Combined effect of hydroethanolic extracts of *Murraya koenigii* and *Phyllanthus niruri* leaves on paracetamol and ethanol-induced toxicity in HepG2 cell line

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The present study is an attempt to determine the combined hepatoprotective potential of hydroethanolic leaf extracts of *Murraya koenigii* and *Phyllanthus niruri* against paracetamol (PCM) and ethanol-induced toxicity in human hepatoma HepG2 cell line. Toxicity in cells was induced by treatment with 15 mM PCM and 50 mM ethanol for 24 h as manifested by a significant ($P < 0.05$) decrease in cell viability, increase in the leakage of serum glutamate oxaloacetate transaminase and serum glutamate pyruvate in culture medium, increase in lipid peroxidation and reduction in reduced glutathione in cell lysate. These alterations were significantly ameliorated when cells were treated with a combination of hydroethanolic leaf extracts of *M. koenigii* and *P. niruri,* and silymarin during both prophylactic and curative studies. Both post-treatment (curative) and pre-treatment (prophylactic) with the combination of plant extracts were able show effective hepatoprotection. This was also evident during morphological studies. The combination of plant extracts thus holds immense potential for future use as a hepatoprotectant.

Keywords: Ethanol, hepatoprotection, HepG2 cell line, *Murraya koenigii*, paracetamol, *Phyllanthus niruri*.

LIVER ailments claim several thousands of lives every year around the globe. Despite the advances in modern medicine, it offers limited success in providing a cure for hepatic disorders further accompanied with severe side effects as aftermaths of the treatment[1]. In the absence of effective modern drugs for liver disorders, today scientists face a serious challenge to explore the hepatoprotective potential of plants based on their traditional use. *Murraya koenigii* commonly known as curry tree and *Phyllanthus niruri* locally known as 'bhui amlaki' have long been used in traditional medicine for the cure of different ailments.

Leaves of *M. koenigii*, a member of the family Rutaceae are used as a spice and condiment in India because of their rich aromatic flavour[2]. They form an integral part of the Indian cuisine. In traditional medicine *M. koenigii* has been used for the treatment of diarrhoea, dysentery, nausea, eruptions and insect bites and as a stimulant, stomachic, antipyretic and analgesic[3]. Recent studies have established antioxidant[4], antidiabetic[5], anticarcinogenic[6], antimicrobial[7] and hepatoprotective[8] potential of curry leaves. Phytochemical studies have revealed the presence of organic constituents such as coumarins, terpenoids, alkaloids and essential oils in *M. koenigii* leaves[9].

P. niruri, a small herbaceous weed belonging to the family Euphorbiaceae has long been used as an important medicinal plant in traditional medicine for the cure of ailments ranging from jaundice, hepatitis, stomach ache, gonorrhoea, asthma, urolithic disease, fever, malaria, vaginitis to tuberculosis[10]. Present-day research has elucidated its antioxidant[11], hepatoprotective[12], antiviral[13], hypolipidaemic[14], anticarcinogenic[15] and antidiabetic[16] potential. The plant extracts have been reported to possess alkaloids, flavonoids, tannins, lignans, polyphenols, triterpenes, sterols and volatile oils[17].

Individually *M. koenigii* and *P. niruri* plant extracts have been reported to possess hepatoprotective potential, but the effect of a combination of extracts has not been reported. Earlier work has determined the hepatoprotective potential of aqueous leaf extract of *M. koenigii* against ethanol-induced damage in HepG2 cell line[18]. Hepatoprotective potential of whole plant extract for different *Phyllanthus* species has been studied against ter-butyl hydroxide-induced cytotoxicity in HepG2 cell line[19]. The present study was aimed at determining the combined hepatoprotective potential of hydroethanolic leaf extracts from *M. koenigii* and *P. niruri* against paracetamol (PCM) and ethanol-induced toxicity in HepG2 cell line.

The leaf samples for *M. koenigii* and *P. niruri* were obtained from Medicinal and Aromatic Plant Research Development Centre (MRDC), Pantnagar, US Nagar, India and taxonomically authenticated from the Department of Biological Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, US Nagar, India.

The leaves were shade-dried and on complete drying were ground to make a fine powder. Alcohol water extract (AWE; 1 : 1) was prepared from dried leaf powder using the used method of Sharma *et al.*[20], with slight modifications. One gram of shade-dried powder was added to 50 ml of ethyl alcohol : water (1 : 1) and the solution was homogenized. The resultant suspension was centrifuged at 11,000 rpm for 10 min at 4°C. The supernatant was filtered using Whatman No. 1 filter paper. AWE was rotary-evaporated at 40°C and later freeze-dried in a lyophilizer.

HepG2 cells (human hepatocellular carcinoma cell line, passage no. 3), obtained from the National Centre for Cell Sciences, Pune, India were maintained in culture in 25 cm^2 polystyrene flasks (Nunc) with minimum

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essential medium (MEM) (Sigma, USA) containing 10% FBS (Cellclone), 1% antibiotic–antimycotic solution, 1 mM sodium pyruvate and 1.5 g/l sodium bicarbonate under an atmosphere of 5% CO_2 at 37°C until confluent. Continuous cultures were maintained by subculturing every 6 days at 10^5 cells/25 cm^2 flask by trypsinization. HepG2 cells in exponential growth phase, i.e. after 24 h of growth post-subculturing in pre-confluent state were used for the experiments.

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]-based cytotoxicity assay was used to determine the IC_{50} of PCM, ethanol, AWE of *M. koenigii* leaves (AWEMK), AWE of *P. niruri* leaves (AWEPN) and silymarin[21].

For prophylactic study HepG2 cells were grown in six-well plates seeded at a concentration of 1×10^5 cells per ml (2 ml/well) for 24 h at 37°C. Cells were preconditioned with different plant extracts (alone and in combination) and silymarin for 24 h followed by treatment with 15 mM PCM and 50 mM ethanol for 24 h. The experimental groups were carried out in triplicate as follows:

Group I: Vehicle control – cells + media as vehicle control.
Group II: Negative control – cells + 15 mM PCM + 50 mM ethanol.
Group III: Positive control – cells + 15 mM PCM + 50 mM ethanol + 10 μg/ml silymarin.
Group IV: AWE of *M. koenigii* – cells + 15 mM PCM + 50 mM ethanol + 100 μg/ml AWEMK.
Group V: AWE of *P. niruri* – cells + 15 mM PCM + 50 mM ethanol + 20 μg/ml AWEPN.
Group VI: Combination of AWEs – cells + 15 mM PCM + 50 mM ethanol + 50 μg/ml AWEMK + 10 μg/ml AWEPN.

For curative study, cells were grown in six-well plates seeded at a concentration of 1×10^5 cells per ml (2 ml/well) for 24 h at 37°C. Damage was induced by treating the cells with 15 mM PCM and 50 mM ethanol for 24 h followed by treatment with different plant extracts (alone and in combination) and silymarin for 24 h. The experimental groups and doses used for plant extracts and silymarin were similar to the prophylactic study.

For cytoprotection assay, HepG2 cells were grown in 96-well plates for 24 h at a concentration of 5×10^4 cells per ml (10^4 cells/well, 200 μl/well) at 37°C. Cell viability was determined for both prophylactic and curative studies using the MTT assay and results were expressed as % cytoprotection[22].

% Cytoprotection = % Viability of treatment group – % Viability of negative control.

Biomarker enzymes serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were spectrophotometrically assessed in spent media as an indicator of cellular damage by kinetic method kits (Erba Mannheim, Germany).

For measurement of lipid peroxidation (LPO) and reduced glutathione (GSH), the cells were grown in six well plates at a concentration of 1×10^5 cells per ml (2 ml/well) for 24 h at 37°C. The cells were treated in triplicate with plant extracts and toxicants as described earlier. After treatment the cells were trypsinized and the pellet was washed twice with PBS at 4°C. The cell pellet (1×10^7 treated cells/ml of lysis buffer) was then lysed in cell lysis buffer (50 mM HEPES buffer, pH 7.0, 150 mM NaCl, 1 mM Na_2EDTA, 1% Triton X-100) by repeated pipetting. The homogenate was then centrifuged (10,000 rpm, 4°C, 10 min) and the supernatant (cell extract) was used for further experiments. Total protein was estimated in the cell extract using Bradford's method[23].

The total glutathione level was quantified using Ellman's method[24]. Total glutathione was determined by kinetic method from a standard curve of reduced glutathione. The results are expressed in nmol/mg of protein.

The extent of lipid peroxidation was estimated by the levels of malondialdehyde (MDA) measured using the thiobarbituric acid reactive substances (TBARS) assay at 535 nm (ref. 25). The results are expressed as nmol/mg of protein using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Morphology of the HepG2 cells was observed to evaluate the extent of degenerative changes and recovery for both prophylactic and curative treatment.

Results are reported as mean ± SD or SEM. Total variation present in a group was determined by one-way analysis of variance (ANOVA), and Student's *t*-test was used to determine significance[26]. The graphs were prepared using the software GraphPad Prism 5.

The present study reveals the hepatoprotective potential of AWEs of *M. koenigii* and *P. niruri* against PCM and ethanol-induced toxicity in HepG2 cell line.

The percentage of yields of prepared AWE for *M. koenigii* leaves was found to be 19.7 ± 0.4, whereas for *P. niruri* leaves it was 20.2 ± 2.4. An earlier study has reported a per cent yield of 12 for AWE of *M. koenigii* leaves[7].

HepG2 cells retain several specialized functions which are characteristic of normal hepatocytes and hence are used as a model for extensive toxicity studies of the liver[27]. The IC_{50} values for silymarin, AWEPN and AWEMK were found to be 200, 600 and 800 μg/ml respectively. HepG2 cells showed growth equivalent to normal untreated cells at a concentration of 10 μg/ml silymarin, 20 μg/ml AWEPN, 100 μg/ml AWEMK and 50 μg/ml AWEMK and 10 μg/ml AWEPN in combination (see data provided in Supplementary Material online). The IC_{50} values for PCM and ethanol were found to be 15 and 100 mM respectively, whereas it was found to be 15 mM PCM and 50 mM ethanol in combination.

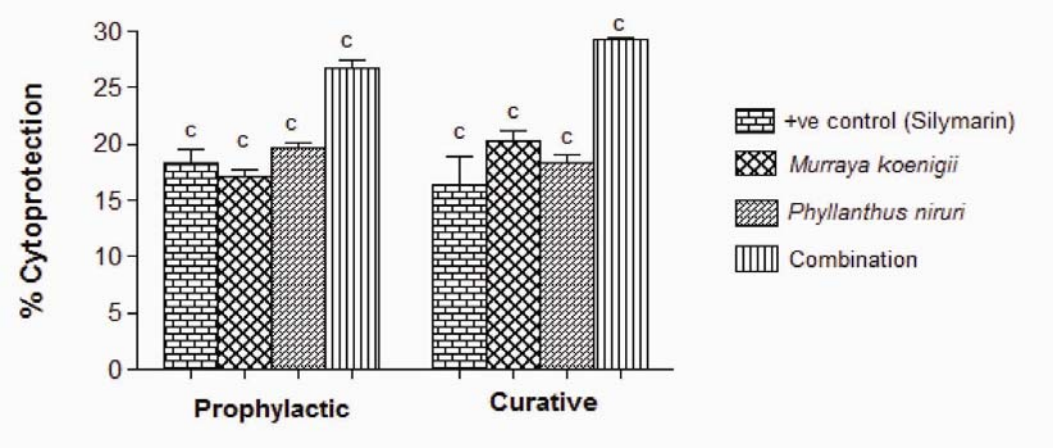

Figure 1. Cytoprotective effects of preconditioning and post-treatment of alcohol water extract (AWE) of *Murraya koenigii* and *Phyllanthus niruri* against paracetamol- and ethanol-induced cytotoxicity in HepG2 cells for 24 h. Percentage of cytoprotection is calculated as: Percentage of viability of cells treated with plant extracts – percentage of viability of cells treated with paracetamol and ethanol. Each value represents mean ± SEM ($n = 3$). P value versus negative control (PCM + ethanol): c < 0.01.

PCM toxicity involves initial metabolism of paracetamol into a reactive metabolite NAPQI (*N*-acetyl-*p*-benzoquinone imine) followed by its binding to cellular proteins, especially to mitochondrial proteins. In later stages this protein binding induces mitochondrial oxidant stress which eventually leads to necrotic cell death[28]. The major enzyme involved in metabolism of PCM is Cyp2E1 and ethanol acts as its inducer, henceforth enhancing the damage induced by PCM[29].

In the prophylactic study it was observed that a combination of extracts gave significant cytoprotection (27%) in comparison to 16% cytoprotection provided by silymarin (Figure 1). The cytoprotection provided by the plant extracts during preincubation may be due to (i) their interference with the metabolic activation of paracetamol by Cyp450 enzymes; (ii) by interfering with the binding of NAPQI to cellular proteins at the initial steps of paracetamol toxicity, or (iii) because of their antioxidant properties which avoid mitochondrial oxidative stress in later stages. Akanitapichat *et al.*[22], have shown that preincubation with 50 and 100 µg/ml of eggplant extracts was effectively able to protect HepG2 cells against the cytotoxicity caused by 300 µM of tert-butyl hydroperoxide (t-BuOOH)[30]. They demonstrated that phenolic antioxidants present in the eggplant extracts are responsible for their hepatoprotective effect against t-BuOOH-induced toxicity.

