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Effective Role of *Terminalia arjuna* Reduced Gold Nanoparticles on Reproductive Dysfunction Induced by Acetaminophen in Male Wistar Rat

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Abstract
Acetaminophen is a commonly used analgesic and antipyretic agent, which causes liver and kidney damage when it is taken in high doses. It also has an adverse effect on reproductive system. *Terminalia arjuna* has a beneficial effect on hepato-protective properties. In this study, effect of green synthesized gold nanoparticles using *Terminalia arjuna* (AuNPs) has been evaluated for prevention of reproductive disorder in male Wistar rats. Then, experiment was conducted on 24 healthy male Wistar rats. Biochemical test such as sperm count, sperm viability, seminal fructose level, testicular cholesterol, superoxide dismutase (SOD), catalase (CAT), and histopathological analysis revealed that co-administration of green synthesized AuNPs along with acetaminophen showed effective significant recovery in the reproductive disorder caused due to acetaminophen toxicity. Overall, the results emphasized the promising effect of green synthesized AuNPs against acetaminophen-induced toxicity in male Wistar rats.

Keywords Acetaminophen · AuNPs · *Terminalia arjuna* · Sperm count · Sperm viability

1 Introduction

Nanomedicine is a branch of science that uses nanotechnology to monitor, maintain, treat, prevent diseases, and control the biological systems at the nanometer size range, using engineered nanostructures and nano devices [1]. Nanomedicines have huge capacity to bring benefits to wide areas of research and application.

Among the metallic nanoparticles, especially, the gold nanoparticles act as a novel drug carrier in the wide areas of particle-based drug delivery system. Gold nanoparticles have huge applications in biomedicine and molecular biology, which involves biomedical [2] and cell imaging [3], peptide delivery [4], biosensing [5], and drug delivery [6]. It is preferable to use nontoxic reagents to improve the biocompatibility of gold nanoparticles. A protecting agent is also required for gold nanoparticle synthesis, whose role is to adsorb onto the surface of the newly formed nanoparticles to prevent particle agglomeration and further growth. Several publications suggest new synthesis approach of gold nanoparticles in which green reduction and protection agents were used [7]. Most of these reducing and stabilizing agents were obtained from plants [8], bacteria, algae, and fungi [9].

Terminalia arjuna, a member of Combretaceae family, is grabbing the attention of researchers by its multibeneficial properties of its plant parts like leaf and barks. The bark of *Terminalia arjuna* was used in ayurveda for the purpose of healing cardiovascular health and oxidative stress–mediated disorders [10].

Acetaminophen or paracetamol is chemically named N-acetyl-p-aminophenol. Paracetamol is a commonly used medicine or drug in mild fever and pain. Paracetamol does not act as non-steroidal anti-inflammatory drugs (NSAIDs). Paracetamol mainly aims on inhibition of cyclooxygenase (COX)-mediated production of prostaglandins, which are involved in inflammation [11]. Acetaminophen is well tolerated and safe when taken in usual therapeutic dose. Although it is used as analgesic (pain reliever) and antipyretic (fever reducer), it has a number of side effects like nausea, stomach pain, loss of appetite, itching, clay-colored stools, or even jaundice. Most instances of paracetamol toxicity resulted from large,

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single overdose [12]. Paracetamol is known to have adverse effects on liver, kidney [13], and also on the reproductive system [14] affecting human health.

Therefore, the present study has been focused on the preventive effect of green synthesized gold nanoparticles using aqueous bark extract of *Terminalia arjuna* (AuNPs) on reproductive dysfunction of male Wistar rats. Ultimately, this study is helpful to find out an effective drug delivery of herbal gold nanoparticles towards the prevention of the male reproductive organs from toxicity induced by acetaminophen. This approach would be an alternative harmless therapy against antioxidant markers and to maintain proper reproductive functions.

