the treatment compared to the control group rats. Since there was no significant alteration within the histology of varied organs at the higher dose level and low dose treated group. The histopathological interpretation of varied organs of the higher dose level treated group were represented in figure.

Effect of Chandraprabha vati on brain, liver and kidney histology

The brain, liver and urinary organ of normal control and Chandraprabha vati treated groups (50, and 500 mg/kg) showed normal structure. However, 500 mg/kg treated Chandraprabha vati treated group showed expected toxicities, i.e. necrosis of neurons in brain, inflamed periportal zone in liver and disruption of epithelial tissue in proximal convoluted tubules in urinary organ these was under the considerable range.

Effect of Chandraprabha vati on liver

Effect of Chandraprabha vati on kidney histology: The kidney of normal control and Chandraprabha vati treated groups (50 and 500 mg/kg) showed normal cytoarchitecture (Figure 19).

Effect of Chandraprabha vati on kidney

Effect of Chandraprabha vati on kidney histology: The kidney of normal control and Chandraprabha vati treated groups (50 and 500 mg/kg) showed normal cytoarchitecture (Figure 20).

Effect of chronic administration of Chandraprabha vati on rat’s excretory organ microscopic anatomy. (a) The kidneys of normal control show traditional histologic design. (b) and (d) Chandraprabha vati brand-1 and a pair of (50mg/kg/day), showing no changes. (c) and (e) Chandraprabha vati brand-1 and a pair of (500mg/kg/day) treated rats showing minute vascular, chronic and death changes within the liver.
The treatment with Chandraprabha vati was successful in reverting the medicine parameters and biochemical changes shows that the thrombocyte count was considerably increased in rats at high dose treated groups. However, the modification within the Hb count falls within the low normal range. The decrease within the hematocrit count might be because of hemodynamic interaction of the Chandraprabha vati constituents throughout its bio phase.

The biochemical parameters disclosed that the albumen and total protein considerably fluctuated, this might flow from to a protein anabolic impact that is any proved by gentle to moderate decrease all told biochemical parameters. The on top of mentioned parameters and these changes are within the conventional vary. the safety profile of Chandraprabha vati is well understood by the correlation of physical and biochemical parameters.

Thus, we state that the Chandraprabha vati is at a dose of 500mg/kg evidenced with its safety in the biological system. The histopathology of brain, kidney and liver supports the toxicity studies

Acute and sub-acute oral toxicity has been advocated as basic tests for assessing safety and performed according the rules of OECD. The acute toxicity take a look at disclosed that Chandraprabha vati is safe up to five hundred mg/kg on single treatment. In sub-acute toxicity study, though the body weights were reduced throughout breed period, there have been no changes of body weights and food consumption in male and feminine rats or between treatment and control groups.

All figures data was expressed as Mean ± SD, *p<0.05 when compared with control group

CONCLUSION

The Chandraprabha vati was well tolerated and failed to turn out any general organ or general toxicity up to a most dose of five hundred mg/kg/day over a amount of twenty eight days. there have been no important effects on biochemical or organ weights and no histologic changes thought of to be associated with treatment. there have been no consistent, adverse, or clinically relevant changes in haematology, clinical biochemistry parameters (except albumen and total protein) on twenty eighth day (terminal time point), simple protein and total protein content were considerably reduced within the treatment teams whereas compared with the management cluster however the decrease in these values were among the normal vary. there have been no determined adverse effects for Chandraprabha vati during this study at the dose levels fifty, and 500mg/kg/day.

From the encouraging results obtained from the current study, more experiments have to be compelled to conduct to evaluate the potential of

DISCUSSION

Effect of Chandraprabha vati on kidney (a) Control (b) Brand-1 Chandraprabha vati (50mg/kg) (c) Brand-1 Chandraprabha vati (500mg/kg) (d) Brand-2 Chandraprabha vati (50mg/kg) (e) Brand-2 Chandraprabha vati (500mg/kg)

Effect of Chandraprabha vati on brain histology

The brain of normal control and Chandraprabha vati treated groups (50 and 500 mg/kg) showed normal cytoarchitecture (Figure 21).

Effect of Chandraprabha vati on rat’s brain histology. Light micrograph of (a) control rat brain showing normal design. (b) and (d) Chandraprabha vati brand-1 and a pair of (50mg/kg/day) treated rats showing no histopathological changes. (c) and (e) Chandraprabha vati brand-1 and a pair of (500mg/kg/day) treated rats showing, a minute amendment in congestion of blood vessels and necrosis of Purkinje cells of the cerebellum.
Chandraprabha vati in deadly conditions, and therefore the formulation are going to be helpful for patients in future.

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DISCLOSURES

Name: B. Syed salman

Contribution: This author helped write the manuscript. B. Syed salman has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.

REFERENCES