Post-treatment of cells with a combination of plant extracts after challenging them with paracetamol and ethanol for 24 h, showed a significant cytoprotection (29.2%) compared to 16.3% for treatment with silymarin (Figure 1). Curative study ensures that cytoprotection by plant extracts occurs as a result of their antioxidant potential to quench reactive oxygen species (ROS), thus avoiding mitochondrial oxidative stress and eventually cell death. It has already been established that delayed treatment with antioxidants which scavenge ROS inhibits paracetamol-induced toxicity without relevant effect on metabolic activation and protein binding of NAPQI[31].

Curative treatment with plant extracts was found to be more potent than the prophylactic treatment, as is evident from Figure 1.

The cells on treatment with PCM and ethanol showed a significant ($P < 0.05$) increase in the levels of SGOT and SGPT leakage in the culture medium for both prophylactic and curative studies in comparison to the untreated normal cells. The marked increase in the levels of SGOT and SGPT signifies damage to the structural integrity of hepatocellular plasma membrane, thus leading to their leakage from cytoplasm into culture medium[32].

During the curative study post-treatment of HepG2 cells with silymarin, AWEMK, AWEPN and a combination of AWEs for 24 h was able to significantly ($P < 0.05$) restore the increased levels of SGOT and SGPT to near normalcy compared to PCM and ethanol-treated cells (Figure 2 *a* and *b*). The combination of extracts showed 71% decrease in SGOT and 47% decrease in SGPT levels in comparison to PCM and ethanol-treated cells of Group II. Peng *et al.*[32] have shown that post-treatment with the fractions isolated from *Ganoderma resinaceum* was able to protect HepG2 cells against oxidative damage induced by hydrogen peroxide (H_2O_2)[33]. The three fractions – ganoderesin B, ganoderol B and lucidone A – showed inhibitory effects against the increase of SGPT and SGOT levels in HepG2 cell culture medium induced by H_2O_2 compared to a control group treated only with H_2O_2.

Preincubation with plant extracts and silymarin was able to significantly ($P < 0.05$) ameliorate the increase in the levels of SGOT and SGPT (Figure 2 *a* and *b*). The

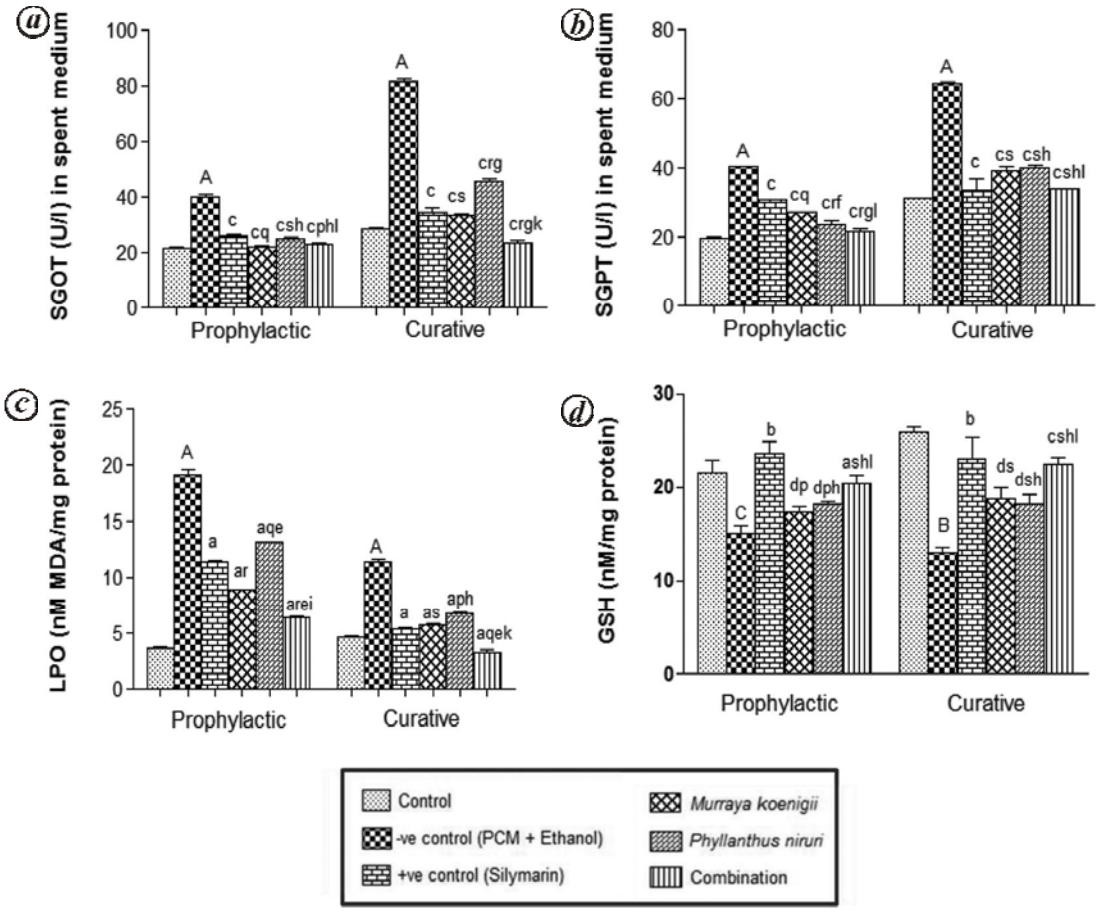

Figure 2. Effect of silymarin and plant extracts AWE of *M. koenigii* leaves (AWEMK), AWE of *P. niruri* leaves (AWEPN and combination of AWEs) on biochemical and antioxidant parameters against paracetamol (PCM) and ethanol-induced toxicity (prophylactic and curative study). ***a–d***, Representation of serum glutamate oxaloacetate transaminase. ***b***, Serum glutamate pyruvate transaminase. ***c***, Lipid peroxidation. ***d***, Reduced glutathione. All values represent mean ± SEM ($n = 3$). *P* value versus vehicle control: A <0.001; B <0.01; C <0.05. *P* value versus negative control: a <0.05; b < 0.01; c < 0.001; d, Not significant. *P* value versus positive control: p < 0.05; q < 0.01; r < 0.001; s, Not significant. *P* value versus AWEMK: e <0.05; f <0.01; g <0.001; h, Not significant. *P* value versus AWEPN: i <0.05; j <0.01; k <0.001; l, Not significant.

combination of extracts showed maximum per cent decrease in the elevated levels of SGOT (44) and SGPT (46) in spent medium.

Both studies showed that a combination of extracts possessed significant ($P < 0.01$) hepatoprotective effect, emphasizing its membrane-stabilizing property.

GSH plays an important role of intracellular antioxidant in cells and prevents damage by ROS to important cellular components by reducing them. During paracetamol-induced toxicity excess binding to NAPQI leads to depletion of GSH. This depletion in turn leads to excessive generation of ROS which ultimately leads to LPO and increase in the levels of MDA[34].

During prophylactic study preincubation of cells with silymarin, AWEMK, AWEPN and a combination of AWEs for 24 h was able to ameliorate the increase in MDA content and decrease in GSH content (Figure 2 *c* and *d*) in comparison to Group II. Cells preincubated with silymarin showed 57% increase in GSH content, and 36% increase when preincubated with a combination of AWEs in comparison to Group II. Furthermore, the cells preconditioned with silymarin showed 41% decrease whereas preconditioning with a combination of AWEs showed 67% decrease in MDA levels. This protection against PCM and ethanol-induced oxidative stress is in agreement with a similar study[35], where preconditioning of HepG2 cells with plant extracts of *Lavandula coronopifolia* (10–50 µg/ml) for 24 h was able to alleviate the increase in LPO and decrease in GSH induced by ethanol.

Post-treatment of cells with silymarin, AWEMK, AWEPN and a combination of AWEs for 24 h was able to significantly ($P < 0.05$), alleviate this increase in MDA and decrease in GSH content (Figure 2 *c* and *d*). Post-treatment with silymarin caused 76% increase in GSH content, whereas treatment with a combination of AWEs showed 72% increase in GSH content in comparison to

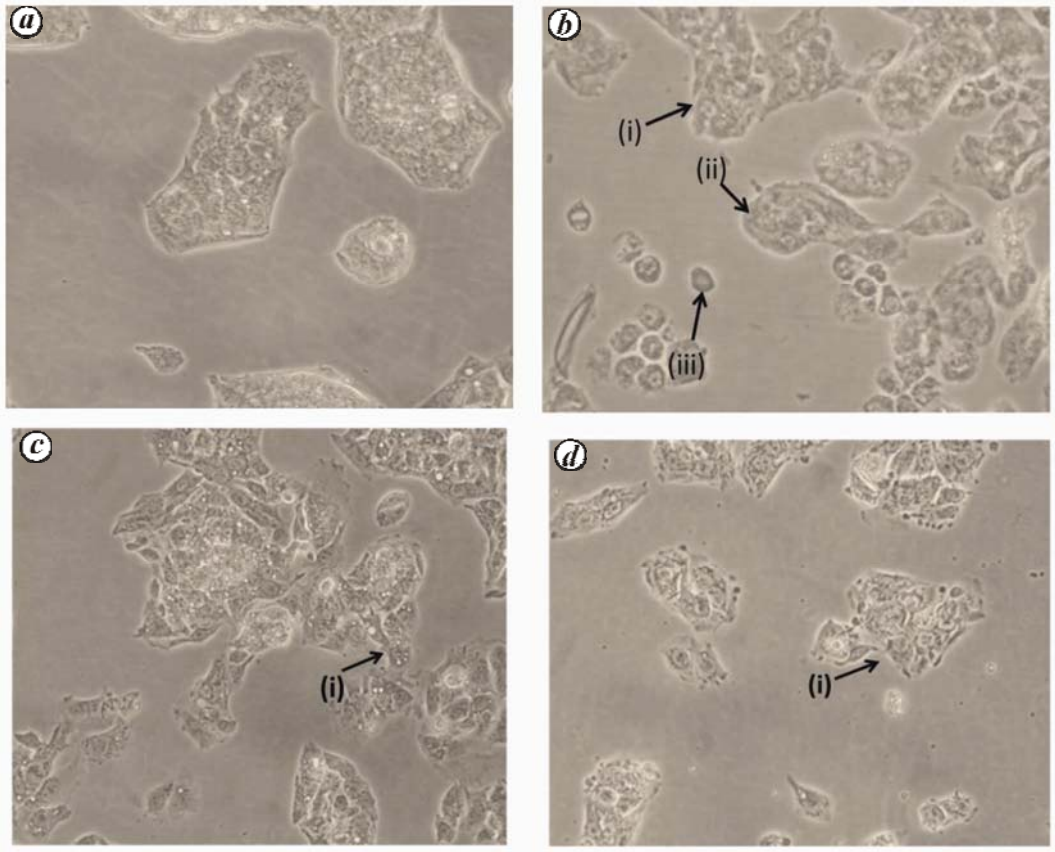

Figure 3. Effect of different treatments on HepG2 cell morphology. ***a***, Normal untreated cells showing regular morphology. ***b***, Cells treated with PCM (15 mM) and ethanol (50 mM) showing (i) excessive granulation, (ii) clumping and (iii) detachment. ***c***, Curative treatment of cells with combination of AWEMK and AWEPN shows repair by restoring morphology similar to normal untreated cells (i). ***d***, Prophylactic treatment with a combination of AWEMK and AWEPN protects the cells from damage induced by PCM and ethanol as is evident with restoration of cell structure (i).

Group II. This was accompanied with 42% decrease in MDA content in silymarin treated cells, and 65% decrease in MDA content in cells treated with a combination of AWEs.

The restoration of normal levels of GSH and decrease in the amount of MDA generated in both prophylactic and curative studies signifies the antioxidant nature of plant extracts and thus their ability to revert damage caused by free radicals. The plant extracts, i.e. AWEMK and AWEPN were found to possess significant antioxidant potential during DPPH radical scavenging assay (data not given). Previous work done has established the presence of flavonoids, phenols, tannins or lignans in leaf extracts of *M. koenigii*[36] and *P. niruri*, which have been known for their rich antioxidant and hepatoprotective properties[37]. Phyllanthin and hypophyllanthin found in *Phyllanthus* species have been reported to be hepatoprotective against carbon tetrachloride (CCl_4)-induced cytotoxicity in hepatocytes[38]. As shown in the present study, a combination of extracts provides significant ($P < 0.05$) hepatoprotection in comparison to individual plant extracts and silymarin treatment. This may be due to combinatorial effect of phytochemicals present in the plant extracts. Further studies at the biochemical and molecular level are required to determine the extent of effect of phytoconstituents at the cellular level.