2 Results

This study was conducted to investigate the preventive effect of green synthesized gold nanoparticles using *Terminalia arjuna* (AuNPs) on the reproductive functions of male Wistar rats. Acetaminophen (500 mg/kg/day) for 14 days was administered to induce toxicity effect on the male reproductive system. The preventive activity of the AuNPs was observed when administered once daily at a dose of 175 μg/kg/day. Different reproductive markers such as epididymal sperm count, sperm viability, estimation of seminal fructose, estimation of testicular cholesterol, estimation of antioxidant enzyme like superoxide dismutase (SOD) and catalase (CAT), estimation of toxicity markers GOT (glutamate oxaloacetate transaminase), and GPT (glutamate pyruvate transaminase) were studied and histopathological analysis was also done.

2.1 Epididymal Sperm Count

Sperm count significantly decreased in group 2 (acetaminophen-treated group) in respect to group 1 (control group). In group 3 (co-administration with *Terminalia arjuna* bark extract and acetaminophen), sperm count was significantly decreased when compared with group 1 but significantly increased when compared with group 2. Sperm count was significantly increased in group 4 (co-administration with AuNPs and acetaminophen) in comparison to group 2 and group 3 (Fig. 1a).

2.2 Sperm Viability

The sperm viability in all the groups was significantly different form each other. Sperm viability significantly decreased in acetaminophen-treated group compared with that of control group but there was significantly increase in sperm viability in case of group 4 treated rats (AuNPs co-administered with acetaminophen) in respect to group 2 and group 3 (Fig. 1b).

2.3 Seminal Vesicular Fructose Level

In Fig. 1c, it was observed that fructose level was significantly decreased in acetaminophen-induced group compared with the control group. In case of group 3 (co-administration of *Terminalia arjuna* bark extract and acetaminophen), fructose level was significantly decrease compared with group 1 but significantly increased when compared with group 2 but fructose level was significantly increased in group 4 (AuNPs co-administered with acetaminophen) in respect to group 2 and group 3.

2.4 Testicular Cholesterol

Testicular cholesterol significantly decreased in acetaminophen-treated group in respect to control group. In group 3 (co-administration with bark extract of *Terminalia arjuna* and acetaminophen), testicular cholesterol was significantly decreased when compared with group 1 but significantly increased when compared with group 2. Testicular cholesterol showed significant increase in group 4 rats treated with AuNPs co-administered with acetaminophen in respect to acetaminophen and *Terminalia arjuna*–treated groups (Fig. 1d).

2.5 Antioxidant Enzyme Level

Antioxidant enzymes such as SOD and CAT significantly decreased in acetaminophen-treated groups compared with the control group. In group 4 rats treated with AuNPs co-administered with acetaminophen, the SOD and CAT level was significantly increased in respect to acetaminophen and *Terminalia arjuna*–treated groups (Fig. 1e, f).

2.6 Estimation of GOT and GPT

In tissue homogenate of testis, the GOT and GPT level significantly increased in acetaminophen-treated rats compared with control group. In group 4 rats treated with AuNPs co-administered with acetaminophen, the GOT and GPT level was significantly decreased in respect to that of acetaminophen and *Terminalia arjuna*–treated groups (Fig. 1 g, h).

2.7 Histopathological Analysis

Normally, in a transverse section of testis tissue, the seminiferous tubules were observed. In the tubules spermatogonia, interstitial cells of Leydig and Sertoli cells were observed. In group 1 (Fig. 2a), it was observed that the spermatogonia were very much densely arranged, the spermatids were embedded closer to the lumen, and supportive Sertoli cells were condensed. In group 2 (Fig. 2b), it was observed that the dense organization of the spermatogonia has been hampered. The