Earlier work has also shown that five phenolic compounds, namely luteolin, quercetin, rosmarinic acid, luteolin-7-glucoside and caffeic acid were able to protect HepG2 cells from oxidative stress against tert-butylhydroperoxide (t-BHP) by restoring the decreased levels of GSH[39]. It has been further reported that treatment with caffeic acid, rosmarinic acid, their combination and aqueous *Perilla frutescens* leaf extract enhances the intracellular GSH level and exhibits a significant decrease in MDA levels in HepG2 cells treated with 0.3 mM t-BHP[40]. Several other studies have shown the hepatoprotective potential of herbal extracts or products in HepG2 cells[41,42].

The curative effect of a combination of AWEs was found to be more potent in comparison to the prophylactic effect, elucidating the fact that plant extracts interfered in the steps involved after generation of NAPQI from PCM. The better curative effect can be presumed to

have potentiated from the efficient replenishment of GSH and antioxidant nature of AWEs in comparison to the prophylactic study where early exposure to plant extracts interferes with the initial steps of metabolism of PCM to NAPQI by Cyp 450. Further evaluation at the molecular level needs to be carried out to demonstrate the actual molecular events participating in this phenomenon.

The hepatoprotective potential of plant extracts and silymarin was further substantiated by evaluating the morphology of HepG2 cells. Treatment with PCM (15 mM) and ethanol (50 mM) caused granulation and detachment of cells (Figure 3 *b*). During the prophylactic study, preconditioning with a combination of AWEMK and AWEPN was able to protect the cells against PCM and ethanol-induced cytotoxicity. The cells were found to show normal morphology with intact cell membranes (Figure 3 *c*). Curative treatment with plant extracts was able to show effective cytoprotection against paracetamol and ethanol-induced cell damage (Figure 3 *d*). Thus, the morphology study is supportive of effective hepatoprotective potential of a combination of AWEMK and AWEPN.

It has been previously reported that cytotoxicity induced by ethanol in HepG2 cells was reversed by water extract, tannins and carbazole alkaloids isolated from *M. koenigii* leaves. The plant extracts were able to restore normal cell morphology similar to untreated cells, showing no ballooning as is visible in ethanol-exposed cells[19].

This study signifies the hepatoprotective potential of AWEs of *M. koenigii* and *P. niruri* (alone and in combination) during both prophylactic and curative studies. The antioxidant-rich plant extracts were able to show significant ($P < 0.05$) per cent cytoprotection, reduction in leakage of SGOT and SGPT, decrease in MDA content and increase in GSH content in comparison to PCM and ethanol-treated cells. The plant extracts showed effective protection against radical damage accompanied with membrane stabilization. The combination of extracts showed immense potential as a hepatoprotectant with curative treatment showing better results in comparison to prophylactic treatment. Thus, it holds great promise for use as a polyherbal formulation for treatment of liver ailments.

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Toxicity study in mice fed with corn produced in soil containing tannery sludge vermicompost and irrigated with domestic wastewater

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Growing food in unconventional systems such as those using irrigation with domestic wastewater and the use of potentially toxic waste has generated resistance from producers and consumers. Here, we evaluate the possible physical and biochemical damage to Swiss mice fed for 13 weeks with corn produced in soil containing tannery sludge vermicompost and irrigated with wastewater from domestic sewage. The corn was offered as an additional food to standard rodent chow at a daily concentration of 15 g/kg of body mass. The results showed no changes in body weight of the animals during the experimental period. The consumption of grain and weight gain of the animals was stable. The total protein, albumin, globulin and alkaline phosphatase levels did not differ among experimental groups. In addition, macroscopic analysis of the liver of the animals showed no sign of injury or disorders. Thus, we preliminarily conclude that the maize produced in this way is innocuous to animals. However, further studies are needed to evaluate other variables not measured in the present study which can contribute to food security and the nutrition of the corn thus produced.

Keywords: Agro-industrial waste, animal models, toxicity, wastewater.

INDUSTRIAL processes and human activities generate waste that can be harmful to the environment and human health[1]. Such waste is a serious threat to the present quality of life[2] and is typified in the wastes generated from processing of bovine leather.

While these activities do generate significant profits and contribute to the economic and social development of a country, they also produce significant amounts of waste. This problem is intensified, especially when one considers that in many tanning industries waste/effluent

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Table 1. Experimental groups established in the present study

Experimental group	ID	Description
Control	G1	Composed of animals fed only standard rodent chow
Group corn commercial[a]	G2	Composed of animals fed standard rodent chow supplemented with commercial corn grain (Anchieta®). This was the second control group because the corn used here was commercially available.
Group chemical fertilization[b] – water supply[c]	G3	Composed of animals fed standard rodent chow supplemented with corn[f] grains produced by plants grown in soil with conventional chemical fertilizer and irrigated with water supply. This group was the third control group and used corn grown in the same soil and conditions of corn used for G4 and G5.
Group chemical fertilization[b] – domestic wastewater[d]	G4	Composed of animals fed standard rodent chow supplemented with corn[f] produced by plants grown in soil with conventional chemical fertilization, but irrigated with wastewater from sewage.
Tannery sludge vermicompost group[e]	G5	Composed of animals fed standard rodent chow supplemented with grain corn[f] produced by plants grown in soil containing tannery sludge vermicompost mixed with manure (20% of tannery sludge and 80% of cattle manure) plus phosphate fertilizer and irrigated with domestic wastewater.

ID, Identification of the experimental groups.

[a]The conditions for the cultivation of corn and seed variety were not identified in the packaging.

[b]The nitrogen, phosphorus and potassium (NPP) used in the cultivation of corn were calculated based on the nutritional needs of the crop, the nutrient concentrations in the soil (Oxisoil Typical) and yield expectation of 10 Mg per ha according to ref. 22. The NPP sources were urea (CH_4N_2O), superphosphate (P_2O_5) and potash (K_2O), respectively.

[c]Irrigation water used in the cultivation of corn offered in this experimental group was from the water supply system *Instituto Federal Goiano* (Urutaí, GO, Brazil) treated in a water treatment plant institution itself.

[d]Wastewater used for irrigation of corn offered in this experimental group was from a domestic wastewater stabilization pond located on the premises of Instituto Federal Goiano (Urutaí, GO, Brazil).

[e]The corn kernels were from the treatment with the highest productivity in the study of Malafaia[22].

[f]Cultivar LG 6036 (LG Sementes®).

produced is disposed of in an improper manner or put in 'dumps', landfills or industrial landfills (localized within the industrial land). This accumulation and concentration of potentially toxic material lead to a high risk of environmental contamination[3–7].

Fixing this problem can be a win-win situation. Indeed, there is active research on new ways to dispose and recover waste. Various studies have shown the benefits of tannery sludge in different agricultural crops such as sugarcane ratoon[8], conilon coffee[9], beans[10], lettuce[11,12], soybean[13], wheat, lettuce and radish[14] and maize[3,15–22]. Concurrently, there are great demands for the use of sewage wastewater in agriculture because it can act as a fertilizer and improve yield[23]. Several studies have shown the advantages of water in agriculture because it adds nutrients that are beneficial for plant growth[23,24–30].

Despite the evidence that both the waste produced in tanning industries and wastewater can be useful in agriculture, there is still resistance from farmers with few practical applications[23]. Part of this resistance is related to food and nutrition security linked to food produced in soils enriched with waste or potentially contaminated with wastewater sewage. These foods could then be risky.

Here, we evaluate the possible biochemical and weight damage to Swiss mice fed corn produced in soil containing tannery sludge vermicompost and irrigated with household wastewater.

We used female Swiss mice (50-day-old) from the Biotério Central of the Universidade Federal de Goiás (Goiânia, GO, Brazil) that were maintained in the Biotério of the Laboratory of Biological Research, Instituto Federal Goiano (Urutaí, GO, Brazil). The animals were subjected to light/dark normal cycle (12/12 h) and kept in collective cages with a polycarbonate ventilated shelf (Alesco®) under controlled temperature (22–25°C).

The treatments consisted of grains offered as additional food to animals fed a standard rodent chow (Nuvilab CR1-Nuvital®), as described in Table 1. All the animals of the experimental group received rodent diet ad libitum, ensuring appropriate nutrition. Therefore, corn was an additional food to the standard diet. The grain was obtained from the work Malafaia[22]. We used 50 Swiss mice and distributed them in five groups (n = 10 per group).

Tables 2 and 3 present the chemical and physicochemical characteristics of the soil, tannery sludge vermicompost and irrigation water used in corn farming.

Each day the animals received 15 g/kg body weight of corn as mentioned in the literature[31–34]. The amount of grain given to the animal's weight was measured daily for the offering of the selected grain concentration (15 g/kg body weight). Corn was kept in sterile containers with no processing or treatment.

We monitored the animals for 13 weeks. This was reasonable considering the lack of standard protocols for assessing food security and nutrition from non-conventional crops. This period has been used in previous studies designed to assess the safety of genetically modified subchronic food testing[35].

To evaluate possible adverse physical effects from the corn, we evaluated body mass (g) with results expressed in weekly averages. At the end of the experiment, we reported animal weight gain, grain consumption, as well as

serum total protein, globulin, albumin and alkaline phosphatase concentrations.

Serum total protein concentration was calculated using the biuret method utilizing the commercial kit Labtest Diagnostic SA®, Cat. 99 (Lagoa Santa, MG, Brazil). For assessment of serum albumin concentration, we used the bromocresol green method with the commercial kit Labtest Diagnostic SA®, Cat. 19 (Lagoa Santa, MG, Brazil). From the total protein and albumin concentrations, we calculated globulin concentration. For this, we used the following equation: globulin = total protein globulin – albumin. Alkaline phosphatase measurement was done using a method described by Bowers and McComb with the commercial kit Labtest Diagnostic SA®, Cat. 79 (Lagoa Santa, MG, Brazil). For these experiments, the animals were fasted for at least 12 h and 2 ml of blood was collected via the brachial artery. The blood was placed in 5 ml tubes containing anticoagulant.

Prior to blood collection, the animals were anesthetized with tribromoethanol 2% (1 ml/100 g, i.p.) followed by cervical dislocation. The livers were removed for macroscopic analysis of possible changes in them. The livers were evaluated for colour as microscopic capsular cutting surfaces. We noted the presence or absence of nodulation (colour, quantity, size and distribution) or any sign of liver damage that could be related to toxicity.

Initially, data were subjected to normality test of Anderson–Darling, Kolmogorov-Smirnov, Cramér-von Mises, Kuiper, Watson and Lilliefors, followed by the homogeneity test using Levene variance (ASSISTAT software, version 7.7 beta (copy distributed free)). All data were normally distributed a 5% probability ($P > 0.15$). Statistical differences were calculated for comparison analysis. The body mass data were subjected to analysis of variance (ANOVA) for repeated measures ANOVA followed by the Duncan test at 5% probability. For data on biochemical assessments, we used the simple one-way ANOVA followed by Duncan test at 5% probability.

The methodology of this study was considered consistent with the ethical principles for animal experimentation and approved by the Committee on Animal Research and Ethics of the Instituto Federal Goiano (GO, Brazil) (protocol no. 003/2014).

We observed body mass for 13 weeks (Figure 1 *a*). This period was chosen due to lack of standard protocols for assessing food security and nutrition from non-conventional crops. Thus, we based our study on those that assessed the toxicity of substances, tea, drinks in

Table 2. Main characteristics of the initial soil and tannery sludge vermicomposting used in the present study. Urutaí, GO, Brazil, 2014

	Results	
Diversify	Ground	Vermicomposting (Lc20)*
pH ($CaCl_2$)	5.30	8.8
N (%)	0.11	1.5
P (Melich – mg dm^{-3})	5.00	700.0
K (mg dm^{-3})	240.00	18,000.0
Ca (cmolc dm^{-3})	2.60	14.0
Mg (cmolc dm^{-3})	0.80	18.0
Ca + Mg (cmolc dm^{-3})	3.40	32.0
Al (cmolc dm^{-3})	0.00	0.0
H + Al (cmolc dm^{-3})	2.20	0.0
CTC (cmolc dm^{-3})	6.20	82.4
Na (mg dm^{-3})	8.00	1.000.0
Cu (mg dm^{-3})	2.50	5.0
Fe (mg dm^{-3})	63.00	244.0
Mn (mg dm^{-3})	47.00	68.0
Zn (mg dm^{-3})	4.40	36.0
Organic matter (%)	2.30	29.9
Sat Al (%)	0.00	0.0
Sat base (%)	65.00	100.0
Ca/Mg (%)	3.30	0.8
Ca/CTC (%)	42.00	17.0
Mg/CTC (%)	13.00	22.0
K/CTC (%)	10.00	56.0
H + Al/CTC (%)	35.00	0.0
Clay (%)	27.00	–
Silt (%)	15.00	–
Sand (%)	58.00	–
Electrical conductivity (μS cm^{-3})	184.00	1.170,0
Total organic carbon (%)	1.30	17.3
Particle density (g cm^{-3})	2.45	–
Cr (mg dm^{-3})	<5.00	<5.0

*Vermicomposting (Lc20): Sludge vermicompost tannery made up of 20% type liming tannery sludge and 80% cattle manure.