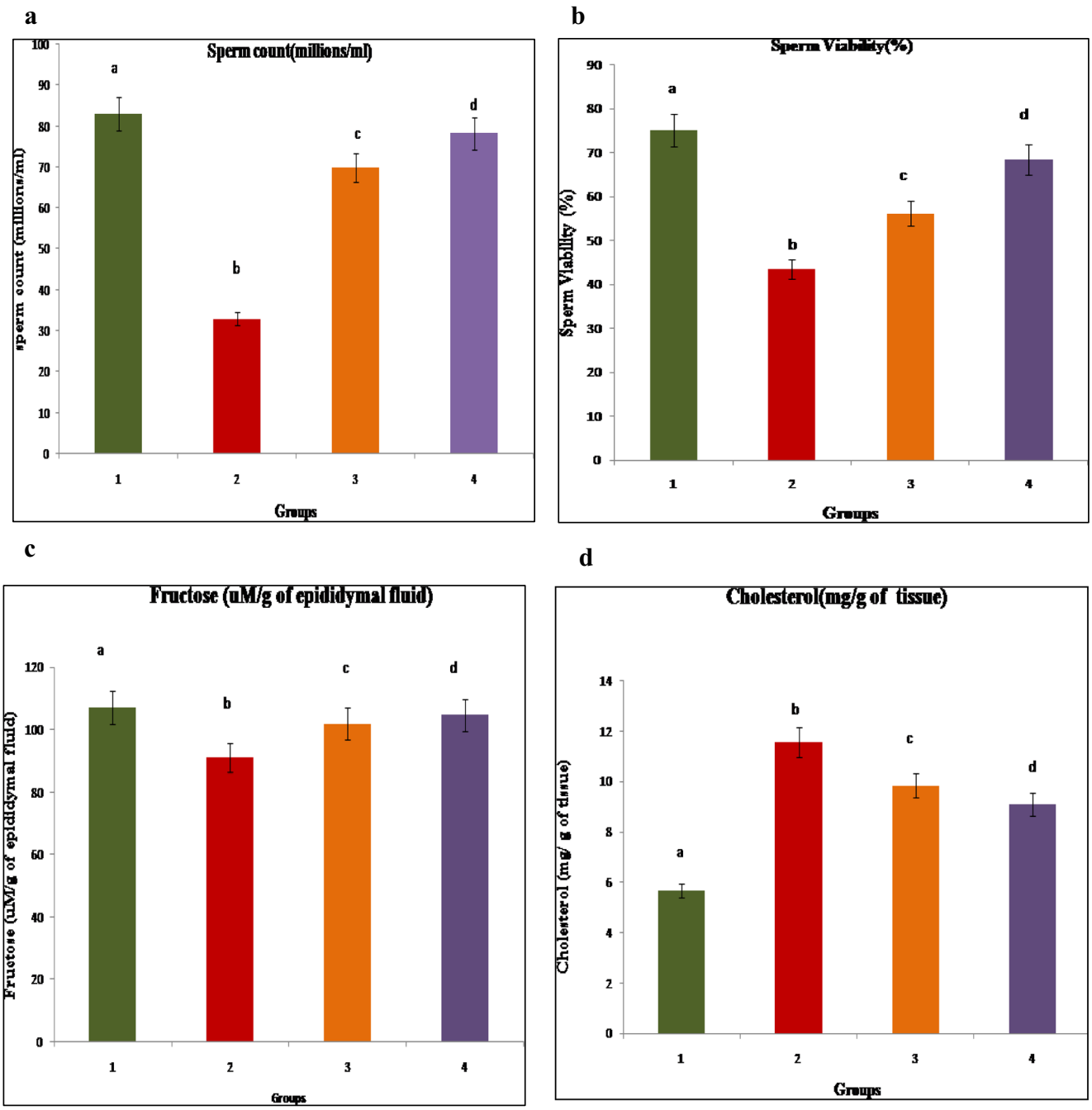

Fig. 1 Graphical representation of (a) sperm count, (b) sperm viability, (c) seminal fructose level, (d) testicular cholesterol, (e) SOD, (f) catalase, (g) GOT, (h) GPT level of experimental groups. Data are expressed as mean ± SE ($n = 6$). ANOVA followed by multiple two-tail t test and data with different superscripts (a, b, c, d) in a specific vertical column differ from each other significantly ($p < 0.05$). Groups, 1: control, 2: acetaminophen, 3: acetaminophen + aqueous extract of *Terminalia arjuna*, 4: acetaminophen + green synthesized gold nanoparticles using *Terminalia arjuna* (AuNPs)