Table 3. Physical, chemical and physico-chemical characterization of the irrigation water used in the present study

Parameters	Supply water*	Wastewater*
pH at 25°C	7.20	6.75
Turbidity (UNT)	8.00	931.00
Dissolved Fe (mg l^{-1})	0.68	1.77
Total N (mg l^{-1})	3.00	54.95
Organic N	ND	3.95
Ammoniacal N (mg l^{-1})	0.17	43.00
Nitrate (mg l^{-1})	0.30	8.00
Electric conductivity at 25°C (μs cm^{-1})	49.10	738.00
Total P (mg l^{-1})	0.22	10.29
Orthophosphate (mg l^{-1})	0.50	23.60
BOD (mg l^{-1})	0.70	733.33
Total solids (mg l^{-1})	80.00	1,790.00
Dissolved Cu (mg l^{-1})	1.00	1.00
Zn (mg l^{-1})	0.03	0.07
Na (mg l^{-1})	5.25	83.50
Dissolved Mn (mg l^{-1})	1.20	2.20
Dissolved Mg (mg l^{-1})	2.43	4.86
Ca (mg l^{-1})	4.00	20.04
S (mg l^{-1})	2.00	2.66
K (mg l^{-1})	1.64	26.00
TOC (mg l^{-1})	18.36	46.00

ND, Parameter not analysed. BOD, Biochemical oxygen demand; TOC, Total organic carbon.
*Values shown are the mean of three samples collected during the experimental period.

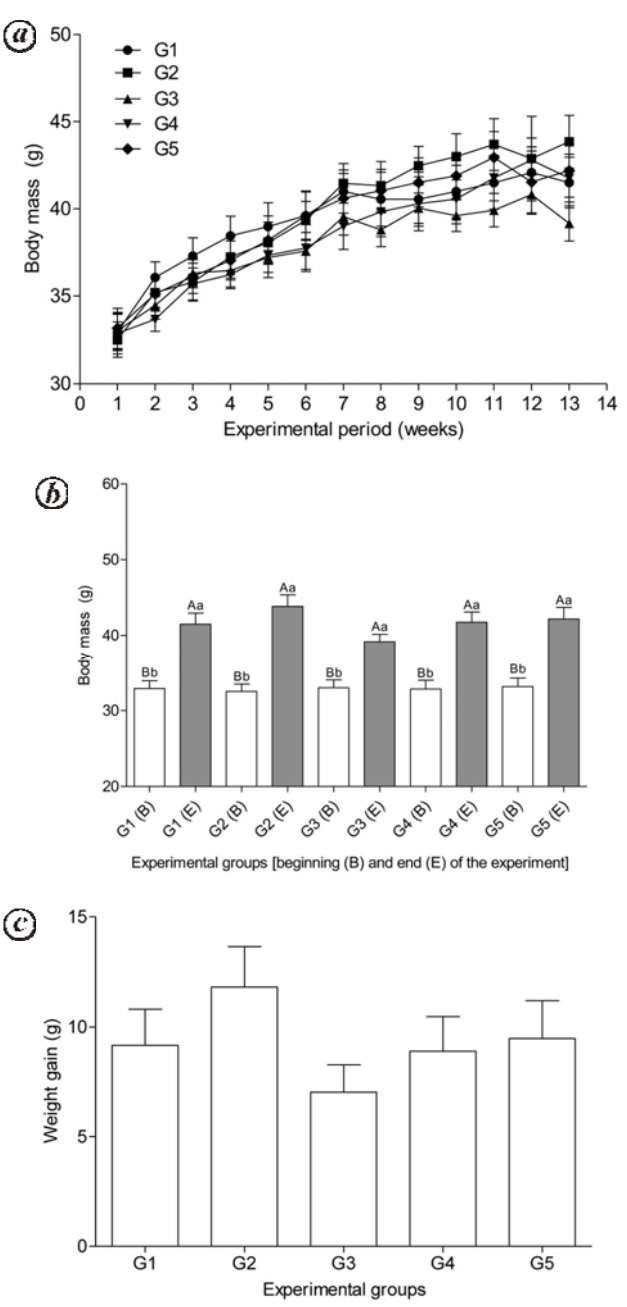

Figure 1. ***a***, Change in body weight throughout the experiment; ***b***, initial and final mass; ***c***, weight gain in Swiss mice fed with corn grain produced under different conditions, as an additional food to the standard rodent diet. In (***a***) the points on the curve represent the means ± SEM of the groups at each week of the trial. The body mass data were analyzed by analysis of variance for repeated measures ANOVA followed by the Duncan test at 5% probability. In (***b***) and (***c***), the bars represent the mean + SEM. We used one way ANOVA at the 5% probability level. In (***b***) different lowercase letters indicate statistical differences between initial and final values of each group with Student's *t* test at 5% probability. Equal capital letters indicate that there is no statistical difference between groups by one-way ANOVA at 5% probability while comparing them from the beginning to the end of the experiment. In (***c***) we show one-way ANOVA at 5% probability. G1, Control group; G2, Group commercial corn; G3, Chemical fertilizer group – water supply; G4, Chemical fertilizer group – wastewater; and G5, Tannery sludge vermicompost group (see Table 1 for details). $n = 10$ for G1, G4 and G5 and $n = 9$ for G2 and G3. Two animals of G2 and G3 died during the experiment due to unknown causes.

general or conventionally produced food[36–42]. These studies in general have considered that 13 weeks corresponds to sub-chronic exposure.

All groups gained weight at the end of the experiment compared to their initial mass (Figure 1 *b*). This is due to the growth and natural development of the animals. There was no difference in the amount of weight gained between the different groups (Figure 1 *c*) ($F_{(4,43)} = 1.027$, $P = 0.404$). These results show that corn consumption did not affect mouse growth and development.

To our knowledge, there have been no previous studies on the effects of corn grown in soil containing vermicompost and domestic wastewater on mouse health. Júnior *et al.*[43] have shown that weight gain is an early indicator of toxicity. Several studies have reported that weight parameters in animal studies can assess the effect of substances in the body for medical, food and environmental toxicology[44–48].

There were no differences between the groups in terms of maize consumption (Figure 2) ($F_{(4,45)} = 0.597$, $P = 0.668$). This was expected because the amount of grain supplied depended on the body weight of the animals. This did not differ during the beginning, middle or end of the experimental period.

There was no difference between the experimental groups for total protein ($F_{(4,43)} = 0.487$, $P = 0.744$), albumin ($F_{(4,43)} = 2.248$, $P = 0.079$), globulin ($F_{(4,43)} = 0.784$, $P = 0.541$) (Figure 3 *a*) and the enzyme alkaline phosphatase ($F_{(4,40)} = 2.780$, $P = 0.102$) (Figure 3 *b*). These results show that the additional intake of corn did not change these biochemical parameters.

These results are similar to recent reports on Swiss mice[49,50] indicating that corn supplements do not change these parameters. It is known, for example, that total protein levels provide insight into the health of an organism[51].

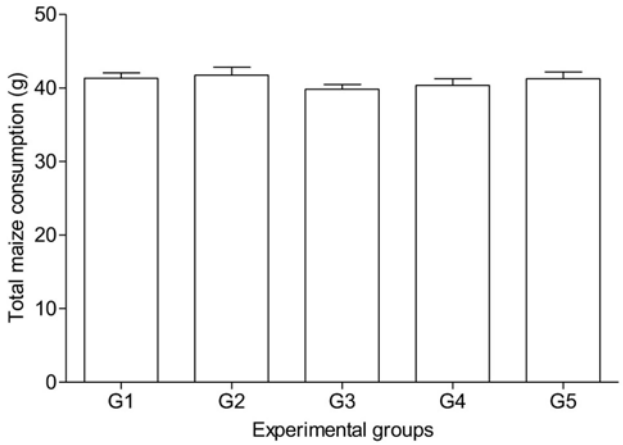

Figure 2. Total consumption of grains during the experimental period. The bars represent the mean + SEM of independent experiment. We used one-way ANOVA at 5% probability level. G1–G5, same as in Figure 1. $n = 10$ for G1, G4 and G5 and $n = 9$ for G2 and G3. Two animals of G2 and G3 died during the experiment due to unknown causes.

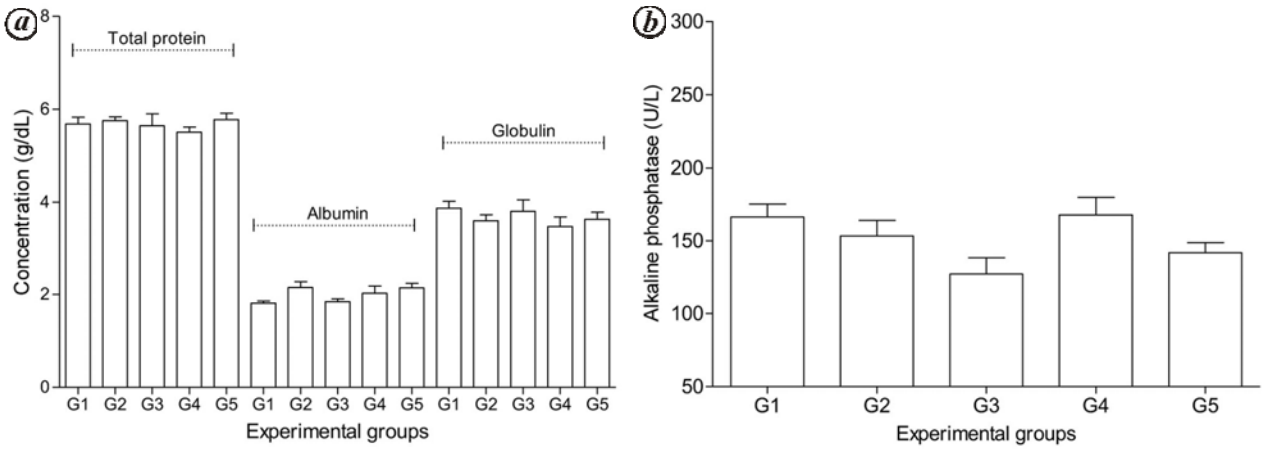

Figure 3. ***a***, Serum concentrations of total protein, albumin, globulin; ***b***, concentration of the enzyme alkaline phosphatase from Swiss mice fed with corn grown under different conditions. The bars represent the mean + SEM of independent experiment. We used one way ANOVA at the 5% probability level. G1–G5, same as in Figure 1. $n = 10$ for G1, G4 and G5 and $n = 9$ for G2 and G3. Two animals of G2 and G3 died during the experiment due to unknown causes. $n = 8$ for G1 (two alkaline phosphatase outliers have been removed), $n = 9$ for the G2 and G3 and $n = 10$ for G4 and G5 groups.

Albumin and alkaline phosphatase enzyme are biomarkers of liver injury[52]. Albumin is the most abundant protein in the plasma and extracellular fluids[53], and is one of the most frequently used variables to compose prognostic indexes. It is the best single marker of complications[54]. Hypoalbuminuria may be correlated to chronic liver damage[52] as well as kidney damage.

Alkaline phosphatase and in particular phosphohydrolase is an enzyme found in many tissues with the highest concentrations in liver, biliary tract epithelium and bone[52]. Generally, any active liver disease may increase the phosphatase values, but drastic increases in enzyme levels occur in the case of biliary tract obstruction that may be induced by drugs or toxic substances in water or food intake. Therefore, this enzyme may be an important marker of activity in the plasma membrane and endoplasmic reticulum during pharmacological treatments and in some pathological conditions. Increases in serum alkaline phosphatase activity can occur during cholestasis as well as intra- and extrahepatically via drugs or hormones or by osteoblastic hyperactivity and necrotic processes[55]. Therefore, our data indicate that animals of different groups show no obvious biochemical signs of liver toxicity.

Macroscopic analysis of the liver of animals showed no signs of liver injury. Qualitative analysis revealed that the livers of the animals were healthy and normal.

Therefore, we can conclude that despite the tannery sludge vermicompost and domestic wastewater containing high concentrations of various elements or increased physical and chemical indicators (Tables 2 and 3), they caused no biological effects that indicate toxicity in animals. Although we have not studied the bioavailability of the elements in soil and in plants, in particular, probably those elements were not in bioavailable forms for maize plants, preventing their absorption and, consequently, their translocation to the corn grains.

Studies in the literature with genetically modified (GM) foods point to the concern of the effects – is it not fully known. Results involving the supply of GM laboratory animal diets have been troublesome, as discussed by Smith[56].

Studies have found that the abnormal and damaged cells of the small intestine of mice fed with transgenic potatoes carriers of bacterial gene that produces insecticidal *Bt* (*Bacillus thuringiensis*)[57]. Stomach intestinal epithelial cells from mice fed with potato lectin *Galanthus nivalis* (GNA), which is a type of insecticide[58], also had increased growth. These results are important because cell proliferation can be a precursor to cancer.

With regard to liver damage, different authors have reported different types of damage depending on the feed[59–62]. This requires further studies given the complexity of the organic system and the variety of treatments offered to animals.

This preliminary study offers preliminary evidence that the consumption of maize produced with tannery sludge worm compost and sewage wastewater does not cause toxicity relative to controls. Thus, this approach may be an important food supply that also reduces agro-industrial waste and increases water reuse.

However, it is essential to consider that this study is not exhaustive and further research is needed to safely assess the risks of corn meal produced under experimental conditions of this study. Future work will use different concentrations of maize, alone or as a food supplement, different feeding periods as well as other animal models and strains.