spermatids are slightly lower in number which makes the lumen look larger. The Sertoli cells layer is not so dense compared with the control group. In Fig. 2c (group 3), it was observed that the arrangement of spermatogonia were condensed compared with that of the acetaminophen-treated group (group 2). The observed size of lumen looks smaller due to the increased spermatid tails. The density of Sertoli cells has been increased compared with the acetaminophen group. In group 4 (Fig. 2d), it was observed that the density of the Sertoli cell has been increased which is very much similar to the control group. The density of spermatogonia is also increased around the base of the seminiferous tubule. Due to the increased growth of the spermatids, the lumen looks smaller quite similar to the control group.

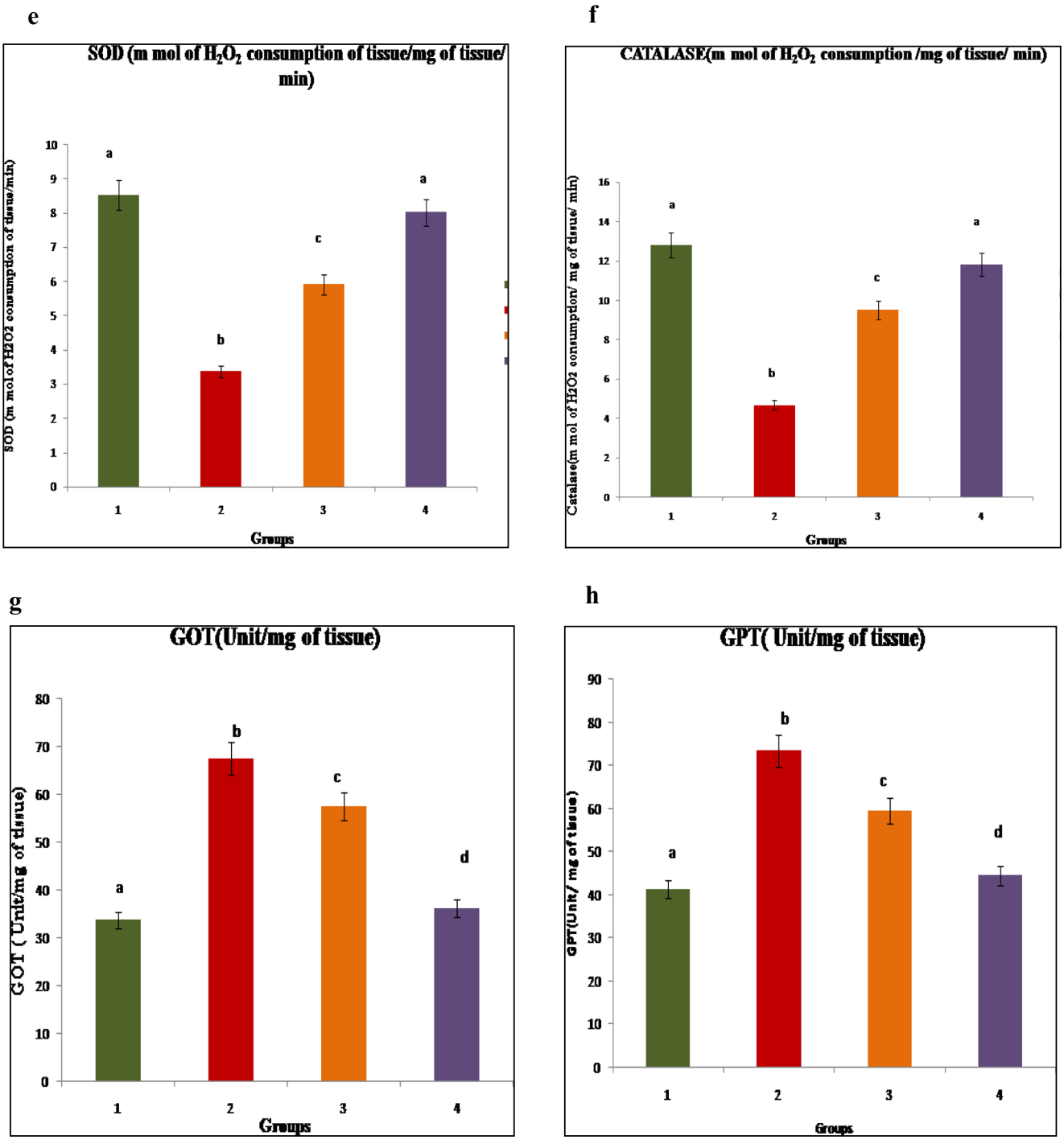

Fig. 1 continued.

3 Discussion

It has been investigated that administration of paracetamol in male Wistar rats significantly decreases the testicular function and it also disrupts the production of testosterone produced by the Leydig cells [14]. Moreover, acetaminophen reduces the semen quality and sperm quantity by triggering apoptosis of the germ cells. In this study, the male albino rats were treated with acetaminophen at 500 mg/kg for 14 days to induce reproductive disorder. To study the protective effect of green synthesized gold nanoparticles using bark extract of *Terminalia arjuna* (AuNPs) on reproductive function, rats were co-administered with AuNPs (175 μg/kg for 14 days) and acetaminophen. In this study, it was noted that after the administration of acetaminophen, the sperm count in group 2 rats significantly reduced. This inhibition in sperm count was