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Interventions to reduce drudgery of workers in the traditional method of harvesting Makhana (*Euryale ferox* salisb.) seeds from ponds

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Makhana (*Euryale ferox* salisb.) is a seed produced from an aquatic crop, which normally grows in water bodies like ponds. In the traditional way of harvesting, a worker goes deep into the pond, lies down, holds his breath and drags the mud with both hands towards a bamboo pole called 'kaara', which is later sieved using a bamboo screen called 'ganjaa'. During this operation mud enters into the ears, eyes, nose and mouth of the worker. Also, the workers are affected by skin-related diseases due to unhygienic working environment. Therefore, an intervention was made and an improved system was developed which consists of a floating platform providing support to a 10 l cylinder having compressed air, 10 m hose pipe with regulator and a mini diving kit having suit with cap, mask and content guage. A comparative study was conducted

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using both traditional system (T1) as well as improved system (T2) of harvesting Makhana seeds from ponds. The results indicate that the average output is only 3.8 kg/h with T1 system, whereas it is 11.3 kg/h with T2 system. The overall discomfort rate is 8.3 in case of T1 system, whereas it is 4.2 in case of T2 system. Also, the body parts discomfort score is higher (78.8) with T1 system compared to T2 system (48.2). The harvesting of Makhana seeds using the improved system involves less drudgery in comparison to traditional system with significantly higher work output.

Keywords: Drudgery, harvesting, Makhana seeds, traditional system.

MAKHANA (*Euryale ferox* salisb.), also known as gorgon nut or fox nut, is a seed produced from an aquatic crop which belongs to the family Nymphaeaceae. It normally grows in stagnant water bodies like ponds, low depressions, lakes, etc. (Figure 1 *a*). About, 80% of total Makhana in India comes from Darbhanga, Madhubani, Purnia and Katihar districts of Bihar[1]. It is mostly cultivated in lowland ponds of Bihar, Odisha, Assam and West Bengal. It is well suited to tropical and sub-tropical climate with 20–35°C temperature, 50–90% humidity and about 100–250 cm rainfall[2]. It has been reported that about 13,000 ha area is under Makhana cultivation in the country[3]. A single Makhana plant produces about 8–9 leaves and flowers arranged alternately, intermingled together (Figure 1 *b*). The leaves are large and round (about 1–2 m in diameter) and float on water, with a leaf stalk attached at the centre of the lower surface. The upper surface of leaf is green, while the underside is purple in colour. The surfaces are covered with sharp prickles/thorns. The roots are long, fleshy and fibrous in nature and generally in 2–3 clusters with a number of air pockets; the seeds are round and lumpy, and about 0.5–1.5 cm in diameter. The flowers are bright purple in colour (Figure 1 *c*). Raw Makhana is a good source of carbohydrates, proteins and minerals containing 76.9% carbohydrate, 12.8% moisture, 9.7% protein, 0.9% phosphorus, 0.5% minerals, 0.1% fat, 0.02% calcium and 0.0014% iron[1,4]. It also has medicinal value and is recommended for treating respiratory, circulatory, digestive, excretory and reproductive disorders[4].

In ponds, 10% of the Makhana plants germinate from the leftover seed of the previous crop. About 80 kg of seeds is normally required for 1 ha of pond area. The depth of the pond varies from 1.2 to 2.4 m, with average depth about 1.8 m. Sprouting takes place during December and January and the Makhana plant comes to the upper surface of the pond during March. Normally, 1 × 1 m spacing (row-to-row and plant-to-plant) is maintained by thinning-off extra plants. Also, during March and April, young and healthy plants are collected from nearby ponds and transplanted at an interval of 1 × 1 m spacing for filling the gaps. The entire pond water surface gets covered with big, expansive and prickly leaves during April and May. After 2–3 months of transplanting, bright purple flowers begin to appear on the pond surface. The flowers change to fruits (Figure 1 *d*) and the fruits then burst inside the water after 30–45 days of flowering. The fresh seeds float on the water surface for 2–3 days before settling down at the bottom, where the red arils of fresh seeds become seasoned or decomposed and turn black in colour at the time of harvesting. Each flower after fruiting produces 8–13 seeds and a single plant produces about 100 seeds. In a pond system, there are about 10,000 plants/ha and seed yield in the traditional system is around 1.8–2.0 t/ha (ref. 1).

Makhana cultivation has several constraints, some of them being no ownership of the pond, drudgery in operation, lack of credit facility, lack of scientific knowledge of cultivation, lack of improved varieties, short lease period and labour-intensive process.

In this communication, we study the environmental concern and drudgery of the workers involved in the traditional way of harvesting Makhana from the ponds in Darbhanga district, Bihar. Makhana harvesting is the only source of income for the Mallah community in the district. Thus the harvesting practice of Makhana has to be improved to reduce their drudgery and improve their livelihoods.

Harvesting refers to collection of scattered Makhana seeds from the bottom surface of the pond. The collection of seeds in the pond system is done during August–October in the morning around 6.00–11.00 am.

First all the parts of the plant are cut and allowed to decompose in the pond, only then do the workers get inside the pond. The pond environment is unhygienic due to mud, thornes/prickles, insects, etc. A worker has to go deep into the pond and hold his breath for a long time. In the traditional system, a bamboo pole locally called 'kaara' is fixed in the pond and the worker goes to the bottom, lies down and drags the mud near the pole with both palms. He covers a radius of his height around the periphery of the pole. A heap of mud is formed near the base of the bamboo pole which is later sieved with locally made bamboo screen called 'ganjaa' (Figure 1 *f*). The black coloured seeds are taken through the water on top and put in earthen pots (Figure 1 *g*). During this operation, mud enters into the ears, eyes, nose and mouth of the worker. Many a times the workers suffer from skin problems like rashes, itching, etc. and injuries due to the presence of sharp thornes/prickles, leech bite, etc. (Figure 1 *h* and *i*).

About 50–60% of the cost of cultivation goes in paying the labour charges for harvesting. Table 1 provides the economics of Makhana cultivation in the traditional system. Normally in ponds, harvesting is done in 2–3 phases. Initially, the charges paid begin with Rs 15–20/kg for the first harvest, Rs 30–40/kg for the second harvest and Rs 50–60/kg for the third harvest and so on if the harvesting phase continues. The increase in the amount paid for

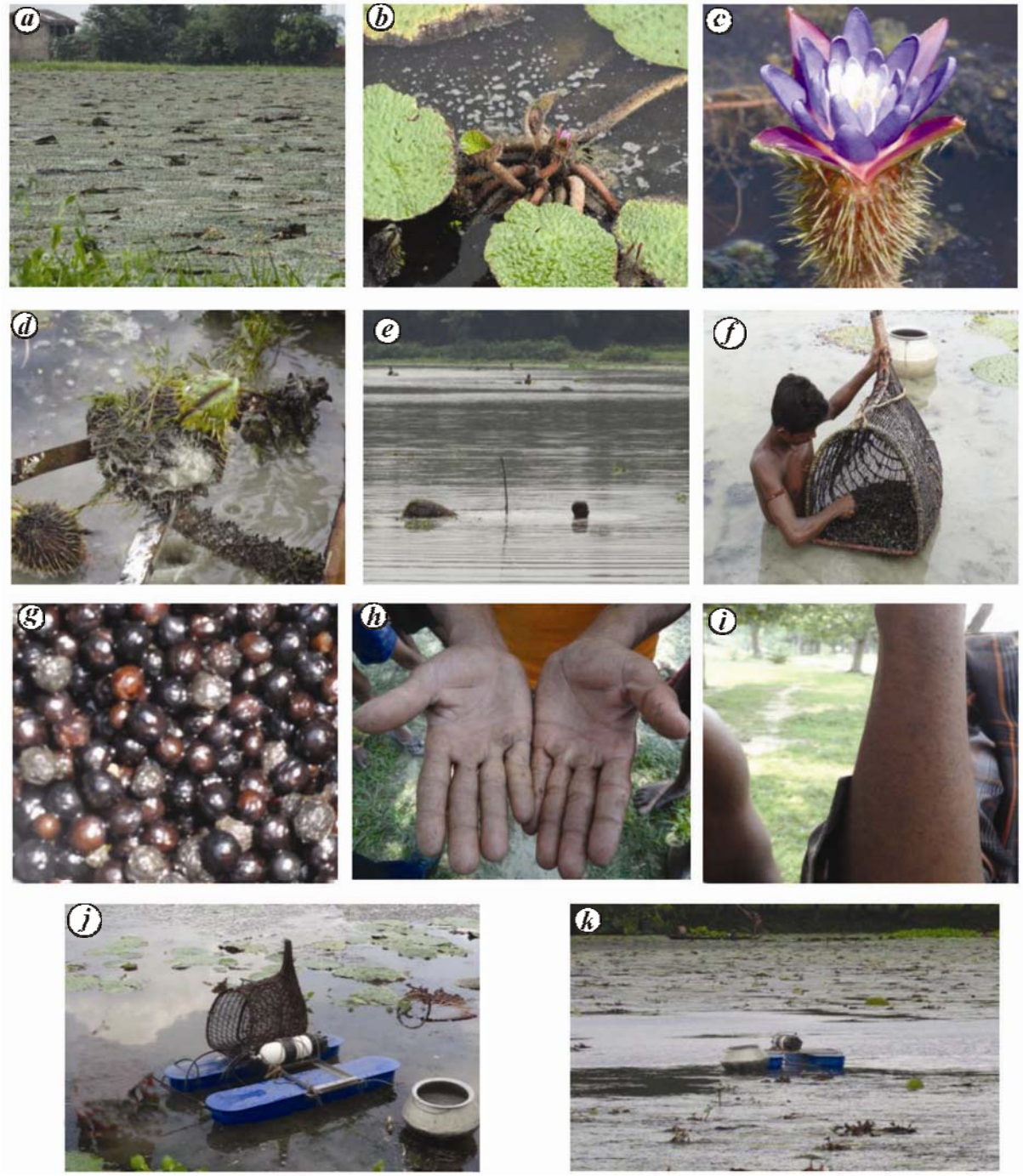


Figure 1. ***a***, Makhana crop in pond. ***b***, Makhana plant. ***c***, Makhana flower. ***d***, Makhana flower changing to fruit. ***e***, Harvesting of Makhana in pond. ***f***, Traditional tool for harvesting Makhana. ***g***, Makhana seeds. ***h***, Palm of Makhana harvester affected by thornes. ***i***, Skin disease in Makhana harvester. ***j***, Improved system for Makhana harvesting. ***k***, Makhana harvesting using improved system.

harvesting is because the collection of seed decreases with increase in harvesting phase.

According to the workers, limit to breathing time inside the pond is the main problem followed by mud entering their eyes, ears, nose and mouth. To eradicate these problems, an improved system has been developed by the Central Institute of Agricultural Engineering, Bhopal and Regional Centre for Makhana, Darbhanga.

The improved system consists of a floating platform having 10 l cylinder of compressed air with a regulator strapped on it, 10 m hose pipe with a regulator and a mini diving kit having suit with cap and mask for use by the workers (Figure 1 *j* and *k*). In this system, the worker is supplemented with filtered air through a 10 m long hose. As the worker is safe and comfortable, the output is higher compared to the traditional system. Also, there are

no injuries and skin-related problems as he is protected by the diving kit.

Ten healthy subjects in the age group of 25–62 years with no previous history of occupational injury were randomly selected for the study from the villages near Darbhanga district. The age, height and weight of the selected subjects were recorded. The subjects were familiarized with the protocol before the data were collected.

The subjects were given sufficient rest before conducting the experiment. After calibration, they were made to do harvesting operation using both the methods, i.e. by traditional system (T1) and improved system (T2). At the beginning and end of each experiment, the subjects were given 30 min rest so that all the physiological parameters regained their normal levels. Each operation was carried out for 1 h duration.

The overall discomfort rate (ODR) and body parts discomfort score (BPDS) were measured after the experiment. After 1 h of experiment, each subject was asked to sit on a chair and quantify his ODR for the work he had just finished. For this purpose, a ten-point visual analogue discomfort scale (VADS) was used (0 – no discomfort and 10 – extreme discomfort)[5]. The subject was asked to indicate the point on the scale which represented his current level of overall discomfort by sliding a pointer on it. ODR given by each subject was averaged to get the mean value. BPDS was measured by the score-based technique[6]. For this, the whole body was divided into 12 parts and a similar body mapping was done by thermocol for rating the perceived exertion of the subject. Each subject was asked about the discomfort felt in his body parts. The total body parts score for a subject is the sum of all individual scores of his body parts. The body discomfort score of all the subjects were added and averaged to get mean score.

A comparative study was conducted for the traditional system as well as improved system for harvesting Makhana seeds from ponds in Dharamsar pokhar and Sursura pokhar of Darbhanga; and Nanoura and Rajokhar pokhar of village-Keoti, Darbhanga. The data obtained were analysed using two-tailed *t*-test to find out whether the mean values of the two groups differed significantly.