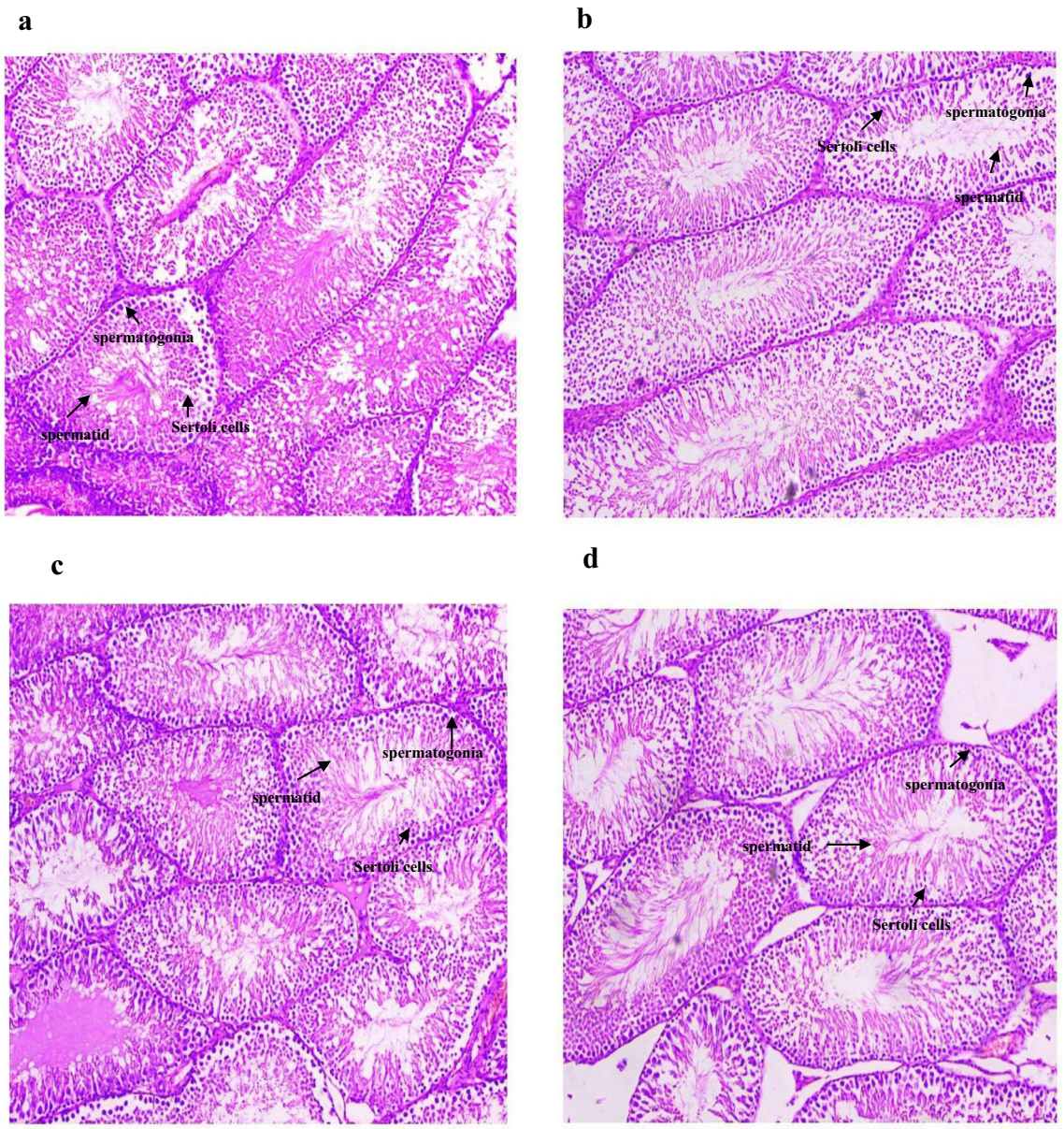

Fig. 2 Histological structure of testis tissue. Section a group 1: control group, arrows indicating that spermatogonia were densely arranged, the spermatids were embedded closer to the lumen, and supportive Sertoli cells were condensed. Section b group 2: acetaminophen-treated group, arrows indicating that dense organization of spermatogonia is disorganized, Sertoli cells and spermatids are slightly lower in number which makes the lumen look larger. Section c group 3: acetaminophen + aqueous bark extract of *Terminalia arjuna*–treated group, arrows indicating that spermatogonia were slightly condensed, Sertoli cells and spermatids are slightly greater. Section d group 4: acetaminophen + green synthesized gold nanoparticles using *Terminalia arjuna* (AuNPs)–treated group, arrows indicating that spermatogonia were densely arranged, density of the Sertoli cells and spermatids has been increased so the lumen looks smaller quite similar to the control group

may be due to low testicular androgenesis as testosterone is one of the most important regulators for the sperm production. In case of group 4, AuNPs-treated rats, the sperm count has been significantly elevated from that of the acetaminophen and *Terminalia arjuna*–treated groups, similar to that of the control group. It can be said that due to the protective action of AuNPs in the male reproductive system, it also increases the number of viable sperms. Sperm viability significantly decreased in acetaminophen-treated group in respect to control group, due to diminution in epididymal sperm count but increased significantly in AuNPs-treated group due effective increase in the sperm count.

Significant reduction of seminal vesicular fructose is noted when the animals were treated with acetaminophen. The decrease in seminal fructose level may be due to the inhibition in androgenesis as fructose quantity in seminal plasma is regulated by testosterone level. The seminal fructose level also significantly increased after the administration of AuNPs.

Thus, AuNPs helps to elevate the fructose level that can be related to the motility of the sperm as the sperms gets energy from fructose.

Cholesterol is the main precursor of androgen and testosterone; it is the final product of androgenesis. Testosterone is one of the prime regulators of sex organ growth and development. Sperm cells produce testosterone from cholesterol. Cholesterol is important for spermatogenesis. If sperm count is less then sperm cell cannot use cholesterol for the production of testosterone and it results in high cholesterol level. In acetaminophen-treated group, cholesterol level significantly increased; it may be due to decrease in the sperm count. A significant decrease in the cholesterol level was noted after treatment with AuNPs, which may be due to increase in the sperm count.

During toxicity, the free radicals are generated at the site of damage tissue and modulate the SOD and Catalase activity; as a result, the depletion of antioxidant enzymes activity and accumulation of superoxide radical was noted that causes damage to the testis. SOD and CAT are the most important antioxidant enzymes involved in ameliorating the effects of oxygen metabolism. Acetaminophen overdose may defeat the antioxidant defense mechanism in testicular tissue and result in a significant decrease in the SOD and CAT activities which is noted in group 2 animals. SOD and CAT level was significantly increased in AuNPS-treated group from that of the acetaminophen and *Terminalia arjuna*–treated group, quite similar to that of control group. Hence, AuNPs helps to prevent free radical generation at the site of tissue thus maintaining proper reproductive function.

The toxicity level of the testicular tissues were measured which showed increased toxicity in acetaminophen-treated rats which was marked by increase in GOT and GPT levels. After the AuNPs treatment GOT, GPT level was significantly decreased. Thus, AuNPs have an effective role in protecting of the testicular tissue from toxicity.

From the histopathological analysis, it was observed that epididymal sperm count decreases as a result density of the spermatogonia also reduced in acetaminophen-treated rats compared with the control. But in group 3, the density of the spermatids was increased than acetaminophen-treated group. The histological architecture of testis tissue in group 4 is a quite similar to that of the control group due to the preventive role of AuNPs. It was noted that in group 4, the Sertoli cells are condensed and spermatogonia were densely arranged around the base of the seminiferous tubule. Thus, from the study, it revealed that AuNPs might be effective in protection from acetaminophen-induced reproductive dysfunction in male Wistar rats.

4 Conclusion

The findings suggest that there is an adverse effect of acetaminophen on the reproductive system of the male Wistar rats but there is an effective role of AuNPs in preventing reproductive dysfunction induced by acetaminophen. This herbal nanoparticle is very much useful to restore the reproductive functions without having any side effects or toxicity. Thus, on the basis of this study, it can be concluded that the toxic effect of acetaminophen on the reproductive functions can be prevented by AuNPs which might serve as a harmless therapeutic approach.

5 Materials and Methods

5.1 Synthesis and Characterization of Green Synthesized Gold Nanoparticles Using *Terminalia arjuna*

AuNPs were synthesized and characterized according to Mitra et al. In 100 ml of distilled water, 1 g of *Terminalia arjuna* bark powdered was dissolved and incubated at 50–60° C for 15 min. Then, filtrate was collected by using Whatmann No. 1 filter paper. Then, 10 ml of aliquot was added to 100 ml of 1 mM HAuCl4 solution and the reaction was stirred for 10 min at 60–70° C for AuNPs synthesis. The change in the color from pale yellow to ruby red color within a minute indicated the formation of green synthesized gold nanoparticles [15].

5.2 Animal Selection and Care

Throughout the experiment, 24 adult male Wistar strain pathogen-free healthy rats were obtained from authorized Chakraborty Animal suppliers, Kolkata (M/S Chakraborty Enterprise Registration no.:1443/PO/b/11/CPCSEA) the study. The rats were12 weeks old having 90 ± 20 g weight. During experimental periods, the rats were maintained under standard laboratory condition that includes 22 ± 4° C and 50–10% humidity with proper supplement of food and water. The animals were housed in specially designed cages (6 rats per cage). The standard diet was provided and water *ad libitum*.

The rats were divided into 4 groups (6 in each group). Group 1 was named "control," group 2 was named "acetaminophen," group 3 was named "*Terminalia arjuna*" (plant extracts), and group 4 was named "AuNPs" (green synthesized gold nanoparticles using *Terminalia arjuna*).