The harvesting of Makhana from ponds is a highly drudgery-prone operation. The unit activities involved in harvesting include going down to the bottom of the pond, lying down and sweeping the mud with both palms, collecting seed with ganjaa, washing them inside the water, bringing the ganjaa along with seeds to the top of the pond, and putting the seeds in an earthen pot floating on water. This action is repeated till the pot gets filled.

In the traditional system, a worker has to come to the surface of the pond about 2–8 times in a minute. But, using the improved system the worker can stay inside water up to 1 h and needs to come to the surface only 3–4 times for empting the seeds into the pot.

The mean age, height and weight of the selected subjects were 40.8 years, 1652 mm and 52.5 kg respectively. The harvesting trials were of 1 h duration for both T1 and T2 systems and ten subjects participated in the trial. Table 2 provides mean output of the harvesting trial conducted.

The results show that in 1 h a worker can harvest about 11.3 kg of Makhana seeds with ease using the developed system, whereas in the traditional method he can harvest about 3.8 kg of Makhana seeds. The *t*-test of the two systems shows that it is significant at 1% level. The results show that there is higher output in case of T2 system over T1 system due to the fact that using air cylinder with diving suit made the workers safe and comfortable. The workers can do harvesting with ease for 1 h without any extra effort. Whereas in case of T1 system, a worker needs to take 8–10 dips in 1 min, where a single dip lasts 6–8 sec only. Also, the body is exposed to sharp thornes/prickles, rashes, leech bite, etc.

Table 3 gives the ODR of the harvesting trial conducted.

The *t*-test shows that it is significant at 1% level. ODR was measured on a 0–10 point scale with VADS for assessing overall body discomfort (0–10 scale) (Figure 2). The average ODR (0–10) of subjects after harvesting

Table 1. Economics of Makhana cultivation using traditional system[1]

Item	Cost (Rs/ha)
Cost of cultivation (*A*)	
Rent of pond/year	15,000
Seed required (80 kg/ha @ Rs 70/kg)	5600
Interculture like thinning (12 labourers)	1440
Harvesting	27,000
Transportation charges	1000
Total cost	50,040
Output: (average seed yield – 1.8 t/ha and rate Rs 55/kg) (*B*)	99,000
Net return/ha (*A* – *B*)	48,960

Table 2. *t*-Test of output data of harvesting with T1 and T2 systems

Harvesting system	Mean	Standard deviation	Standard error	*t* Value
T1	3.87	0.68	0.22	10.74***
T2	11.35	2.52	0.80	

Table 3. *t*-Test of overall discomfort rate data of harvesting with T1 and T2 systems

Harvesting system	Mean	Standard deviation	Standard error	*t* Value
T1	8.3	0.82	0.26	10.82***
T2	4.2	1.03	0.33	

Table 4. *t*-Test of body parts discomfort score data of harvesting with T1 and T2 systems

Harvesting system	Mean	Standard deviation	Standard error	*t* Value
T1	78.8	4.47	1.41	21.49***
T2	48.2	4.92	1.55	

Table 5. Techno-economics involved in harvesting of Makhana with improved system (T2)

Item	Improved system (T2)
Cost of equipment (Rs) (mini diving kit – two units, breathing air cylinder – one and floating platform and accessories)	5,00,000.00
Life (years)	10
Annual use/year (h)	450
Fixed cost	
Depreciation (Rs)	45,000.00
Interest @ 12% (Rs/year)	33,000.00
Taxes, insurance and shelter @ 2% (Rs/year)	10,000.00
Total fixed cost (Rs/year)	88,000.00
Variable cost	
Labour cost (Rs/h)	100.00
Repair and maintenance @ 5% (Rs/h)	55.56
Electricity cost (Rs/h)	6.00
Total variable cost (Rs/h)	161.56
Custom hiring charges per unit (Rs/h)	400.00
Break-even point (h)	369
Payback period (years)	4.8
Benefit–cost ratio	2.48

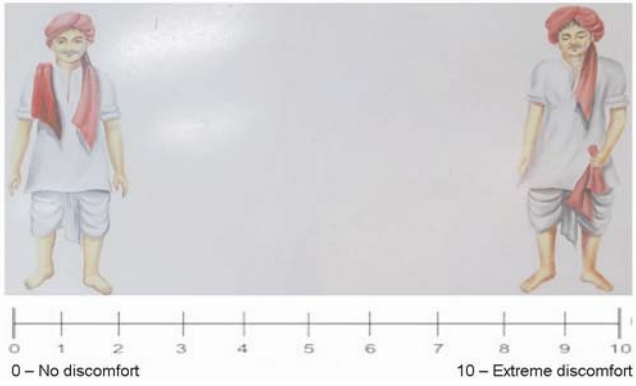
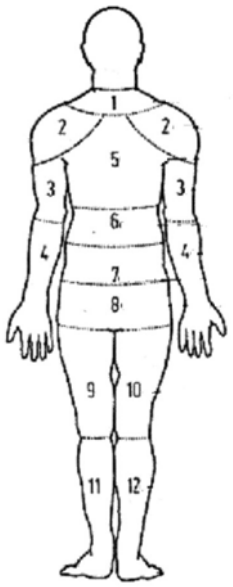


Figure 2. Visual analogue discomfort scale for assessing overall body discomfort (0–10 scale).

Makhana by T1 and T2 systems was found to be 8.3 and 4.2 respectively. ODR is more in T1 system compared to T2 system because of frequent diving in and out of water and the worker's body being exposed to cold water, sharp thornes/prickles, mud, insects, etc.

The BPDS of subjects after harvesting Makhana by T1 and T2 systems was found to be 78.8 and 48.2 respectively (Table 4). The *t*-test of the two system shows that it is significant at 1% level.



Sl. no.	Body part
1	Neck
2.	Shoulders
3.	Upper arms
4.	Lower arms
5.	Upper back
6.	Mid back
7.	Lower back
8.	Buttocks
9.	Left thigh
10.	Right thigh
11.	Left leg
12.	Right leg

Figure 3. Body part postural discomfort score[6].

The total cost of the improved system (T2) was about Rs 5 lakhs. The major cost of the system was for the air compressor which is required for filling filtered air into the cylinder. The equipment can be procured by the self-help groups/non-government organizations/Krishi Vigyan Kendra/pond owners of the area and can be made available to the divers/workers involved in harvesting Makhana seeds as and when required. Table 5 shows the costs involved in the improved system (T2). It can be observed that the break-even point is about 369 h and payback period is 4.8 years with benefit–cost ratio of 2.48.

In conclusion, an improved system has been developed for Makhana collection. With this improved system, the workers can remain under water for 1 h with ease and perform their work efficiently without any drudgery and occupational health problems. The results of 1 h harvesting trial conducted at four ponds with 10 subject show positive sign of using the improved system. The data indicate that the mean output is 11.3 kg/h with the T2 system and it was only 3.8 kg/h with the T1 system. The ODR on 0–10 scale is 4.2 in case of T2 system and it is 8.3 in case of T1 system. Also, the BPDS is lower with T2 (48.2) system compared to T1 (78.8) system. The techno-economics of the improved system was calculated; the break-even point comes out to be 369 h and payback period is 4.8 years with benefit–cost ratio of about 2.48.

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Salbardi–Belkher inland basin: a new site of Lameta sedimentation at the border of districts Amravati, Maharashtra and Betul, Madhya Pradesh, Central India

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The Late Cretaceous infratrappean Lameta sediments in central and western India are known from five inland basins, viz. (i) Nand–Dongargaon, (ii) Jabalpur, (iii) Balasinor–Jhabua, (iv) Ambikapur–Amarkantak and (v) Sagar. Among these, the successions in the first three basins is well studied. The dinosaurian remains from the formations of these inland basins serve as a significant tool for regional reconstructions of palaeogeographic and palaeoenvironmental conditions during Lameta sedimentation. Here, a new inland basin with good outcrops of Lameta sediments having dinosaurian skeletal remains egg nests and eggs is documented. Considering the lithofacies and dinosaurian remains from this new inland basin, it is evident that Lameta sedimentation during the Late Cretaceous was not restricted to only five inland basins documented earlier, but was taking place contemporaneously in an additional inland basin in between Balasinor–Jhabua in the west and Nand–Dongargaon basin in the east. We propose the name of this new site as Salbardi–Belkher inland basin. This newly identified basin lying at the border of Maharashtra and Madhya Pradesh also redefines the existing palaeogeographic limits of Lameta sedimentation, including dinosaur inhabitation.

Keywords: Dinosaurian remains, fluvio-lacustrine succession, infratrappean sediments, inland basins.

THE infratrappean Lameta Formation, disconformably overlying the Gondwana or Precambrian rocks, is mainly exposed in Jablapur district, Madhya Pradesh (MP); Nagpur and Chandrapur districts, Maharashtra and Anjar and Kheda districts, Gujarat, besides the scattered occurrences in Saugor (Sagar) and Amarkantak districts, MP (Figure 1). The Lameta beds are mostly considered to be fluvial-lacustrine in nature. However, there is a debate about the type area succession at Jabalpur regarding its marine[1–4] versus non-marine[5–8] nature. In general, major lithologies of the Formation are represented by variously coloured argillaceous sedimentary rocks, medium to fine-grained sandstones and silicified, brecciated and nodular limestones, which may show variations in stratigraphic position, in the lithocolumns exposed at various localities depending upon the nature of depositional environment than on time of deposition[3,9]. Despite remarkable similarity in lithological sequence, the successions in various areas also show a common characteristic in having dinosaurian remains in the form of bones, egg nests and eggs. Based on lithological succession and fossil remains, Mohabey[9] identified five inland sub-basins in which Lameta sedimentation took place, viz. (i) Nand–Dongargaon, (ii) Jabalpur, (iii) Balasinor–Jhabua, (iv) Ambikapur–Amarkantak and (v) Sagar (Figure 1).

The present study documents a new region in which Lameta sediments are exposed. Good sections exposing Lameta beds in this region occur near Bairam (lat. 21°22′25″N; long. 77°37′23″E), Belkher (lat. 21°21′48″N; long. 77°31′23″E), Pandhri (lat. 21°22′02″N; long. 77°32′54″E) and Salbardi (lat. 21°25′15″N; long. 78°00′00″E), besides 3–4 small, isolated exposures in nearby locations. These exposures are spread over an aerial distance of 10–40 km. In two localities, dinosaur bones and eggs are preserved. Comparing the depositional environment set-up and dinosaurian remains of the studied areas with those of the other five inland basins[9], we propose a new inland basin for Lameta sedimentation called Salbardi–Belkher inland basin, exposed in an area which is presently covered partially by districts Amravati in Maharashtra and Betul in MP.

Small sedimentary inliers consisting of Lameta Formation, overlying the Upper Gondwana sedimentary rocks are exposed in the Deccan Trap region (inset Figure 1). Quartzo-feldspathic gneiss of Archaean age forms the basement for Gondwana sedimentary rocks, which rest on it nonconformably. Broadly, the Lameta Formation is represented by sandstone, clay-marl and limestone litho-units,

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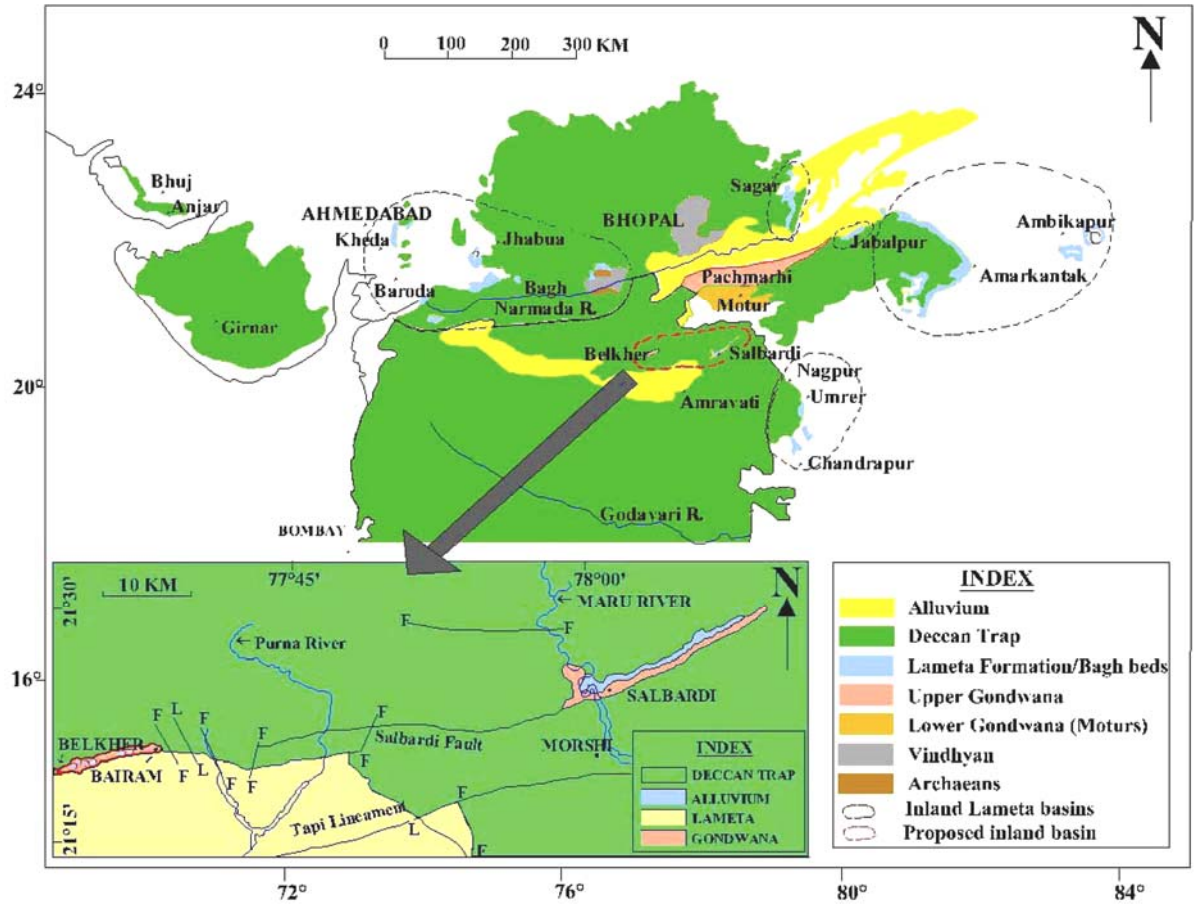