5.3 Treatment Schedule

In the experimental design, the animals were divided into four groups. Group 1 (control) received no treatment. Group 2 received acetaminophen (500 mg/kg/day) through intraperitoneally. Group 3 received intraperitoneal infusion of acetaminophen co-administered with *Terminalia arjuna* bark extracts (175 μg/kg/day). Group 4 received acetaminophen with co-

administration of AuNPs (175 μg/kg/day) intraperitoneally. The treatments were conducted for 14 days and all groups were fed standard diet and water ad libitum. Animal treatment for the experimental studies was performed as per the Animal Ethical Committee guidelines (Reference number: 04/IAEC (3)/S/RNLKWC/2018) and were maintained as per Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA), Government of India (Registration no.:1905/PO/Re/S/2016/CPCSEA). All experimental protocols have been approved by the Constitutional of Institutional Animals Ethics Committee (IAEC) of Raja Narendra Lal Khan Women's College (Autonomous), Midnapore-721102, West Bengal, under registered Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA).

After the treatment schedule on the 15th day, the rats were anesthetized by the help of chloroform and then sacrificed. After that caudal epididymis, seminal vesicle and testis were collected from each rat and were first perfused with PBS and then stored at − 20° C. For preparation of the seminal vesicle and testis tissue homogenate, the tissues were separately homogenized in the ice-cold buffer containing 0.25 M sucrose, 1 Mm EDTA, and 1 mM tris HCl, pH 7.4. The seminal vesicle homogenate was centrifuged at 2500×*g* for 10 min at 4° C and testis homogenate was centrifuged at 1000×*g* for 10 min at 4° C, and the supernatant was stored at − 20° C for the biochemical estimation of different parameters [16].

5.4 Biochemical Tests

Epididymis sperm count [17] was done from freshly collected caudal epididymis by dilution of epididymal fluid with the help of phosphate buffer and hemocytometer. Sperm viability test [18] was conducted by staining freshly collected epididymal fluid with Eosin Y followed by Nigrosin and observing under microscope. Fructose level of the seminal vesicle was done by reaction of indole reagent in acidic media [19]. The testicular cholesterol was determined by using commercially available cholesterol kit [20] using semi auto analyzer. Superoxide dismutase (SOD) activity was determined by the ability of the testicular enzymes to inhibit the auto oxidation of pyrogallol in a UV spectrophotometer [21]. Catalase (CAT) activity of the testicular tissue was determined by the decomposition of H2O2 on the tissue supernatant [22]. Toxicity marker of the testicular tissue was determined by GOT (glutamate oxaloacetate transaminase) and GPT (glutamate pyruvate transaminase) from the collected testis [23].

5.5 Histopathological Assessments

The tissues were collected, fixed in 10% formalin solution, then dehydrated in graded (50–100%) alcohols followed by clearing in xylene. Then, paraffin embedding was done at 58° C for 4 to 5 h followed by paraffin block preparation. Paraffin sections of 5 μm were prepared using rotary microtome. Then, the sections were deparaffinized with xylene, stained with hematoxylin-eosin, followed by mounting with DPX with a coverslip [24]. Prepared slides were observed for histopathological alterations under light microscopy (Olympus model, Japan).

5.6 Statistical Analysis

The data were calculated and statistical analysis was done by using statistical package, Origin 6.1 Northampton, Mass, USA. The statistically collected data was calculated and were expressed as mean ± SE, $n = 6$. Comparison was done between the means of control and with all experimental groups, by ANOVA followed by multiple two-tail t test. Bars for a specific data differ from each other significantly ($p < 0.05$).

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Compliance with Ethical Standards

Ethics Approval Approval letter have been attached in the supplementary file.

Consent for Publication All authors have given approval for the submission of the manuscript.

Competing Interests The authors declare that they have no competing interests.

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