Figure 1. Map showing the proposed inland basin of Lameta sedimentation in central and western India as proposed by Mohabey[18] along with location and extent of newly proposed Salbardi–Belkher inland basin. (Inset) Geological map of the study area having locations and extents of Lameta sediments exposed at Bairam, Belkher and Salbardi areas.

which disconformably overlie arenaceous–argillaceous sediments of the Upper Gondwana Group, with good preservation of current generated structures. Upper Jurassic to Early Cretaceous age of the Upper Gondwana successions exposed at Bairam and Belkher areas has been suggested on the basis of gymnosperm and pteridophyte remains[10]. The lithological similarity between the Gondwana sediments exposed in Salbardi area with the previous two localities, suggests the same age and hence is considered to be a co-eval lithounit[11]. Greyish-black, hard, massive and vesicular to amygdaloidal traps overlie the Lameta Formation.

The succession is studied in three sections in the study area, namely Bairam, Belkher and Salbardi. The successions exposed at these sections are approximately 39, 47 and 35 m thick respectively, and show almost similar lithological set-up. The lithocolumn at Bairam area is represented by clay-marl, arenaceous and calcareous sediments which can be broadly divided into lower, middle and upper litho-units (Figure 2). The lower unit is 12 m thick clay-marl of brownish–yellowish–greenish colour. Occasionally, faint laminations and discontinuous thin beds of siliceous limestone are also observed in them. Overlying 13 m, areno-argillaceous column constitutes the middle unit, of which the lower 2 m is greyish-brown, medium to coarse-grained sandstone. It is succeeded by 9 m thick, light grey clay, having abundant concretions and 5–20 cm thick, discontinuous beds of light-green siliceous limestone. The top 2 m is dark brown, medium to fine-grained sandstone having *Thalassinoides* burrows. The upper unit is 14 m thick calcareous sediment consisting of nodular limestone and chertified limestone. The former is bluish grey, hard and compact rock having nodular tendency and also contains irregular clasts of chert and jasper. The chertified limestone is light grey, hard and compact, having poor tendency of flat bedding. Chert is mostly in the form of horizontal discontinuous beds.

The litho-section at Belkher area is comparatively well exposed attaining a thickness of about 47 m. Broadly, it exhibits sandstone, clay-marl and limestone units (Figure 2). The lower 21 m column is areno-argillaceous, consisting of medium to fine-grained, hard and compact, yellowish-orange and greyish-brown sandstone bed, and dark

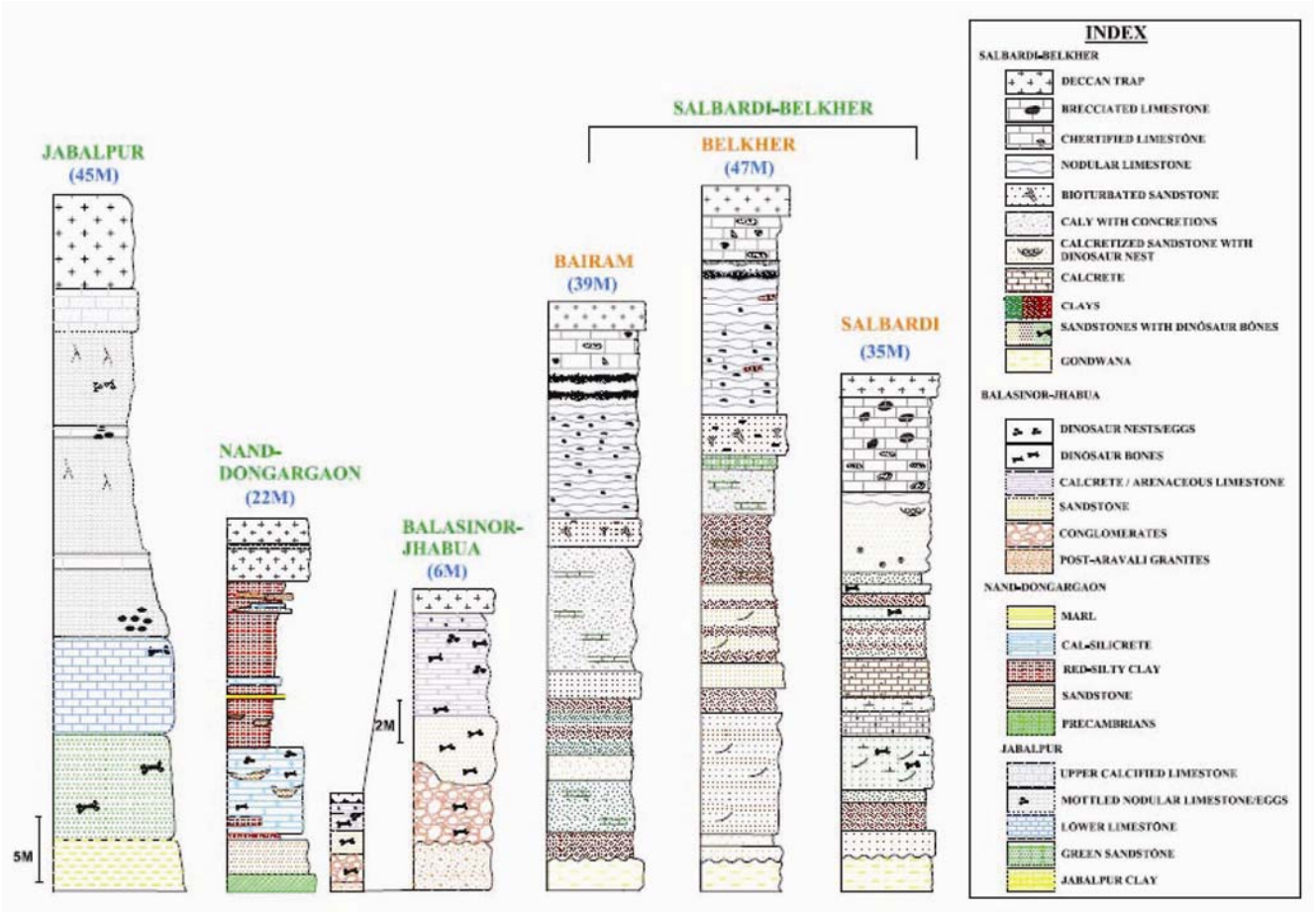

Figure 2. Comparative details of lithological architecture of Lameta successions exposed in various inland basins.

yellowish-orange to dark reddish-brown, massive to faintly laminated clayey beds. The middle 10 m column consists of dark brown to light grey-coloured clay with thin patches of siliceous limestone. Discontinuous beds of greyish-black to medium green-coloured, subangular to subrounded concretions of 3–15 cm diameter are also present. The litho-unit is distinctly overlain by 2 m thick, dark brown, hard and compact sandstone bed showing vertical to inclined burrows of *Thalassinoides*. The upper 14 m thick column consists of nodular and chertified limestone similar to the one observed in Bairam.

The litho-section at Salbardi is about 35 m thick consisting of areno-arglillaceous and calcareous sediments (Figure 2). This locality is significant because of good preservation of dinosaurian fossils. The lowermost 9 m column is areno-argillaceous and consists of dark reddish-brown to medium green, medium to coarse-grained, hard and compact, parallel-bedded to cross-bedded sandstone ranging in thickness from 2 to 4 m, occasionally interbedded with same coloured clay horizons. Calcrete, clay and calcretized sandstone constitutes the middle 18 m thick column. Calcrete is of two types, i.e. light grey rhizomorphic and light brown nodular, constituting 2 and 4 m columns respectively. Calcrete is overlain by 7 m thick column showing alternations of greenish-grey to dark brown clay in the lower part, whereas the remaining 5 m upper column is green-coloured, medium-grained, hard and compact sandstone having skeletal fragments of dinosaurs. It is succeeded by 5 m thick, greyish-yellow, hard and compact calcretized sandstone showing well-preserved dinosaur egg nest. The uppermost 8 m succession is nodular chertified limestone similar to the ones described earlier from the adjacent localities, except that the topmost 2 m thick intraformational brecciated limestone here is reddish-brown, hard and compact with subangular to subrounded clasts of green sandstone, nodular limestone and certified limestone.

The succession at Salbardi is found to be productive for dinosaurian remains. It contains fragmentary bones preserved in light green-coloured, medium-grained sandstone. Srivastava and Mankar[12] have reported fragmentary remains of right ulna of *Titanosaurus colberti*. Egg nest and eggs belonging to *Megaloolithus* oogenus of Megaloolithidae oofamily have also been reported from the same locality. These are preserved in calcretized sandstone beds overlying green-coloured sandstone having skeletal fragments[13]. Fragmentary bones and

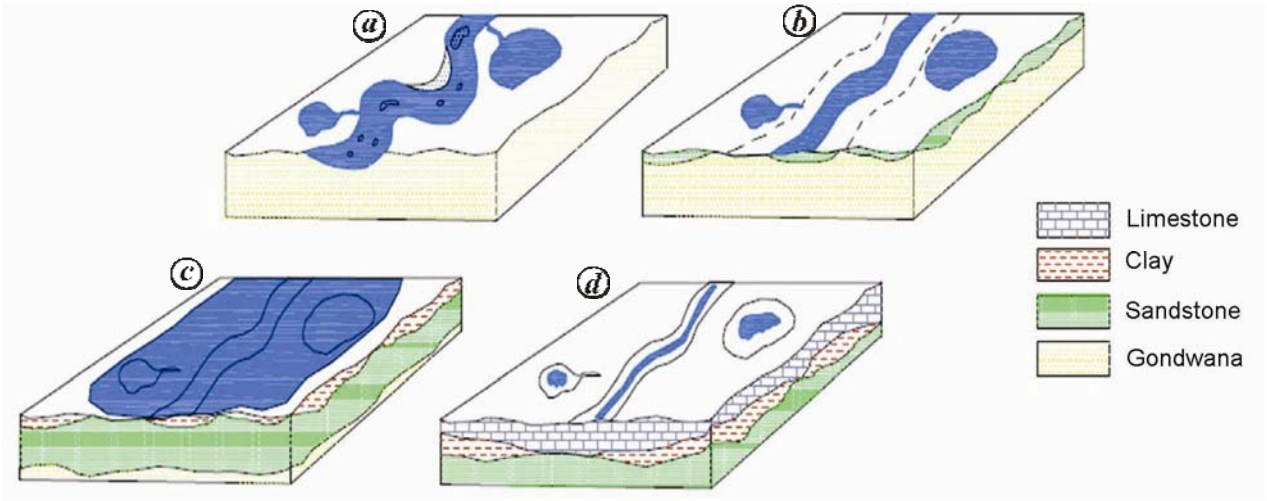

Figure 3. Schematic model of the depositional environment for Lameta sediments. ***a***, Closures of the Gondwana deposition followed by the initiation of Lameta sedimentation as channel floor, point-bar deposits. ***b***, Development of lakes allowing deposition of fine-grained argillaceous sediments and change of river course adding new depositional sites. ***c***, A high stand of water level flooding the entire area, including lakes during which large sheet-like deposits have taken place. ***d***, Low stand of water due to hot and dry climatic conditions causing reduction of sites for fluvial–lacustrine deposition that ultimately terminated due to volcanic activity. Pedogenic activities might have taken place at any stage, but were more pronounced during B stage (Srivastava and Mankar[16]).

occasional coprolite occurrences have also been recorded from the succession of Pandhri area.

Reconstruction of depositional environments in detail for the successions exposed at Bairam, Belkher and Salbardi areas and a model showing the same on the basis of rock types and lithofacies analysis have already been reported[14–16]. Three major lithofacies associations, viz. arenaceous, argillaceous and calcareous have been identified. The arenaceous lithofacies association includes (i) massive sandstone, (ii) green sandstone, (iii) thinly bedded, yellowish-orange and greyish-brown sandstone, (iv) coarse-grained sandstone, and (v) dark brown bioturbated sandstone. The argillaceous association consists of (i) yellowish–brownish–greenish clay–siltstone and (ii) light grey silty clay with concretions. The calcareous association is represented by (i) calcrete, (ii) nodular limestone, (iii) chertified limestone, and (iv) intraformational brecciated limestone[16].

The lithofacies assemblages indicate a fluvial–lacustrine environment of deposition, in which temporal variations in depositional setting and climatic condition have been observed (Figure 3). The lower arenaceous part of the succession mostly represented by thinly bedded, parallel laminated units shows low to moderate energy conditions. Deposition of argillaceous sediments interbedded with cross- and parallel-bedded sandstones shows fluctuation in the energy condition of the depositing medium. Calcrete formation point to seasonal variability of energy condition of the river system during arid climate, and also formation of detached lakes and water bodies during low water condition. The bioturbated sandstone indicates short-span high growth of benthic fauna and complete churning of the sediments by benthonic community during clam and quiet water conditions.

The predominance of carbonate sedimentation in the upper part of the succession shows a major change in the chemical set-up and prevalence of alkaline environment of deposition. Sheet flood environments of deposition, similar to that reported from Jabalpur area[6], are evident by horizontally bedded units of nodular and cherty limestone.

The Lameta Formation, in the form of discontinuous patches is exposed at several places in central and western India. These deposits are considered to have been formed in five inland basins as determined on the basis of their lithological correlatability and dinosaurian remains[9]. A brief account of each is given below.

Jabalpur: The scattered patches of Lameta sediments exposed in and around Jabalpur area are considered to be deposited in a smaller basin compared to the others. However, Jabalpur Lameta beds are considered to be more significant due to rich preservation of dinosaurian remains and also due to controversy about their environment of deposition as to whether they are marine or non-marine deposits. Dinosaur remains consist of rich assemblage belonging to caudal vertebrae, tooth, coprolites and skull of Sauropoda, Theropoda and Ornithopoda, i.e. *Titanosaurus indicus* and *Antarctosaurus septentrionalis* (sauropods); *Indosuchus raptorius*, *I. matleyi*, *Lametasaurus indicus*, *Composuchus solus*, *Laevisuchus indicus*, *Jubbalppuria tenuis*, *Dryptosauroides (?)grandis*, *Ornithomimoides mobilis*, *O. barasimlensis* (Theropoda) and *Brachypodasaurus gravis* (Ornithopoda)[17–24]. Egg nests and eggs are represented by *Megaloolithus dhoridungriensis* and *M. phensaniensis* of Megaloolithidae oofamily and *Elipsoolithus khedaensis* of Elipsoolithidae oofamily[9,25–27]. Various successions exposed at different places in this inland basin are extensively studied for environment of deposition, trace fossils, palaeo-floral and faunal

Table 1. A generalized idea about the lithological set-up and depositional environments of Lameta sediments in various inland basins

		Jabalpur inland basin		Nand-Dongargaon inland basin	Balasinor-Jhabua inland basin	Salbardi-Belkher inland basin
Major lithounits		(1) Upper sandstone (2) Upper limestone (3) Mottled nodular marl (4) Lower limestone (5) Green sandstone		(1) Red green silly clays associated with sandstone (2) Channel related sandstone with calcretized in upper part (3) Yellow laminated clays and interbedded with limestone, marlite and septarian concretion bands (4) Calcrete (5) Gray nodular marls	(1) Arenaceous limestone (2) Calcareous sandstone (3) Grits (4) Conglomerate	(1) Intraformational brecciated limestone (2) Chertified limestone (3) Nodular limestone (4) Clay-marl with concretions (5) Yellowish-greenish-reddish clay-marl (6) Bioturbated sandstone (7) Greyish-yellowish-brownish sandstone (8) Green sandstone
	Marine	Singh[28]	Saha *et al*.[4]			
Depositional environment		(1) Intertidal (2) Intertidal (3) Tidal flat (4) Tidal flat (5) Estuarine	(1) Intertidal-supratidal (2) Intertidal channels on the marshes (3) Marsh (4) Lagoon (5) Estuary	–	–	–
	Non-marine	Tandon *et al*.,[6]		Mohabey[9]	Mohabey[27]	Srivastava and Mankar[16]
		(1) Sheet flood (2) Sheet wash-pedogenically modified (3) Palustrine flat (4) Braided stream	Fluvial under semi-arid, pedogenically modified and flat-palustrine system	(l) Overbank (2) Channel (3) Overbank (4) Lacustrine (5) Flood plain (6) Back-swamp	(1) Palustrine environment (2) Fluvial or mostly lacustrine environment	(1) Gravity flow (2) Flood plain (3) Flood plain (4) Pedogenic (5) Lacustrine (6) Fluvial (7) Point bar (8) Channel floor
				Alluvial-limnic under semi-arid condition		Fluvial-lacustrine under semi arid condition

assemblage, including dinosaur remains. The overall succession is represented by green sandstone, lower limestone, mottled nodular marl, upper limestone and upper sandstone (Figure 2 and Table 1). The 18m thick succession at type locality consists of Lower limestone, Mottled nodular marl and Upper limestone[28], which was further redefined by Saha *et al.*[4], with an increase in total thickness up to 21 m. The succession at Chui Hill ranges in thickness from 39–45 m, however, the Upper sandstone is not exposed[4,28]. The succession at Bara Shimla Hill exhibits all the five major lithounits constituting a column of about 34 m thickness[28]. Whether Jabalpur basin was marine or nonmarine, has been a matter of debate. Marine environment has been suggested on the basis of green sandstone and trace fossils[28], petrology, algal structures, glauconitic beds, extensive crab burrows, including *Thalassinoides* and lithological architecture[28–30]. Recently, Shukla and Srivastava[3] and Saha *et al.*[4], suggested marine environment of deposition, including intertidal to supratidal channels, marsh, estuary and lagoon sub-environments based on ichnofossils, lithofacies architecture and nesting habit of lizards.

The non-marine fluvial–lacustrine environment is proposed by many workers on the basis of fossil biota, lithological characteristics, occurrence of various types of calcretes and dinosaurian remains[5–7,9].

Nand–Dongargaon: This basin is well studied for its lithological set-up and dinosaurian remains[9,31,32]. The basin occupies an area of about 700 sq. km and shows good exposures at Pisdura, Dongargaon, Nand and Kotabala areas of Chandrapur district, and Rajulwari, Pahami and Shivapur areas of Nagpur district. The basement is mostly of Precambrian rocks; however, in the northern part of Nand area, the Gondwana sedimentary rocks underlie the Lameta beds. In general, the succession is a 20 m thick, calc-argillaceous litho-unit, deposited in alluvial–limnic environment under semi-arid condition[31] (Figure 2 and Table 1). Mohabey[9] broadly classified four different major lithounits, viz. (i) red and green silty clay associated with sandstone, (ii) channel-related sandstone (trough and cross-bedded), which is calcretized in the upper part, (iii) yellow laminated clay and shale interbedded with limestone, marlite and septarian concretion bands, and (iv) grey nodular marls. On the basis of lithology, sedimentary texture and structure and faunal content, four subenvironments of deposition have been proposed, viz. (i) channel, (ii) overbank, (iii) paludal and (iv) limnic[9,31–33]. Dinosaur remains consists of *T. indicus*, *T. blandfordi*, *T. colberti* and *Laplatasurus madagascariensis*[20,34–36]. Egg nests and eggs include *Megaloolithus matleyi* and *M. megadermus*[37,38]. Recently, the southern sector of Nand–Dongargaon basin has been studied in detail by establishing detailed lithofacies architecture in the succession[32]. Six lithofacies were identified, viz. (i) clay–siltstone lithifacies, (ii) limestone–carbonate mud lithofacies, (iii) septerian concretionary lithofacies, (iv) varved clays lithofacies, (v) fibrous radaxial calcite cryptalgal lithofacies and (vi) sandy-gravel lithofacies showing lacustrine environment of deposition.

Balasinor–Jhabua: This inland basin is significant because of rich preservation of skeletal remains, egg nests and eggs of dinosaurs at Balasinor and Rahioli localities of Kheda and Panchmahal districts in Gujarat. The Lameta sediments in these two adjoining districts lie over a strike length of ca. 40 km over a width of 15 km (ref. 39). At Balasinor, 4–12 m thick succession rests unconformably over the Godhra granites/phyllites of the Aravalli Supergroup (Figure 2 and Table 1). The lower 2–6 m litho-column is an indurated, pebbly-conglomeratic unit showing channel and scour-fill structures with pebbles of quartz and cherty quartzite cemented together by calcareous and siliceous material. Bedding is not distinct; however, rare instances of size gradation of the clasts can be noticed. This litho-unit shows rich preservation of reddishbrown dinosaurian skeletal fragments. It grades upwards into 2 m thick, pebbly, poorly sorted sandstone, which also shows rare occurrences of skeletal remains. Bedding is indistinct; upward fining of grain size is prominent. It grades to grey and brown-coloured siliceous limestone with calcareous claystone and brown marl. The limestone is highly variable and may be mottled, nodular and brecciated with ample amounts of lithoclasts, peloids, and subangular to subrounded detrital quartzs[39]. This litho-unit is considered to be a deposit of palustrine environment due to its fine-grained, massive and micritic nature as well the presence of discontinuous cracks and bioturbation[40]. The other significant exposure lying at Rahioli is sandy calcrete preserving complete eggs and nests of dinosaurs. It is interpreted to be the deposit of sheet wash into a palustrine environment[25,27]. Dinosaur remains consist of *Antarctosaurs septentrionalis* (sauropods) and *Rajasaurus narmadensis* (thereopods)[19,21,41,42]. Nests and eggs includes *Megaolithus rahioliensis*, *M. phensaniesis*, *M. khempuensis*, *M. megadermus* and *M. balasinorensis*[19,25,40,43]. Singh and Tondon[44] using oxygen and carbon isotope analysis interpreted fluvial or mostly lacustrine environment of deposition, particularly for the egg-bearing horizons.

Saugor (Sagar) and Amarkantak–Ambikapur: Both these inland basins lack adequate published data, despite occurrence of good exposures of Lameta sediments with dinosaurian remains. As such, these inland basins are less discussed in the original paper by Mohabey[9]. The Sagar basin with small geographical extent, lying northwest of Jabalpur area, consists of small lithocolumns of gritty sandstone, calcareous clay, chert and mottled limestone having pebbles of jasper and chert. At places, it is calcareous having pockets of chert and jasper[45]. The Amarkantak–Ambikapur basin with a large geographical area is still not well studied[9].

As mentioned earlier, fluvio-lacustarine Lameta sediments of central and western India are considered to be

deposited in five inland basins[9]. The successions exposed in these basins are mainly represented by calc–marl–argillaceous and arenaceous lithofacies. These lithofacies are represented in almost all the successions; however, they vary in thickness and abundance of rock formation in stratigraphic column (Figure 2 and Table 1). Apart from lithological similarities, these sediments also have a common character of preservation of skeletal fragments, eggs and nests of dinosaurs.

The present report of Lameta rocks from a region having a few isolated patches of comparatively larger dimensions at Bairam, Belkher–Pandhri and Salbardi areas, in addition to 3–4 minor exposures nearby has added a new dimension to the geographical extent of Lameta sedimentation. The successions at these places are fluvio-lacustrine and show dinosaur inhabitation represented by preservation of skeletal remains, egg nests and eggs. We are of the opinion that this entire region was a separate inland basin of Lameta sedimentation, in addition to the earlier five proposed by Mohabey[9]. This new region is being proposed as Salbardi–Belkher inland basin (Figure 1).

The Lameta sedimentation in Salbardi–Belkher inland basin occurred after Gondwana sedimentation ceased. The inland basin allowing Lameta sedimentation shows shallowing and deepening of the depositional site because of periodical recharge by river water. In general, the lower part of the Lameta succession is dominantly represented by arenaceous and argillaceous sediments that show a change in the energy condition in the depositing medium. During the deposition of the arenaceous sediments, the energy condition was moderate in which sand-sized particles could be transported and structures like cross-bedding and parallel-bedding were formed. The argillaceous sediments show low energy condition in which the deposition took place mainly from suspension load. Succession in the upper part is mostly calcareous in nature, showing shallowing of the basin as well as alkaline nature of the depositing medium. It is interpreted that the basin in the initial phase was shallow in the east compared to west. In the east, near Salbardi, lower part of the succession is arenaceous and contains calcrete horizons revealing shallow nature, whereas dominance of argillaceous sediments at Belkher and further at Pandhri in the extreme west indicates low energy condition of deposition in deeper parts of the basin.

Comparison of the new inland basin with other Lameta basins shows that the proposed basin was nearly similar in size as the Jabalpur basin. Similar to Rahioli, Jabalpur and Nand–Dongargaon basins, the dinosaurian remains, including bones and eggs are indicative of channel to point bar deposition under sub-arid condition. With the addition of this new basin, the earlier proposed palaeogeography of Lameta sedimentation gets modified with larger geographical extent.

In conclusion, a new inland basin has been identified for Lameta sedimentation, i.e. the Salbardi–Belkher inland basin. The fluvio-lacustrine successions of this basin are similar to other Lameta successions exposed at various places in central and western India. The palaeoenvironmental set-up and palaeoclimate of all these basins were found to be similar.

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